Pediatric Kidney Disease

Franz Schaefer
Larry A. Greenbaum
Editors

Third Edition

Springer

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A L G r a w a n y

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Preface

We are delighted to welcome you to the new edition of Pediatric Kidney Disease. As in the previous versions of this textbook, our goal has been to bridge the gap between multivolume "library-level" books and numerous pocket handbooks available in our specialty. We hope that Pediatric Kidney Disease will continue to be the standard textbook for reference to busy clinicians, who need to obtain an up-to-date, easy-to-read, review of virtually all kidney disorders that occur in children.

Following a critical review of the previous edition and the downloadable figures of each chapter, the table of contents was revised to optimally reflect the needs and expectations of our readers. One-third of the chapters were written by new authors. For chapters with unchanged authorship, each author was asked to thoroughly update the materials, which has resulted in extensive revisions of most chapters. A number of new topics have been included, such as metabolic disorders affecting the kidney, pediatric kidney tumors, sickle cell nephropathy, diabetic kidney disease, and strategic choices in kidney replacement therapy.

For all chapters, we have requested the authors to ensure the relevance and clinical usefulness of their chapter for busy pediatric and pediatric nephrology clinicians as well as the multidisciplinary team members. We hope that the included material and its presentation are of value and will contribute to the expansion of knowledge in the field of pediatric nephrology.

Heidelberg, Germany — Franz Schaefer
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1 Antenatal Assessment of Kidney Morphology and Function

Khalid Ismaili, Benedetta D. Chiodini, Marie Cassart, and Karim Khelif

Introduction

Due to high patient expectations and demand, obstetrical two-dimensional (2D) ultrasound (US) is now a routine component of the care of pregnant women in most Western countries. In Europe, three sonographic examinations are performed, one in each trimester [1]. In other countries, including the United States and Canada, only one second-mid-trimester examination is performed routinely, with first-trimester or third-trimester examinations performed in case of specific indications [2]. Assessment of the fetal genitourinary tract is part of every routine fetal US examination. Congenital abnormalities of the kidney and urinary tract (CAKUT) make up one of the largest groups of congenital anomalies amenable to neonatal care, affecting 0.2–2% of all newborns [1]. Moreover, dramatic changes have occurred in the management of these children, and nowadays, CAKUTs are mostly found in asymptomatic infants and the treatment applied is mainly preventive with a low rate of surgical procedures performed during the last decade [3]. Also, the antenatal detection and postnatal follow-up have brought new insights into the natural history of many CAKUTs [4, 5].

Fetal Imaging Methods

Ultrasound is the first line imaging technique used in antenatal diagnosis. It has now been nearly four decades since its first use to evaluate the fetus and it is presently accepted as a safe and noninvasive imaging modality. The transducers most commonly used are curvilinear sector transducers (3–8 MHz), which have good penetration of the sound beam and allow for a visualization of the whole fetus. Higher frequency linear transducers (5–10 MHz) or transvaginal probes are used to achieve high resolution scans in near fields. The transducers most frequently used in daily clinical practice are multifrequency probes which allow for harmonic, three-dimensional and Doppler flow imaging. *3D imaging* is mostly used for the study of the face and vertebral column [6], the other organs are well depicted by C

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imaging. Hence, 3D imaging is currently not routinely performed for urinary tract and kidney diseases. *Doppler imaging* is useful to evaluate the vascularization of the organs and the fetal well-being [2].

MR imaging of the fetus is a more recent technique. The examinations are performed on 1.5–3 T magnets. Although no deleterious effects on the fetus have been demonstrated to date [7], MR imaging is generally avoided during organogenesis in the first trimester. *MR imaging* is mostly performed in order to establish a more precise diagnosis where US cannot completely depict the suspected malformation or anomaly. The advantages of MR imaging are the larger field of view and the better contrast resolution which allow for a better characterization of the anatomy of the organs. The indications mostly concern the brain but it is presently extended to the fetal digestive and urinary tracts in specific indications [8]. Depending on local practice, sedation can be given to the mother before the examination to reduce fetal movement. Contrast media are not currently used due to the lack of data concerning potential side effects in pregnant women and fetuses [9]. Fast sequences (20 s) are performed in different planes and can be repeated in case of fetal movements. In urinary tract and kidney diseases the indications of fetal MR imaging are closely circumscribed; they will be illustrated in the next paragraphs.

The Normal Urinary Tract

Bladder

Urine starts to be produced during the ninth week of fetal life. At that time, the urine is collected in the bladder, which can be visualized as a fluid-filled structure within the fetal pelvis. During the second and third trimester, the fetus normally fills and partially or completely empties the bladder approximately every 25 min and the cycle can be monitored during the sonographic examination [10]. The bladder can easily be located by its outline of umbilical arteries, which are identifiable on color Doppler.

Kidneys

Endovaginal probes can be used to visualize fetal anatomic structures earlier than with transabdominal US. Thus, the fetal kidneys can be seen at around 11 weeks endovaginally and around 12–15 weeks with transabdominal probes. During the first trimester, the kidneys appear as hyperechoic oval structures at both sides of the spine (their hyperechogenicity can be compared with that of the liver or spleen) [11]. This echogenicity will progressively decrease and during the third trimester the cortical echogenicity will always be less than that of the liver or spleen. In parallel with the decrease of echogenicity, corticomedullary differentiation will appear at about 14–15 weeks. It should always be visible in fetuses older than 18 weeks (Fig. 1.1). Prominent pyramids should not be misinterpreted as calyceal dilatation.

Growth of the fetal kidneys can be evaluated throughout pregnancy. As a rule, a normal kidney grows at about 1.1 mm per week of gestation.

Evidence of Normally Functioning Urinary Tract

Besides visualization of the bladder and normal kidneys, assessment of the urinary tract should include an evaluation of the amniotic fluid volume. After 14–15 weeks, two thirds of the amniotic fluid is produced by fetal urination and one third by pulmonary fluid. A normal volume of

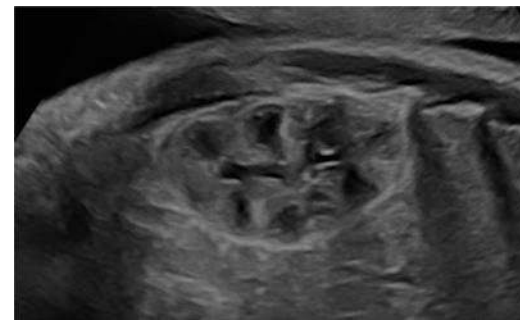

Fig. 1.1 Normal third trimester fetal kidney. Sagittal US scan of the kidney with clear visibility of the corticomedullary differentiation

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amniotic fluid is required for the proper development of the fetal lungs. This can be confirmed by measuring thoracic diameters or thoracic circumference [12].

Ultrasound Findings as Evidence of Abnormal Fetal Kidney and Urinary Tract

Abnormal US appearance of the kidneys as a pathophysiological base of CAKUT have been described extensively [13, 14]. Anomalies of the urinary system detected in utero are numerous; they can include anomalies of the kidney itself, of the collecting system, of the bladder and of the urethra. In addition, they can be isolated or in association with other systems. Therefore, the sonographic examination should be as meticulous as possible in order to visualize the associated features. These findings, among others, will determine the prognosis.

Abnormal Renal Number

Renal agenesis refers to the complete absence of one or both kidneys without identifiable rudimentary tissue [15].

Bilateral renal agenesis is part of Potter's syndrome and is incompatible with extrauterine life. The diagnosis is based on the absence of renal structure and the presence of oligohydramnios after 15 weeks of gestation. Pulmonary hypoplasia is invariably associated and leads to death from respiratory failure soon after birth. In this context, enlarged globular adrenals should not be mistaken for kidneys [16]. The use of color Doppler may help demonstrate the absence of renal arteries and subsequently confirm the diagnosis [17].

Unilateral renal agenesis is more common (1 in 500 pregnancies), usually asymptomatic and incidentally detected. This situation usually has no significant consequence on postnatal life as long as the solitary kidney shows compensatory hypertrophy [18]. The pathogenesis of renal agenesis is mostly due to a failure of the formation of the metanephros. In addition, interruption in vascular supply and regression of a multicystic dysplastic kidney (MCDK) may also lead to renal agenesis in the fetal period [19]. An investigation after birth is necessary to confirm the status of the remnant kidney and to look for possible associated anomalies such as hearing deficits, ear pits, coloboma, cleft lip or palate, single umbilical artery, syndactyly, microphallus, cryptorchidism, duplicated Mullerian structures, and ectopic ureteral insertion [18, 20]. In the long term, blood pressure and urinalysis should be monitored, in order to detect high blood pressure and/or microalbuminuria due to compensatory glomerular hyperfiltration. Children with congenital injury to the solitary kidney are at higher risk of adverse outcome, with a median time to chronic kidney disease of 14.8 years [21].

Abnormal Location of Kidney

Ectopic kidney, especially in the pelvic area, is part of the differential diagnosis of the "empty renal fossa" in the fetus and may represent 42% of these cases [18]. The diagnosis of horseshoe or crossed fused kidneys can also be assessed in utero through the demonstration of renal parenchyme crossing the midline in horseshoe kidney (Fig. 1.2) or attached to the lower pole of the normally positioned contralateral kidney, i.e. crossed fused renal ectopia [22]. An ectopic kidney is usually small and somewhat malrotated with numerous small blood vessels and associated ureteric anomalies. Ectopic kidneys may be asymptomatic, but complications such as ureteral obstruction, infection, and calculi are common [23]. Therefore, at birth, the anomaly has to be confirmed by US or by MRI and a voiding cystourethrography (VCUG) may be useful in complex cases.

Abnormal Renal Echogenicity

Hyperechogenicity of the fetal kidney is defined by comparison with the adjacent liver or spleen. This is difficult to assess in the first and second

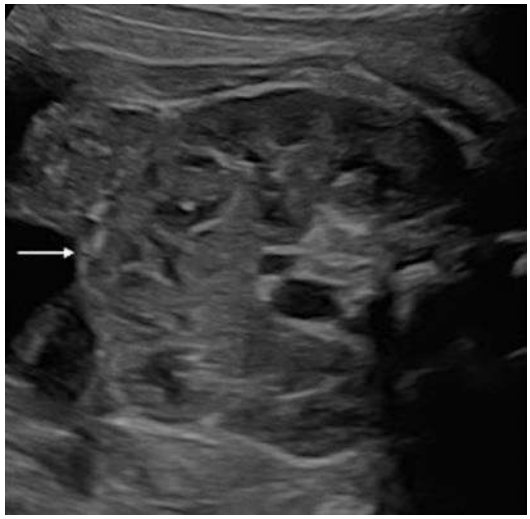

Fig. 1.2 Horseshoe kidney. Axial US scan on the fetal abdomen showing the parenchymal bridge crossing the midline (arrow)

trimester as the kidney is "physiologically" hyperechoic (or isoechoic at the end of the second trimester). It is easier to characterize after 28–32 weeks as the renal cortex by that time should be hypoechoic compared to the liver and spleen [11]. Increased echogenicity of the renal parenchyma is nonspecific and occurs as a response to different changes in renal tissue [24]. Interstitial infiltration, sclerosis and multiple microscopic cortical and medullary cysts may account for hyperechogenicity even in the absence of macrocysts. The detection of hyperechoic kidneys represents a difficult diagnostic challenge and generates significant parental anxiety due to the uncertain prognosis [25, 26]. The differential diagnosis must be based on kidney size, corticomedullary differentiation, the presence of macrocysts, the degree of dilatation of the collecting system and the amount of amniotic fluid [27]. The diagnosis must also take into account the familial history, the presence of associated anomalies and/or aberrant karyotype and genetic diseases [25]. Metabolic disorders should also be added to the long list of causes of hyperechoic kidneys in children (Fig. 1.3): tyrosinemia, galactosemia, fructosemia, mitochondrial disorders, glutaric aciduria, carnitine

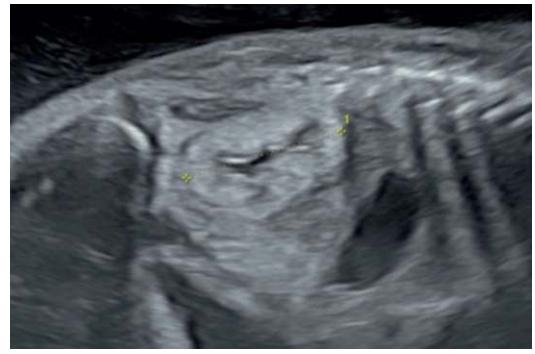

Fig. 1.3 Hyperechoic undifferentiated kidney. Sagittal US scan of the left kidney of a fetus with post natal diagnosis of nephrocalcinosis

palmitoyltransferase II deficiency, congenital disorders of glycosylation, and peroxisomal disorders can all be accompanied by hyperechoic kidneys [28].

So far, the outcome of fetal hyperechoic kidneys can only be accurately predicted in severe cases with significant oligohydramnios [26, 27]. For some patients, the characteristic US patterns will appear after birth or even later. A follow-up is therefore mandatory. It should be stressed that some cases remain unsolved and have to be considered as normal variants [25, 27]. Table 1.1 provides information on the spectrum of renal disorders associated with fetal hyperechoic kidneys.

Abnormal Renal Size

Measurements of the kidneys must be systematic whenever an anomaly of the urinary tract or amniotic fluid volume is suspected. It is therefore important to have standards for renal size and volume measurements covering the complete gestational age range, because renal pathology often presents late in pregnancy [31]. Small kidneys most often correspond to hypodysplasia or damaged kidneys from obstructive uropathy or high-grade vesicoureteral reflux (VUR) [32, 33]. Enlarged kidneys may be related to urinary tract dilatation, renal cystic diseases or tumoral involvement.

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Table 1.1 Conditions associated with hyperechoic kidneys on prenatal ultrasound

	Kidney size	Amniotic fluid volume	Renal cysts	Collecting system	Associated abnormalities	Inheritance	Alternative prenatal diagnosis
Obstruction	−2 to 0 SD	Normal or reduced	Cortical <1 cm	Dilated	No	Sporadic	MRI
Renal vein thrombosis	0 to 2 SD	Normal	No	Not seen	Thrombus in the inferior vena cava	Sporadic	Doppler
ARPKD	2 to 4 SD	Reduced	Small medullary	Not seen	Lung hypoplasia	AR	Genetics
ADPKD	0 to 2 SD	Normal or reduced	Subcapsular and medullar	Not seen	No	AD	Genetics
Glomerulocystic dysplasia	0 to 2 SD	Variable	Small cortical	Not seen	Variable if syndromic	Variable	Genetics (*HNF1B/TCF2*)
Bardet-Biedl syndrome	2 to 4 SD	Variable	No or medullary	Not seen	Polydactyly	AR	Genetics
Beckwith-Wiedeman syndrome	2 SD	Normal or increased	No or medullary	±	Macrosome, omphalocele	AD or dysomy	Genetics
Perlman syndrome	2 SD	Normal or reduced	No	±	Macrosome	AR	–
Infantile Hypercalcemia [29]	0 SD	Normal	No	Not seen	No	AR	Genetics SLC34A1
17q12 microdeletion syndrome [30]	Enlarged	Reduced or elevated	No or multicystic	Not seen			Microarray CGH
Normal variant	0 to 2 SD	Normal or increased	No	±	No	Sporadic	–

ARPKD autosomal recessive polycystic kidney disease, *ADPKD* autosomal dominant polycystic kidney disease, *AD* autosomal dominant, *AR* autosomal recessive, *MRI* magnetic resonance imaging, *HNF-1β* hepatocyte nuclear factor-1β

Urinary Tract Dilatation

Fetal renal pelvis dilatation is a frequent abnormality that has been observed in 4.5% of pregnancies [34]. Pyelectasis is defined as dilatation of the renal pelvis whereas pelvicaliectasis and hydronephrosis include dilatation of calyces. In practice, these terms are interchanged and used as descriptions of a dilated renal collecting system regardless of the etiology [35].

The third-trimester threshold value for the anteroposterior (AP) renal pelvis diameter of 7 mm is certainly the best prenatal criterion both for the screening of urinary tract dilatation and for the selection of patients needing postnatal investigation [35, 36].

There are several theories that account for the visibility of the renal pelvis during pregnancy. The distension of the urinary collecting system may be simply a dynamic and physiologic process [37]. The size of the fetal renal collecting system is highly variable over a 2-h period [38]. The tendency of renal pelvis dilatation to resolve spontaneously is supported by normal postnatal renal appearances reported in 36–80% of cases followed up after birth [39, 40]. However, prenatally detected renal pelvis dilatation may be an indicator of significant urinary tract pathologies [41]. The likelihood of having a clinically significant uropathy is directly proportional to the severity of the hydronephrosis [35]. A summary of the literature describing the postnatal uronephropathies found in neonates who presented with fetal renal pelvis dilatation is given in Table 1.2. The incidence and type of pathology varies considerably between studies, reflecting the differences in prenatal criteria and the variability in postnatal assessment. The two main pathologies found are pelviureteric junction stenosis and VUR. US is the first examination to perform after birth [46]. In babies diagnosed in utero with renal pelvis dilatation, the presence of persistent renal pelvis dilatation or other ultrasonographic abnormalities (such as calyceal or ureteral dilatation, pelvic or ureteral wall thickening and absence of the corticomedullary differentiation) and signs of renal dysplasia (such as small kidney, thinned or hyperechoic cortex or cortical cysts) should determine the need for further investigations [47, 48]. In cases when the urinary tract appears normal on neonatal US examinations, no further evaluation is needed [39]. Based on our own experience [34, 39, 49, 50], we propose an algorithm for a rational postnatal imaging strategy (Fig. 1.4). Using this algorithm, we found that very few abnormal cases escaped the work up and that the risk of complications was very low.

Renal Cysts

Renal cystic diseases should be suspected not only in the case of obvious macrocysts but also in the case of hyperechoic kidneys [51]. Cysts may be present in one or both kidneys. Their origin may be genetic, and they may occur as an isolated anomaly or part of a syndrome. Familial history is of a great importance for the diagnosis [51].

Obstructive renal dysplasia and MCDK are the most common entities in which macrocysts can be detected. Obstructive renal dysplasia is associated with urinary tract obstruction that may have resolved at the time of diagnosis, leaving the cystic sequelae behind as the unique evidence that a urinary flow impairment ever existed [11]. In this condition, the cysts measure less than 1 cm and are located within the hyperechoic cortex (Fig. 1.5) [51]. MCDK is discussed further under Renal Causes of Fetal Renal Abnormalities in this chapter. Although rare, isolated cortical cysts may be seen in utero. They may persist after birth or regress spontaneously [52].

The most frequent genetically transmitted cystic renal diseases are the autosomal dominant polycystic kidney diseases and abnormalities of the hepatocyte nuclear factor-1β (HNF1B) encoded by the *TCF2* gene [53]. HNF1B diseases are typically associated with bilateral cortical renal microcysts as well as other renal parenchymal abnormalities including MCDK and renal dysplasia [53]. HNF1B plays an important role in the early phases of kidney development [54]. Both the type and the severity of the renal disease are variable in children with HNF1B mutations. Their

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Table 1.2 Incidence of uro-nephropathies in neonates with antenatally diagnosed renal pelvis dilatation

Authors	Year	Threshold value of renal pelvis (mm)	Total	Abnormal (%)	UPJS (%)	VUR (%)	Megaureter (%)	Mild dilatation (%)	Duplex kidney (%)	Other (%)	(%) Undergoing surgery
Dudley [42]	1997	5	100	64	3	12	3	43	4	7	3
Jaswon [43]	1999	5	104	45	4	22		8		4	1
Ismaili et al. [39]	2004	4–7	213	39	13	11	7	18[a]	5	3	3
Bouzada [44]	2004	5	100	57	30	2	2		1		11
Vasconcelos [45]	2019	5	624		18	8	6	40	3		26

UPJS uretero-pelvic junction stenosis, *VUR* vesicoureteral reflux

[a] In this study mild and transient dilatations were considered as non-significant findings

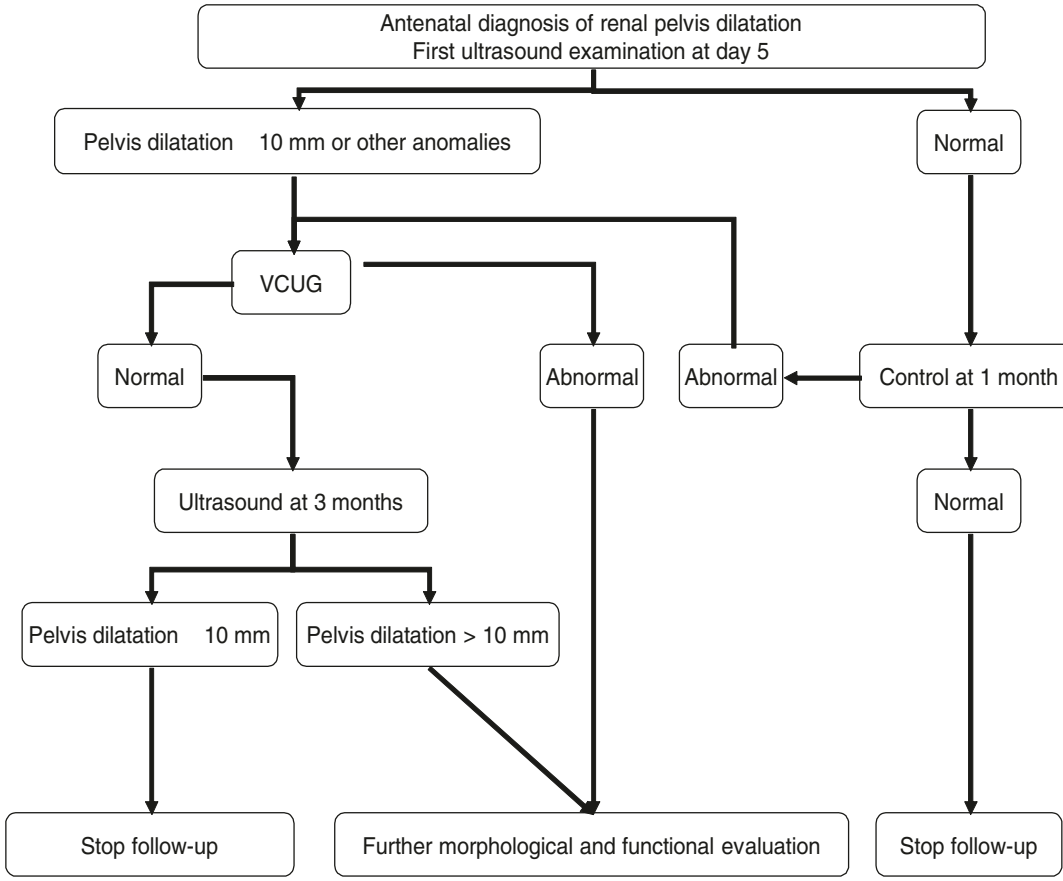

Fig. 1.4 Algorithm of a rational postnatal imaging strategy in infants with fetal renal pelvis dilatation

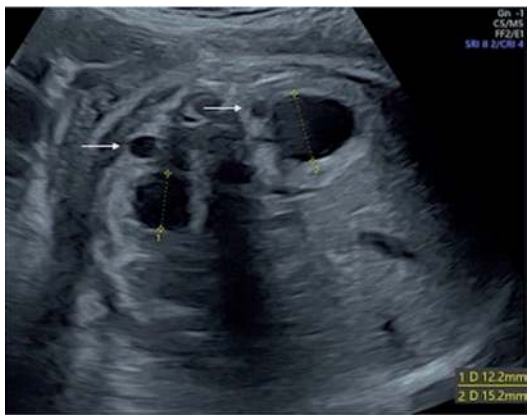

Fig. 1.5 Bilateral obstructive cystic nephropathy. Axial slice on the dilated renal pelvis with hyperechoic and cystic parenchyma (arrows)

range from severe prenatal renal failure to normal renal function in adulthood. There is no obvious correlation between the type of mutation and the type and/or severity of renal disease. Furthermore, the inter- and intrafamilial variability of the phenotype in patients who harbor the same mutation is high, making genetic counseling particularly difficult in these families [55]. Cystic kidneys are also part of many syndromes (Tables 1.1 and 1.3) with many associated anomalies that are sometimes typical of the underlying pathology.

Renal Tumors

Fetal renal tumors occur only rarely. Mesoblastic nephroma represents the most common congenital renal neoplasm [65]. It is a solitary hamartoma with a usually benign course. Mesoblastic nephroma appears as a large, solitary, predominantly solid, retroperitoneal mass arising and not

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Table 1.3 Syndromes with cystic renal disease

	Renal cysts	Associated abnormalities	Inheritance	Reference
Meckel-Gruber syndrome	Medullary	Encephalocele, brain/cardiac anomalies, hepatic ductal dysplasia, cleft lip/palate, polydactyly	AR	[56]
Trisomy 9	Medullary	Mental retardation, intrauterine growth retardation, cardiac anomalies, joint contractures, prominent nose, sloping forehead	Chromosomal	[57]
Trisomy 13	Medullary	Mental retardation, intrauterine growth retardation, cardiac anomalies, cleft lip/palate, polydactyly	Chromosomal	[57]
Trisomy 18	Medullary	Mental retardation, intrauterine growth retardation, cardiac anomalies, small face, micrognathia, overlapping digits	Chromosomal	[57]
Bardet-Biedl syndrome	No or medullary	Polysyndactyly, obesity, mental retardation, pigmented retinopathy, hypogonadism	AR	[58]
Zellweger syndrome	Medullary	Hypotonia, seizures, failure to thrive, distinctive face, hepatosplenomegaly	AR	[59]
Ivemark syndrome	No or medullary	Polysplenia, complex heart disease, midline anomalies, situs inversus	Sporadic, AR	[60]
Beckwith-Wiedeman syndrome	Medullary	Overgrowth, macroglossia, omphalocele, hepatoblastoma, Wilm's tumor	Sporadic, AD	[61]
Jeune's syndrome	Medullary	Narrow chest, short limbs, polydactyly, periglomerular fibrosis	AR	[62]
Tuberous sclerosis	Medullary	Mental retardation, seizures, facial angiofibroma, angiomyolipoma, hypopigmented spots, cardiac rhabdomyomas, cerebral hamartomas	AD	[63]
Alagille syndrome	Renal dysplasia with or without cysts	Cholestasis, peripheral pulmonic stenosis, characteristic face	AD	[64]

AD autosomal dominant, *AR* autosomal recessive

separable from the adjacent kidney. It does not have a well-defined capsule and may sometimes appear as a partially cystic tumor [11]. In case of a tumor with multiple cysts, a MCDK should be considered first. Mesoblastic nephroma frequently coexists with polyhydramnios although the reason for this association remains unclear [65]. Fetal Wilm's tumor is exceptionally rare and may be indistinguishable from mesoblastic nephroma on imaging [66]. Another differential diagnosis is nephroblastomatosis, which appears either as hyperechoic nodule(s) or as a diffusely enlarged hyperechoic kidney [67]. Renal tumors have to be differentiated from adrenal tumors and intra-abdominal sequestrations [68].

Bladder Abnormalities

On fetal US examinations, the bladder should always be seen from the tenth week of gestation. Nonvisualization of the bladder in the setting of oligohydramnios is highly suspicious of a bilateral severe renal abnormality with decreased urine production. It is important to carefully analyze the kidneys in order to exclude agenesis or dysplasia associated with poor outcome.

Nonvisualization of the bladder with an otherwise normal sonogram (kidneys and amniotic fluid) may be due to physiologic bladder emptying cycle in the fetus. Normal repletion should be checked within the following 20 min. Persistent non visualized bladder can be due to its inability to store urine, for example in cases of bladder or cloacal extrophy [69]. In this context, no bladder is seen between the two umbilical arteries. Bladder extrophy or cloacal malformations represent a diagnostic challenge on US, and MRI may help to define the pelvic anatomy of the fetus [70].

Enlarged bladder in the first trimester has a poor prognosis. Most of the cases are secondary

to urethral atresia or stenosis, some are part of a syndrome (such as Prune Belly), or associated with chromosomal anomalies. Later in pregnancy, megabladder is defined as a cephalocaudal diameter superior to 3 cm in the second trimester and to 5 cm in the third trimester. Megabladders are mainly due to outflow obstruction or to a major bilateral reflux [71]. It is often difficult to make a clear distinction between both abnormalities, as they may be associated. An irregular and thickened bladder wall is suggestive of an outflow obstruction. Megacystis-microcolon hypoperistalsis (MMH) syndrome is another differential diagnosis that carries a very poor prognosis [72]. It can be excluded by MRI during the third trimester because the colon is well visualized at that time.

Assessment of Fetal Renal Function

In utero, excretion of nitrogenous waste products and regulation of fetal fluid and electrolytes balance as well as acid-base homeostasis are maintained by the interaction of the placenta and maternal blood [73]. Thus, the placenta functions as an in vivo dialysis unit. Several parameters have been used in the evaluation of renal function in fetal life. However, since fetal homeostasis depends on the integrity of the placenta, it is very difficult to assess the functional status of the fetal kidney. Furthermore, changes in the volume or composition of fetal urine may in many instances reflect the condition of the placenta rather than the condition of the fetal kidney [74]. However, exact diagnosis of the renal abnormalities and accurate prediction of the renal function after birth are important tasks, because during parental counseling parents often want to know if their child will have renal failure and whether or not surgery or other aggressive therapies will be performed. Therefore, in addition to using fetal renal sonography to determine potential fetal renal anatomical abnormalities, it is important to assess function as accurately as possible.

Amniotic Fluid Volume

During the first trimester of gestation the placenta (chorion and amniotic membrane) is the principal source of amniotic fluid, while after 15 weeks the volume of amniotic fluid is maintained by fetal urine production. Therefore, the assessment of the quantity of amniotic fluid after 15 weeks constitutes the initial step in the evaluation of the fetal urinary tract. Abnormal amounts of amniotic fluid must alert the sonographer to search meticulously for renal and urinary tract anomalies [75]. Assessing amniotic fluid volume is difficult and mostly subjective. However, the four-quadrant sum of amniotic fluid pockets (amniotic fluid index) provides a reproducible method for assessing amniotic fluid volume with interobserver and intraobserver variation of 3–7% [76].

Various cut-off criteria have been suggested for definition of oligohydramnios by amniotic fluid index, including less than 3rd percentile [77], or 5th percentile for gestational age [76]. Oligohydramnios of any cause typically compresses and twists the fetus, thus leading to a recurrent pattern of abnormalities that has been called the oligohydramnios sequence [12]. Oligohydramnios may be caused by decreased production of fetal urine from bilateral renal agenesis or dysplasia, or by reduced egress of urine into the amniotic fluid due to urinary obstruction [78]. Other causes may be fetal death, growth retardation, rupture of the membranes, or post-term gestation (Table 1.4).

In cases of bilateral obstructive uropathy, the evaluation of amniotic fluid by the amniotic fluid index seems to be the most reproducible and inexpensive method to predict renal function after birth [79]. An amniotic fluid index less than the 5th percentile is generally associated with an adverse perinatal outcome [76, 79]. Yet, an amniotic fluid index between the 5th and 25th percentiles should be considered as a warning sign since it may be a subtle indication of renal impairment, especially early in gestation when ultrasonographic signs of renal dysplasia may not be present and when fetal urinalysis is not available [79].

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Table 1.4 Causes of oligohydramnios and polyhydramnios

	Origin	Pathologies
Oligohydramnios	Uronephropathy	Bilateral renal agenesis Bilateral renal dysplasia Autosomal recessive polycystic kidney disease Bilateral obstructive uropathy Bilateral high-grade reflux Bladder outlet obstruction
	Other	Premature rupture of membranes Placental insufficiency Fetal death Fetal growth retardation Twin-to-twin transfusion (twin donor) Maternal drug intake: prostaglandin synthase inhibitors, angiotensin-converting enzyme inhibitors, cocaine Postmaturity syndrome
Polyhydramnios	Uronephropathy	Renal tumors, especially mesoblastic nephroma Bartter syndrome Congenital nephrotic syndrome Alloimmune glomerulonephritis
	Other	Maternal diabetes Maternal drug intake: lithium Multiple gestations Twin-to-twin transfusion (twin recipient) Fetal infections: rubella, cytomegalovirus, toxoplasmosis Fetal gastrointestinal obstructions: esophageal atresia, duodenal atresia, gastroschisis Fetal compressive pulmonary disorders: diaphragmatic hernia, pleural effusions, cystic adenomatoid malformations, narrow thoracic cage Neuro-muscular conditions: anencephaly, myotonic dystrophy Cardiac anomalies Hematologic anomalies (fetal anemia) Hydrops fetalis Fetal chromosome abnormalities: trisomy 21, trisomy 18, trisomy 13 Syndromic conditions: Beckwith-Wiedeman syndrome, achondroplasia No evident cause

Finally, one of the most devastating consequences of oligohydramnios, especially before 24 week's gestation, is pulmonary hypoplasia [80]. Traditional explanations suggest that oligohydramnios causes pulmonary hypoplasia either by compression of the fetal thorax [80] or by encouraging lung liquid loss via the trachea [81]. However, since several morphogenetic pathways governing renal development are shared with lung organogenesis, this sequence is put into question. Some reports suggest that abnormal lung dysplasia may precede the advent of oligohydramnios in fetuses with intrinsic defects of renal parenchymal development [82].

Polyhydramnios, also referred to as hydramnios, is defined as a high level of amniotic fluid. Because the normal values for amniotic fluid volumes increase during pregnancy, this definition will depend on the gestational age of the fetus. During the last 2 months of pregnancy, polyhydramnios usually refers to amniotic fluid volumes greater than 1700–1900 mL. Severe cases are associated with much greater fluid volume excesses. The two major causes of polyhydramnios are reduced fetal swallowing or absorption of amniotic fluid and increased fetal urination (Table 1.4). Increased fetal urination is typically observed in maternal diabetes mellitus, but it may be associated with fetal renal diseases as mesoblastic nephroma [65], Bartter syndrome [83], congenital nephrotic syndrome [84] and alloimmune glomerulonephritis [85].

Fetal Urine Biochemical Markers

The healthy fetus produces hypotonic urine. In case of kidney damage, proximal tubular function is harmed and urine osmolality increases. Fetal urine biochemistry was first introduced four decades ago as an additional test to improve prediction of renal function after birth [86]. Thereafter, investigators started to establish gestational age-dependent reference ranges for various biochemical parameters of fetal urine [87]. Rapidly, two important pitfalls emerged in this area of research. Initially, biochemical markers were analyzed either from amniotic fluid or bladder sampling based on the debatable assumption that both liquids have nearly identical composition. Subsequently, a practice has emerged whereby reference ranges are only taken from bladder or fetal urinary tract sampling. The second pitfall involved the pertinence of the use of urinary solutes that are filtered by the glomerulus and reabsorbed by the tubules. These solutes express a tubular damage rather than a compromised GFR and it is therefore questionable if they can accurately predict renal function.

Fetal urine biochemistry is currently used especially in dilated uropathies because of technical difficulties of sampling fetal urines from a nondilated urinary tract, as seen in the majority of nephropathies. β2-microglobulin is the most widely used fetal urinary marker, although other compounds such as calcium, chloride and sodium may also be of interest; prognostic values of these markers are outlined in Table 1.5. Most fetal urine studies agree on some points [88, 89]: (1) fetuses with renal damage (dysplasia) show increased urinary solute concentrations; (2) a combination of β2-microglobulin and cystatin C or chloride exhibits better accuracy than the measurement of any single electrolyte; (3) the predictive accuracy of the proposed parameters is, however, far from perfect.

Novel approaches to fetal urine biochemistry such as proteomics and metabolomics may yield novel markers that may improve the usefulness of this technique in the near future [95]. The ongoing European ANTENATAL study was designed to validate a fetal urine peptide signature proposed by Buffin-Meyer in order to predict postnatal renal function in fetuses with posterior urethral valves [96, 97]. The final results are expected in 2023.

Fetal Blood Sampling

Fetal blood sampling poses probably greater risks than urine sampling, but it allows a more accurate evaluation of fetal glomerular filtration rate (GFR) [94, 98, 99]. In the fetus, creatinine cannot be used as a marker of GFR because it crosses the placenta and is cleared by the mother. This is not the case for α1-microglobulin, β2-microglobulin and cystatin C, which have been used to predict renal function in uropathies and nephropathies (Table 1.6). This technique may be helpful, especially in cases where fetal urine is difficult to sample. It is however unlikely that fetal serum

Table 1.5 Fetal urine biochemical markers of postnatal prognosis

	Normal limits [90]	Good prognosis [91–93]	Moderate renal failure at age 1 year [94]	Poor prognosis (neonatal death or termination of pregnancy) [92–94]
Na^+	75–100 mmol/L	<100 mmol/L	59 mmol/L (54–65)	121 mmol/L (100–140)
Ca^{2+}	2 mmol/L		2 mmol/L (1.5–2.5)	2 mmol/L (1.5–2.5)
Cl^-		<90 mmol/L	57 mmol/L (52–62)	98 mmol/L (85–111)
β2 microglobulin	<4 mg/L	<6 mg/L	6.8 mg/L (4.2–9.4)	19.5 mg/L (11–28)
Cystatin C		<1 mg/L	0.47 mg/L (0.05–4.75)	4.1 mg/L (0.45–13.1)
Protein		0.22 g/L (0.09–0.97)		0.28 g/L (0.06–13.5)
Osmolarity	<200 mOsm/L	<210 mOsm/L		

Table 1.6 Fetal serum biochemical markers of postnatal prognosis

	Controls [98]	Bilateral hypoplasia and dysplasia [99]	Bilateral uropathies [100]
Outcome		Good prognosis	Postnatal renal failure
β2-microglobulin	4.28 mg/L (2.95–6.61)	3.2 mg/L (1.5–3.7)	5.3 mg/L (3.5–7.2)
Cystatin C	1.67 mg/L (1.12–2.06)	1.43 mg/L (1.09–1.86)	1.95 mg/L (1.56–4.60)
α1-microglobulin			60.5 mg/L (31.8–90.1)

α1-microglobulin, β2-microglobulin and cystatin C will overcome the limitations associated with fetal urinalysis [98]. The only clinical useful information emerging from the studies performed to date is that fetal serum β2-microglobulin remains the best marker of renal function; however its helpfulness is questionable outside extreme values (less than 3.5 mg/L good outcome; more than 5 mg/L poor outcome) [100].

Ultrasound-Guided Renal Biopsies

Ultrasound-guided renal biopsy would theoretically allow precise definition of the extent of renal damage in obstructed and primarily dysplastic kidneys [101]. However, this is an invasive procedure with a high rate of failure in obtaining an adequate sample [94]. Furthermore, a focal needle aspiration is not representative of the whole kidney parenchyma since renal dysplasia is patchily distributed.

Specific Renal and Urinary Tract Pathologies

Causes of fetal abnormalities of the kidney and urinary tract may be considered as prerenal, renal and postrenal.

Pre-renal Causes of Fetal Renal Abnormalities

Intrauterine Growth Restriction (IUGR)

Intrauterine growth restriction (IUGR) complicates up to 10% of all pregnancies. It is associated with a perinatal mortality rate that is six to ten times higher when compared to normally grown fetuses and is the second most important cause of perinatal death after preterm delivery. The cause of IUGR is multifactorial. Worldwide, maternal nutritional deficiencies and inadequate utero-placental perfusion are among the most common causes of IUGR.

IUGR caused by placental insufficiency is often associated with oligohydramnios due to reduced urine production rate in these fetuses. This phenomenon is probably due to chronic hypoxemia that leads to the brain-sparing redistribution of oxygenated blood away from non-vital peripheral organs such as the kidneys [73]. As a consequence, fetal renal medullary hyperechogenicity may develop between the 24th and the 37th weeks of gestation due to tubular blockage caused by Tamm-Horsfall protein precipitation, and may be a sign of hypoxic renal insufficiency [102]. IUGR complicated by renal medullary hyperechogenicity suggests a more serious state, because these fetuses have a higher risk of pathological postnatal clinical outcome, such as neonatal mortality (8%), fetal distress leading to cesarean section (36%), transfer to intensive care unit (64%), and perinatal infection (24%) [102]. IUGR not only leads to a low birth weight but it might also reprogram nephrogenesis, which results in a low nephron endowment. According to the hyperfiltration hypothesis, this reduction in renal mass is supposed to lead to glomerular hyperfiltration and hypertension in remnant nephrons with subsequent glomerular injury with proteinuria, systemic hypertension and glomerulosclerosis in adult age [103].

Renal Vein Thrombosis

Renal vein thrombosis is the most common vascular condition in the newborn kidney and represents 0.5/1000 of admissions to neonatal intensive care units [104]. Factors predisposing a neonate

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to renal vein thrombosis include dehydration, sepsis, birth asphyxia, maternal diabetes, polycythemia and the presence of indwelling umbilical venous catheter [104]. In addition, prothrombotic abnormalities may be present in more than 40% of these babies, such as Protein C or S deficiency, Factor V Leiden mutation, Lupus anticoagulant and Antithrombin III deficiency [105].

Renal vein thrombosis may also occur in utero. However, the origin of the thrombosis is not always obvious. Sonographically, the fetal kidney appears somewhat enlarged; the cortex may appear hyperechoic and without corticomedullary differentiation (Fig. 1.6a). Pathognomonic vascular streaks may be visible in the interlobar areas. Thrombus in the inferior vena cava is a common association [11]. Color Doppler US may be used in addition to grey-scale examination in the assessment of renal vein thrombosis. In the early stages of renal vein thrombosis, intrarenal and renal venous flow and pulsatility may be absent and renal arterial diastolic flow may be decreased, with a raised resistive index. Collateral vessels develop very rapidly (Fig. 1.6b) and in most cases there are no consequences on further renal development [106]. After birth, the hyperechoic streaks and the thrombus are calcified. This feature helps differentiate antenatal from postnatal onset of the renal vein thrombosis [106].

The Twin-To-Twin Transfusion Syndrome

The twin-to-twin transfusion syndrome complicates 10–15% of monochorionic twin pregnancies [107]. The etiology of this condition is thought to result from an unbalanced fetal blood supply through the placental vascular shunts, with the larger twin being the recipient and the smaller twin the donor [108]. The twin-to-twin transfusion syndrome is defined by the existence of a oligo-polyhydramnios sequence (that is, the deepest vertical pool being 2 cm or less in the donor's sac and 8 cm or more in the recipient's sac) [107]. Additional phenotypic features in the donor include a small or nonvisible bladder and abnormal umbilical artery Doppler with absent or reverse end-diastolic frequencies. In addition to the neonatal complications of growth restriction, up to 30% of donors have renal failure and/or renal tubular dysgenesis due to the chronic renal hypoperfusion state in utero [107]. In the recipient, confirmatory features include large bladder, cardiac hypertrophy and eventually hydrops. Risk of renal failure in the recipient twin is considerably smaller than in the donor twin. This can be seen as one fetus dying and vascular resistance dropping significantly to cause reversed blood transfusion from the recipient twin to the dead fetus, resulting in hypovolemia and anemia in the live fetus [109].

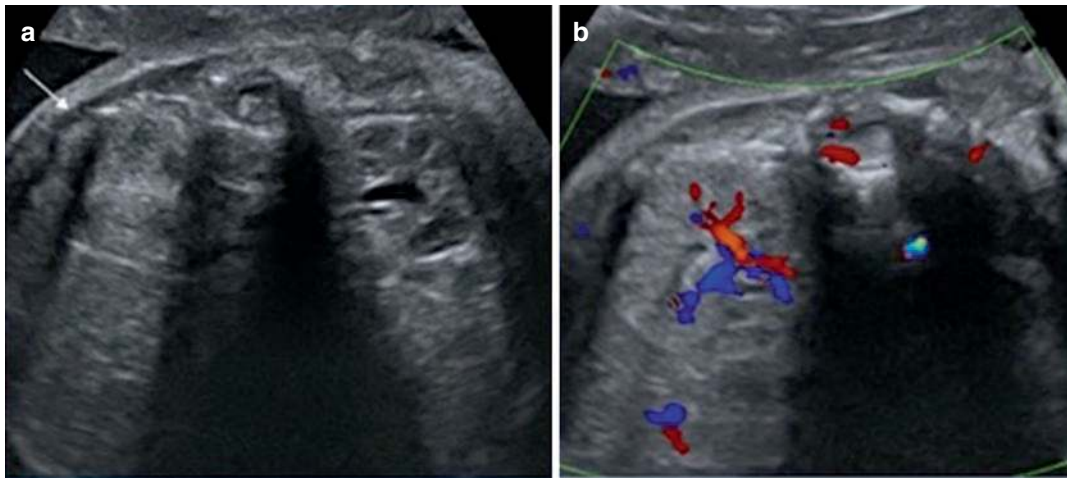

Fig. 1.6 Right renal vein thrombosis. (**a**) Axial US scan showing differences of size and echogenicity of both kidneys. The right kidney is enlarged and hyperechoic (arrow). (**b**) Axial US scan showing normal renal arterial and venous flow on the right kidney attesting of rapid revascularisation

Maternal Alcohol/Drug Intake

Mother's alcohol and/or drug consumption can in certain cases affect fetal kidney function or cause congenital kidney anomalies.

Alcohol

Based on both animal and human studies, it has been suggested that children with fetal alcohol syndrome (FAS) should be screened for renal anomalies [110]. The kidneys of children exposed prenatally to ethanol (with or without FAS) were smaller as compared to children with no evidence of alcohol exposure in utero [110]. Recent studies from Portugal have found an association between alcohol consumption during pregnancy and a decreased kidney function later in childhood, especially in overweight schoolchildren [111].

Renin-Angiotensin System (RAS) Antagonists

Administration of angiotensin converting enzyme inhibitors (ACEIs) and angiotensin type I receptor blockers (ARBs) can severely affect renal development and function at any gestational age. RAS inhibitors lead to tubular dysgenesis, oligohydramnios, growth restriction, neonatal anuria and stillbirth [112–115]. In one study almost 9% of children exposed to ACEIs during the first trimester (but not later in pregnancy) showed major congenital anomalies (cardiovascular, central nervous system and renal malformations) at a rate 2.7 times that among unexposed infants [113]. The renal anomalies are thought to be caused both directly by antagonism of the fetal intrarenal renin-angiotensin system and indirectly by fetoplacental ischemia resulting from maternal hypotension and a drop of fetal-placental blood flow [73]. Pregnancies exposed to RAS blockers and complicated by oligohydramnios are associated with the highest rate of adverse pregnancy outcomes. Combined monitoring of amniotic fluid volume evaluation and fetal serum β2-microglobulin may be helpful in the management of these pregnancies [116].

Nonsteroidal Antiinflammatory Drugs (NSAID)

Cyclooxygenase type 1 (COX-1) inhibitors such as indomethacin, the most common NSAID used as a tocolytic, definitely reduce urine output and may lead to oligohydramnios and renal dysfunction [73]. It was hoped that cyclooxygenase type 2 (COX-2) inhibitors would target COX-2 activity and potentially spare COX-1-specific fetal side effects. However, COX-2 expression is higher in fetal as compared to adult kidneys and may even occur constitutively in fetal kidney tissue [73]. Sulindac and nimesulide administration has therefore been linked both to constriction of the ductus arteriosus and oligohydramnios [117].

Cocaine

Maternal cocaine use adversely influences fetal renal function by hypoperfusion and thus influences the fetal renin-angiotensin system. It is also associated with oligohydramnios as well as other fetal vascular complications leading to higher renal artery resistance index and a significant decrease in urine output [118]. However, and contradicting a widely held belief [119], a prospective, large-scale, blinded, systematic evaluation for congenital anomalies in prenatally cocaine-exposed children did not identify any increase in the number or consistent pattern of genitourinary tract malformations [120].

Immunosuppressive Medications During Pregnancy

Historically, physicians discouraged pregnancy in female kidney transplant recipients, due to concerns for maternal, graft, and fetal health [121]. Nevertheless, a large number of women with transplanted organs have completed pregnancies [122]. Although some immunosuppressants such as corticosteroids, cyclosporine and azathioprine have been shown to be teratogenic in animals, case reports and registry records have not identified any consistent malformation patterns in children of allograft recipients [123–126] (Table 1.7). On the contrary, the use of mycophenolate mofetil (MMF) during pregnancy has been

Table 1.7 In utero malformations caused by the exposure to immunosuppressive drugs used in kidney transplant recipients

Immunosuppressive drugs	Congenital malformations	References
Corticosteroids	In animals: increased risk of cleft palate. In humans: no increased prevalence of congenital malformations even at high doses.	[123]
Calcineurin inhibitors	In animals: skeletal retardation in cyclosporine exposure. In humans: no increased prevalence of congenital malformations.	[124, 125]
Azathioprine	In animals: various congenital malformations. In humans: safe in pregnant women.	[126]
Mycophenolic acid	In animals: wide range of teratogenic and fetotoxic effects; high miscarriage rate. In humans: congenital malformation prevalence of 26% (orofacial defects, hypoplastic nails, short fifth finger, corpus callosum agenesis, myelomeningocele, hydronephrosis, atrial septal defect, and tracheo-esophageal atresia).	[127–129]
Rapamicin inhibitors (mTOR)	In animals: high risk of intra-uterine growth retardation, impaired skeletal ossification, and miscarriage. In humans: few studies, no congenital malformations reported.	[130]
ATG	No data available	[131]
Basiliximab	No data available	[131]
Rituximab	No data available	[131]
Belatacept	No data available	[131]

clearly associated with teratogenicity in humans [127–129] (Table 1.7). Therefore, European best practice guidelines recommend that women receiving MMF switch to another drug and wait at least 6 weeks before attempting to conceive [131, 132].

Primary Congenital Kidney Anomalies

CAKUT is the most common cause of pediatric kidney failure [133]. CAKUT is highly heterogeneous, and the etiologic factors are not completely understood. These conditions are genetically variable and encompass a wide range of anatomical defects, such as renal agenesis, renal hypodysplasia, pelviureteric junction stenosis, and VUR. Mutations in genes causing syndromic disorders, such as *HNF1B* and *PAX2* mutations, are detected in only 5–10% of cases [134]. Familial forms of nonsyndromic disease have also been reported [135], further supporting a genetic determination such as *ACTA2* and *CHRM3* genes in Prune belly syndrome, *HPSE2* and *LRIG2* genes in Ochoa syndrome, among many others (Table 1.8). However, owing to locus heterogeneity and small pedigree size, the genetic cause of most familial or sporadic cases remains unknown [135].

Multicystic Dysplastic Kidney (MCDK)

These kidneys contain bizarrely shaped tubules surrounded by a stroma, that includes undifferentiated and metaplastic cells (for example, smooth muscle and cartilage). According to Liebeschuetz [155] the prevalence of MCDK is about 1 in 2400 live births, which is higher than other reports [156]. MCDK is usually unilateral and presents a typical US pattern: multiple noncommunicating cysts of varying size and nonmedial location of the largest cyst, absence of normal renal sinus echoes, and absence of normal renal parenchyma [157]. MCDK may also develop in the upper part of a duplex system or be located in an ectopic position. Unilateral isolated MCDK carries a good prognosis but careful examination of the contralateral kidney is essential because there is a high incidence of associated pathologies, many of which may not be detected until birth [158]. These associated malformations include ectopic ureteral insertions more commonly in the semi-

Table 1.8 Genes implicated in urinary tract malformations

Urinary tract malformation	Genes implicated	Mutated protein
Bladder extrophy	*ISL1* [136]	Transcription factor potentially involved in formation of the bladder and urethra
Branchio-oto-renal syndrome	*EYA1* [137]	Transcriptional co-activator required for eye morphogenesis
	SIX1 [138]	Homeobox protein similar to *EYA* gene product
Campomelic dysplasia (sex reversal, megaureter)	*SOX9* [139]	Transcription factor modulating smooth muscle in the ureter
Diverse kidney malformations	*TCF2* [140]	Transcription factor widely expressed in renal tract epithelia
Megaureter	*TBX18* [141]	Transcription factor affecting ureter morphogenesis
	TSHZ3 [142]	Transcription factor modulating smooth muscle
Primary VUR, Ehler-Danlos	*TNXB* [143]	Extracellular matrix protein found in the urinary tract
Posterior urethral valves	*BNC2* [144]	Basonuclin 2, a zinc finger containing protein implicated in epithelial maturation
Prune belly syndrome, megacystis microcolon intestinal hypoperistalsis	*ACTA2* [145]	Smooth muscle contractile protein
	ACTG2 [146]	Smooth muscle contractile protein γ2-actin
	CHRM3 [147]	M3, the main acetylcholine receptor in detrusor smooth muscle
	MYH11 [148]	Smooth muscle contractile protein called myosin heavy chain 11
	MYLK [149]	Myosin light chain kinase modifying myosin in smooth muscle cells
	MYOCD [150]	Transcription-related protein needed for the expression of smooth muscle contractile proteins
Renal hypodysplasia, primary VUR	*NRIP1* [151]	Nuclear receptor transcriptional cofactor that modulates retinoic acid transcriptional activity
Small kidneys, VUR, optic nerves malformation	*PAX2* [152]	Transcription factor widely expressed in the developing ureter and kidney
Urofacial-Ochoa syndrome	*HPSE2* [153]	Heparanase 2, a protein probably modulating growth factor signaling in bladder nerves
	LRIG2 [154]	Leucine-rich-repeats and immunoglobulin-like-domains 2 probably modulating growth factor signaling in bladder nerves

nal glands in boys and (hemi)vagina in girls. These ectopic insertions should be searched in utero but are more obvious after birth. An early diagnosis has a relevant clinical impact and leads to better management of the children and planning of further surgery in symptomatic cases [159]. The distinction between MCDK and cystic renal dysplasia associated with urinary tract obstruction may be difficult, especially in the absence of hydronephrosis. This distinction, although helpful in terms of diagnosis, may be somewhat artificial in terms of prognosis, since in either case, the affected kidney has no or minimal functional capacity.

Autosomal Recessive Polycystic Kidney Disease (ARPKD)

ARPKD belongs to the family of cilia-related disorders, has an incidence of 1 in 20,000 live births and may cause fetal and neonatal death in severe cases [160, 161]. Mutations in the *PKHD1* fibrocystin gene are usually demonstrated in this disease [162]. Yet, since some patients survive the neonatal period with few or slight symptoms, different combinations of *PKHD1* gene mutations and its resulting changes in the fibrocystin/

polyductin protein structure may at least partially explain the phenotypic variance [163]. The disease is characterized by marked elongation of the collecting tubules that expand into multiple small cysts. The cystic dilatation of the tubules is variable and predominates in the medulla. The outer cortex is spared since it contains no tubules. The classical in utero pattern of ARPKD includes markedly enlarged (+4 SD) hyperechoic kidneys without corticomedullary differentiation (Fig. 1.7). This appearance can be observed in the second trimester. The patterns may evolve and the size of the kidneys may continuously increase during the third trimester. Oligohydramnios and lung hypoplasia may be present, and therefore the prognosis is extremely poor.

Another presentation of ARPKD is of reversed corticomedullary differentiation with large kidneys (+2 to +4 SD). This finding is probably related to increased interfaces within the medullae and to the presence of material within the dilated tubules [164]. It is an important observation since there are few other causes of reversed corticomedullary differentiation. Liver involvement, typical of the condition, is usually impossible to demonstrate in utero. The differential diagnosis includes the glomerulocystic type of autosomal dominant polycystic kidney disease, Bardet-Biedl syndrome in which polydactyly is present [58] and other rare entities such as bilateral renal tumors, medullary sponge kidney, bilateral nephroblastomatosis, Finnish-type congenital nephrotic syndrome, medullary cystic disease, or congenital metabolic diseases (that is, glycogen storage disease or tyrosinosis). Oligohydramnios and absence of urine within the bladder would suggest ARPKD over all these rare entities.

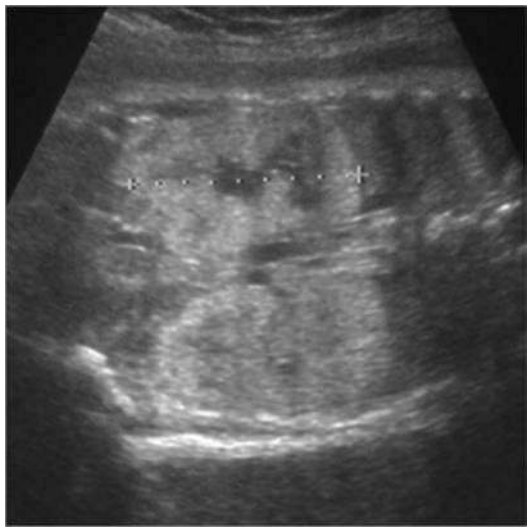

Fig. 1.7 Autosomal recessive polycystic kidney disease. Third trimester-coronal scan through the kidneys that appear large (+3 SD), and hyperechoic

Autosomal Dominant Polycystic Kidney Disease (ADPKD)

ADPKD is a common hereditary kidney disease, with 1/1000 people carrying the mutation. The pathological abnormality consists of a cystic dilatation of all parts of the nephron, which causes the kidneys to enlarge while the cortex and medulla become replaced by cysts, thus leading to end-stage renal failure [164, 165].

There are two major types of ADPKD: type I is caused by mutations in the *PKD1* gene on chromosome 16p13.3 and accounts for 85–90% of cases [164], and type II is caused by mutations in the *PKD2* gene on chromosome 4q21-22 and accounts for 10–15% of cases [165]. Other ciliopathy genes are likely to be involved since some obvious cases have none of these mutations. Although the age of clinical onset of this disorder is typically in the third to fifth decade of life, early manifestations during childhood or young adult age are now well described [166]. It is however unknown whether early diagnosis of ADPKD can enable earlier management and improve outcomes [167].

There may be different presentations in utero. In most cases, the kidneys are not grossly enlarged, but the corticomedullary differentiation is increased due to cortical hyperechogenicity. In this type of ADPKD, cysts are unusual in utero; they will develop after birth. Markedly enlarged kidneys resembling ARPKD are another pattern that can be encountered in utero and suggests either the homozygous presentation of ADPKD or the glomerulocystic type of ADPKD [168]. In these presentations of the disease renal failure may be already present in utero or at birth [163].

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Renal Hypoplasia and Dysplasia

Renal dysplasia refers to abnormal differentiation or organization of cells in the renal parenchyma and is characterized histologically by the presence of primitive ducts and nests of metaplastic cartilage [32, 33]. Hypoplasia is a reduction of the number of nephrons in small kidneys (below −2 SD) (Fig. 1.8) [33]. Hypoplasia may coexist with dysplasia and the diagnosis is inferred from the hyperechoic appearance on US caused by the lack of normal renal parenchyma and structurally abnormal small kidneys [32, 33]. As in most cases the diagnosis is made by US examination, the spectrum of renal dysplasia includes inherited or congenital causes of renal hypoplasia, renal adysplasia, cystic dysplasia, oligomeganephronic hypoplasia, reflux nephropathy and obstructive renal dysplasia [32]. Cases with oligohydramnios have the poorest outcome [169].

A number of developmental genes have been implicated in the pathogenesis of hypodysplastic kidneys [134]: *EYA1* and *SIX1* causing autosomal dominant branchio-oto-renal syndrome [137, 138], *HNF1B/TCF2* associated with autosomal dominant renal cysts and diabetes syndrome [140, 170], and *PAX2* causing autosomal dominant renal-coloboma syndrome [152].

After birth, the prognosis depends on the residual renal function at 6 months of age. Infants with a GFR below 15 mL/min per 1.73 m^2 are at high risk to require early renal replacement therapy [32].

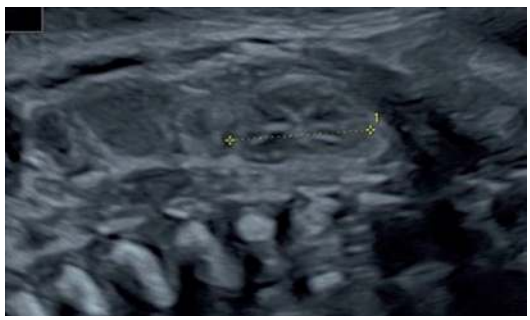

Fig. 1.8 Unilateral renal hypoplasia. Sagittal US scan through a small-sized right kidney: 18 mm at 31 weeks: between calipers

Congenital Nephrotic Syndrome

Congenital nephrotic syndrome (CNS) is defined as proteinuria leading to clinical symptoms in the first 3 months after birth. Infantile nephrotic syndrome manifests later, in the first year of life. However, these definitions are arbitrary as the time of onset ranges from fetal life to few years of age [171]. Moreover, CNS is a heterogeneous group of genetic disorders with variable clinical presentations and progression to end-stage renal failure [172, 173]. In a recent cohort study on renal transplanted infants weighing ±15 kg, CNS represented 35% of cases [174].

Congenital nephrotic syndrome of the Finnish type (CNF) is characterized by autosomal recessive inheritance and is caused by mutations in the nephrin gene (*NPHS1*) [175]. Most infants are born prematurely, with low birth weight for gestational age. The placenta is enlarged, weighing more than 25% of newborn weight. Edema is present at birth or appears within a few days due to severe nephrotic syndrome. In utero, the observation of hydrops fetalis and increased nuchal translucency reflects massive proteinuria [176, 177]. Most of the α-fetoprotein measured in the amniotic fluid is produced by fetal urine [178]. CNF should be suspected in front of an α-fetoprotein concentration exceeding 250–500 mg/L (mean normal values: 16.3 mg/L at 15 weeks of gestation; 8.1 mg/L at 20 weeks of gestation) [179].

Other cases of prenatally diagnosed congenital nephrotic syndrome have been reported, including Podocin gene (*NPHS2*) mutations [180], Pierson syndrome [181, 182], *PLCE1* gene mutations [183], secondary nephrotic syndrome due to CMV or other intrauterine infections [184] and massive proteinuria in offsprings of mothers with homozygous deficiency for the metallomembrane endopeptidase [85].

Postrenal Causes of Fetal Renal Abnormalities

Dilatations of the renal pelvis, calyces and ureters are the principal signs of impaired urinary flow on antenatal ultrasound scanning.

Pelvicoureteric Junction Stenosis

Pelvicoureteric junction stenosis occurs in 13% of children with antenatally diagnosed renal pelvis dilatation [39] and is characterized by obstruction at the level of the junction between the renal pelvis and the ureter. The anatomical basis for obstruction includes intrinsic stenosis/valves, peripelvic fibrosis, or crossing vessels. Sonographic diagnosis depends on the demonstration of a dilated renal pelvis in the absence of any dilatation of ureter or bladder. It should be particularly suspected when severe (greater than 15 mm) dilatation is seen, when the cavities appear round shaped and in the presence of a perirenal urinoma [35, 185] (Fig. 1.9). The anomaly is twice as common on the left side and twice as common in boys than in girls [186]. Prognosis may be poor in bilateral cases associated with oligohydramnios and hyperechoic parenchyma. The postnatal management of these children still remains a controversial topic among the nephro-urologic community [187]. It involves close monitoring of both sonomorphological and functional criteria assessed by radionuclide renogram including renal transit limited to the cortical area, differential function and output function (drainage pattern) [188–191]. Importantly, while the wait-and-see approach has gained wide acceptance, the decision to follow up those neonates conservatively requires some level of vigilance and clear parental consent and cooperation in order to avoid any evitable complications including renal functional deterioration [192].

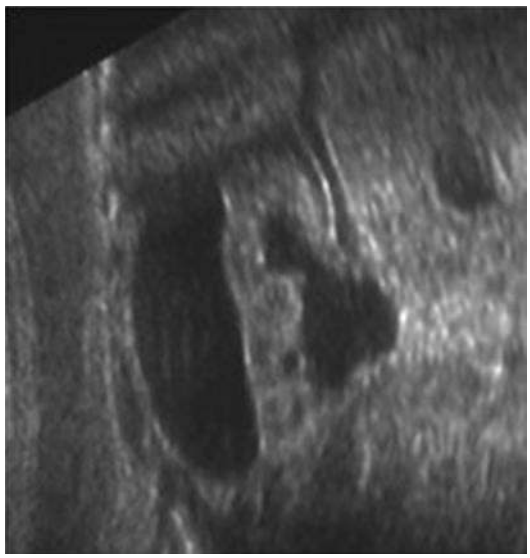

Fig. 1.9 Pelvicaliceal dilatation and perirenal urinoma in a fetus with pelviureteric junction stenosis. Coronal US scan through the right fetal kidney

Vesicoureteric Reflux (VUR)

VUR is defined as the retrograde flow of urine from the bladder upward within the ureter, sometimes extending into the renal pelvis, calyces and collecting ducts. Fetal renal pelvis dilatation can signal the presence of VUR in 11% [39] to 30% [193] of cases with the lower figure being more realistic. Making a precise diagnosis of VUR in utero is difficult. However, intermittent renal collecting system dilatation during real-time scanning (Fig. 1.10) or pelvicaliceal wall thickening are sonographic criteria highly suggestive of this diagnosis [194]. Although some children with high-grade, prenatally diagnosed VUR may have associated renal dysplasia [5, 194], VUR related to fetal renal pelvis dilatation was found in a large and prospective study to be of low-grade in 74% of cases with a high rate of 2-year spontaneous resolution (91%) [5]. Furthermore, in the absence of infections, reflux is not per se associated with progressive kidney injury [5]. The systematic use of VCUG in those babies is therefore questionable, especially since parents are frequently reluctant to consent to an invasive and uncomfortable examination for their child [195].

Uretero-Vesical Junction Obstruction (Megaureter)

In utero, under normal conditions, the ureters are not visualized. Megaureter should be suspected in the presence of a serpentine fluid-filled structure with or without dilatation of the renal pelvis and calices (Fig. 1.11). The ureter may be dilated because of obstruction at the level of the junction between the ureter and the bladder or as a result of nonobstructive causes including high-grade reflux. The differential diagnosis relies on VCUG. Megaureter could also be encountered in fetuses and/or newborns with neurogenic bladder or posterior urethral valves. In those cases, specific treatment strategies should be directed

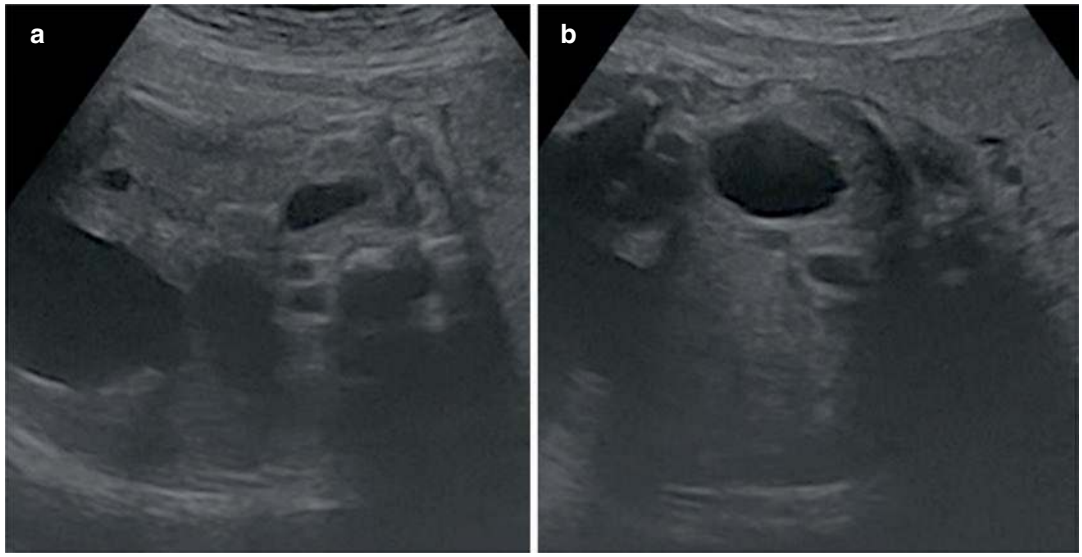

Fig. 1.10 (**a** and **b**) Fetal vesicoureteral reflux. Transverse scans of the fetal abdomen. Intermittent renal collecting system dilatation during the same antenatal ultrasound examination only visible in figure **b**, due to vesicoureteral reflux

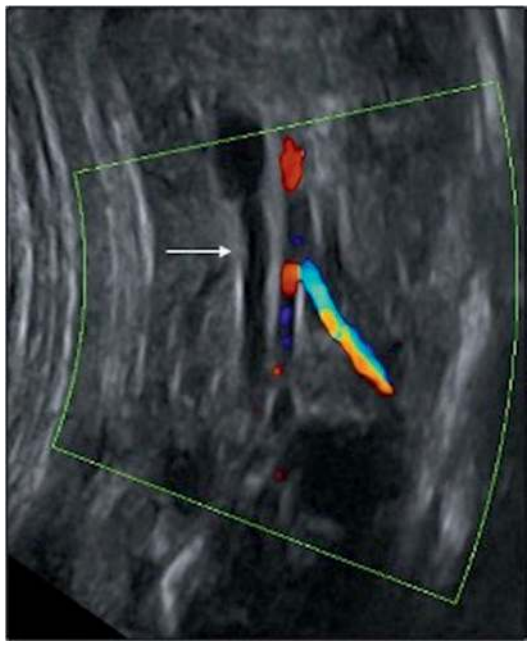

Fig. 1.11 Megaureter. Coronal scan of the fetal abdomen showing a fluid-filled structure (arrows) laterally to the aorta corresponding to a dilated ureter

toward the underlying condition. Prognosis of primary megaureter is generally good since most cases resolve spontaneously between ages 12 and 36 months [196]. However, in children with high-grade hydronephrosis, or a retrovesical ureteral diameter of greater than 1 cm, the condition may resolve slowly and may require surgery [196].

Duplex Kidneys

Duplication of the renal collecting system is characterized by the presence of a kidney having two pelvic structures with two ureters that may be completely or partially separated [197]. Most cases with non-dilated cavities have no renal impairment and should be considered as normal variants [4]. However, a proportion of duplex kidneys may be associated with significant pathology, usually due to the presence of VUR or obstruction. Fetal urinary tract dilatations are related to complicated renal duplication in 4.7% of cases [39]. VUR usually involves only the lower pole ureter in 90% of cases. Compared to single-system reflux, duplex system VUR tends to be of a higher grade with a high incidence of lower pole dysplasia [198]. Obstructive ureteroceles are associated with the upper pole ureter in 80% of cases, although obstruction of the upper pole may also occur secondary to an ectopic insertion or an isolated vesicoureteric junction obstruction (Fig. 1.12a) [198, 199]. In utero, duplex kidneys are highly suspected in the presence of two separate noncommunicating renal

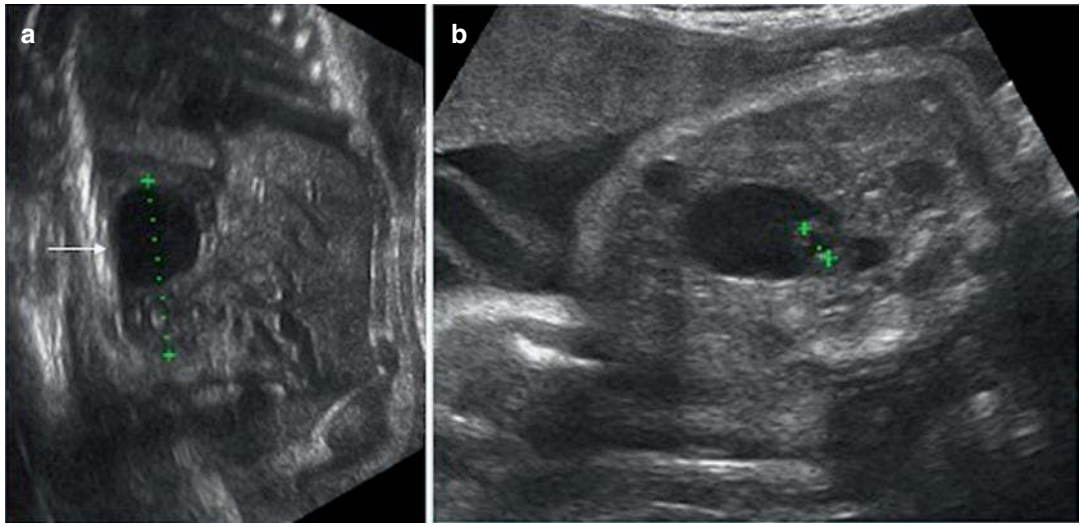

Fig. 1.12 (**a**) Fetal duplex kidney with dilatation of the upper pole (arrow). Sagittal US scan through the fetal kidney. (**b**) Fetal bladder with ureterocele (between crosses). Axial US scan through the fetal bladder

pelves, dilated ureters, cystic structures within one pole, and echogenic cyst in the bladder, representing ureterocele (Fig. 1.12b) [199, 200]. After birth, the classical radiological workup of abnormal duplex kidneys is based on US and VCUG [4]. Most people agree that the surgical approach to complicated duplex systems is largely predicated on the function of the affected renal moiety and the presence or absence of function [4].

Bladder Outlet Obstruction

When bladder obstruction is suspected in the first trimester, the most common causes are Prune Belly syndrome or fibrourethral stenosis, which is mainly associated with chromosomal and multiple congenital anomalies and carries a very poor prognosis [201]. In the second trimester, the most common cause of lower urinary tract obstruction in male fetuses is posterior urethral valves, which are tissue leaflets fanning distally from the prostatic urethra to the external urinary sphincter. The failure of the bladder to empty during an extended examination and the presence of abnormal kidneys and oligohydramnios must raise suspicion of posterior urethral valves. On occasion a megabladder with a thickened wall may be seen (Fig. 1.13a), and the dilated posterior urethra may take the aspect of a keyhole (Fig. 1.13b). In extreme cases in utero bladder rupture may be observed with extravasation of urine resulting in urinary ascites. This phenomenon was thought to be a protective pop-off mechanism, although recent reports provided evidence against this hypothesis [202].

In many cases there is only a partial obstruction, and amniotic fluid volume can be maintained throughout pregnancy. In some cases, spontaneous rupture of valves appears to occur in utero with the reappearance of cyclical emptying of the bladder. The most reliable prognostic indicators of poor renal functional status are presentation before 24 weeks, oligohydramnios, increased cortical echogenicity, and the absence of corticomedullary differentiation [203, 204].

The prognosis in severe cases is often relatively easy to predict, and perinatal death will occur secondary to pulmonary hypoplasia and renal failure [204]. The renal parenchymal lesions may be secondary to the obstruction but also to associated high-grade reflux. In partial obstruction, however, the outcome is less predictable, and late morbidity most commonly takes the form of end-stage renal failure, which affects 15–30% of individuals some time in childhood [205]. Once the prognosis has been determined

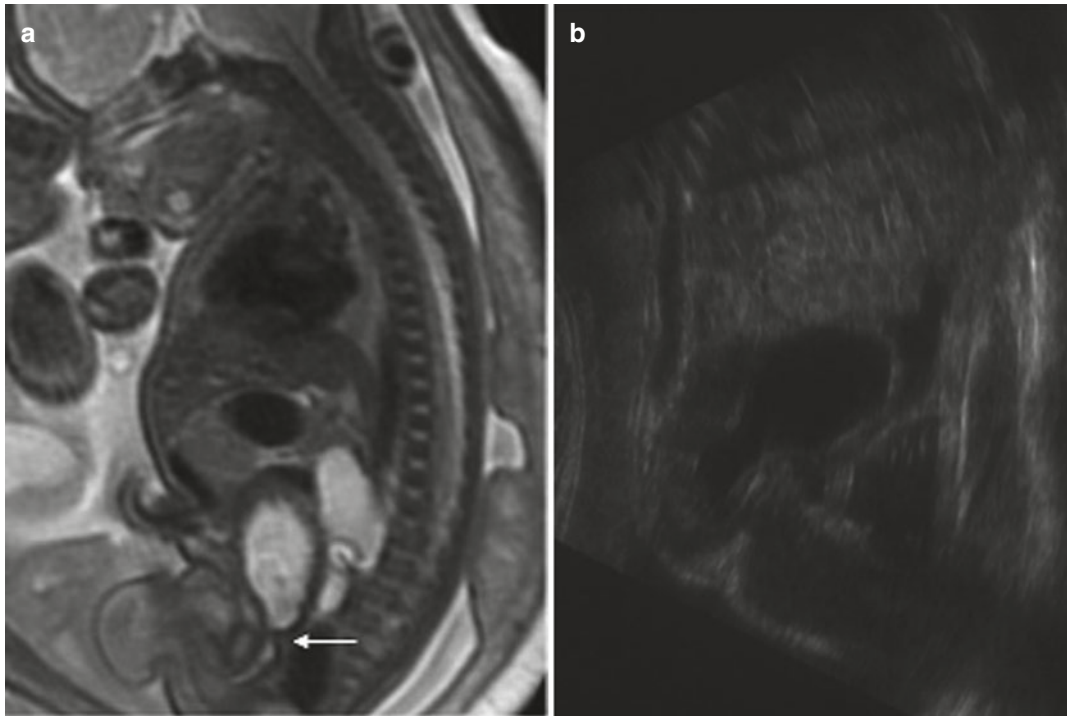

Fig. 1.13 (**a**) Fetal urethral valve with thickened bladder wall. Posterior urethral dilatation (arrow). Sagittal MR image on the fetal pelvis. (**b**) Megabladder. Third trimester scan. Huge enlargement of the fetal bladder due to posterior urethral valves. The key-hole sign is present

as accurately as possible, management of these cases should be performed in a fetal medicine and pediatric surgery reference center. In each new case, the great variability of presentation makes participation of different specialists necessary in the difficult decision-making process. Various options should be considered, including in utero follow-up with planned postnatal management (sustained medical treatment or palliative care), termination of pregnancy, and occasionally, in utero therapy [50].

Fetal Intervention for Lower Urinary Tract Obstruction

There was considerable interest in fetal intervention for obstructive uropathies in the 1980s and 1990s, which was revolutionary at that time. In utero intervention was thought to improve neonatal outcome by restoring amniotic fluid levels, thereby allowing normal pulmonary maturation and eventual renal function [206, 207]. A variety of in utero therapeutic approaches to bladder outflow obstruction have been tried. Expectably, the open surgical technique of **fetal vesicostomy** was not free of risks and has been abandoned due to significant fetal loss, premature uterine contractions and maternal morbidity [206, 207].

Direct endoscopic ablation of urethral valves is a more recent technique and requires the introduction of an endoscope into the fetal bladder, leading to ablation of the valves either by laser, saline irrigation or mechanical disruption using guide wire [208]. Direct visualization of the valves, however, is difficult, and it may be hard to avoid damage to surrounding tissues.

Vesicoamniotic shunting is performed under US guidance using a pigtail shunt, which when inserted leaves one end in the fetal bladder and the other in the amniotic space. This technique, which was first reported in 1982 [209], is

preferred to bladder drainage by serial vesicocentesis. A previous systematic review to assess the effectiveness of bladder drainage (vesico amniotic shunting or vesicocentesis) showed that fetal bladder drainage increased survival [210]. However, the studies identified in this systematic review were small, heterogeneous, observational, and non-randomized trials and so the potential for bias in these results was substantial. In addition, using survival alone as a marker of efficacy in these patients was misleading, since most of the survivors were left with significant renal morbidity. End-stage renal failure was present in 40% of those children who survived [90]. After decades of absence of serious clinical research, Rachel Morris and colleagues orchestrated and published in *The Lancet* in 2013 the results of the PLUTO (Percutaneous vesicoamniotic shunting in Lower Urinary Tract Obstruction) trial, in which fetuses with fetal lower urinary tract obstruction were randomly assigned to either vesicoamniotic shunting or conservative management [211]. Thirty-one women with singleton pregnancies complicated by lower urinary tract obstruction were included in the trial, with 16 allocated to the vesicoamniotic shunt group and 15 to the conservative management group. Unfortunately, the trial was stopped early because of poor recruitment after only about 20% of the planned 150 pregnancies were randomly assigned during a 4-year period. Although the results of PLUTO results have to be interpreted with caution due to the premature termination of the study and the small number of patients [212], postnatal survival was three-times higher in the fetuses receiving vesicoamniotic shunting. However, only two of seven shunted survivors had normal renal function at age 1 year. These results suggest that the chance of newborn babies to survive with normal renal function is very low irrespective of whether or not vesicoamniotic shunting is done. These findings are in line with results from studies in animals, which have shown that renal damage occurs rapidly after the onset of obstruction and might be only partly reversible [213].

In conclusion, the evidence available so far suggests that intrauterine shunting of an obstructed urinary tract will improve perinatal survival but does not carry any advantage with regards to the rescue of postnatal kidney function and long-term renal survival.

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Laboratory Evaluation of Renal Disease in Childhood

2

Damien Noone and Valérie Langlois

Assessment of the Urine

Urinalysis

The American Academy of Pediatrics stopped recommending routine yearly urinalysis as a screening tool for chronic kidney disease (CKD) in otherwise healthy school-aged children over a decade ago [1]. Nonetheless, the value of the urinalysis in the evaluation of kidney disease should not be underestimated in certain patient populations. Important information can be learned from this simple, quick, inexpensive test when used in the appropriate setting. Commercially available reagent strips can be used to screen the urine for pH, specific gravity (SG), protein, blood, glucose, ketones, leukocytes, and nitrates. Urine specimens should be fresh and clean-voided midstream in older children. If analysis cannot be done within 4 h, then the sample needs a preservative and/or to be stored at 4 °C or lower (−20 or −80 °C) to maintain the integrity and prevent degradation of the specimen. The variations in pH and osmolality of the urine can cause particles within the urine to lyse. Various preservatives can be used, e.g., boric acid (changes urinary pH), sodium azide (to prevent bacterial overgrowth), formaldehyde (causes false positive leucocyte esterase), mercury salts and chlorhexidine [2].

Depending on urine concentration, the urine color varies from pale yellow to amber. Red or tea colored urine suggests the presence of blood, hemoglobin, myoglobin, porphyrin, non-pathologic pigments (beets, food color) or certain medications. Blue to green is suggestive of the presence of biliverdin or Pseudomonas infection.

The urine is normally clear, but can be cloudy in the presence of leukocytes, epithelial cells, bacteria, or precipitation of amorphous phosphate or urate. Unusual urine odor can lead to the diagnosis of rare metabolic disorders such as maple syrup urine disease (maple syrup odor), phenylketonuria (musty odor) or hypermethioninemia (fishy odor).

SG reflects the urinary concentrating and diluting capability of the kidney. In normal conditions, it reflects the patient's hydration status. However, with abnormal kidneys, a very low SG may represent a concentrating defect. It may be useful in distinguishing pre-renal states from intrinsic renal disease. SG usually ranges from 1:001 to 1:035 and can be measured using a urinometer or a refractometer, but more commonly using the reagent strips. The reagent strip test is based on pKa change of polyelectrolytes in relation to ionic concentration [3].

Urinary pH usually ranges from 5.0 to 8.5 depending on the acid-base balance of the body and can be estimated using the reagent test strip.

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However, precise measurements need to be obtained using a pH meter with a glass electrode, particularly when <5.5 or >7.5. Urinary pH is important in the diagnosis of renal tubular acidosis and monitoring the treatment for prevention of urinary stones.

Glucose is not usually present in the urine. Glucose is freely filtered at the glomerulus and reabsorbed in the proximal tubule via a sodium-coupled active transport mechanism. Glucosuria can be seen when the serum glucose is above the renal threshold, or due to isolated renal glucosuria or generalized proximal tubular dysfunction (Fanconi syndrome). Normal values for maximal tubular glucose reabsorption (TmG) in children vary from 254 to 401 mg/min/1.73 m^2 [4]. Reagent test strips are usually impregnated with the enzyme glucose oxidase and only detect glucose. Other sugars can be detected by the copper reduction test such as Clinitest Tablet (Ames Co.). A false negative glucose can occur with high dose vitamin C and in the setting of elevated ketones [2].

Ketone bodies are formed during the catabolism of fatty acids and include acetoacetic acid, β-hydroxybutyric acid, and acetone. Most reagent strips for ketones are based on a color reaction with sodium nitroprusside and are sensitive for acetoacetic acid but will not detect β-hydroxybutyric acid or acetone.

Strip tests detect leukocyte esterase, an enzyme found in neutrophils. Nitrites indicate the presence of bacteria capable of reducing dietary nitrate, such as *Escherichia coli*, *Enterobacter*, *Citrobacter*, *Klebsiella*, and *Proteus* species. A positive urinalysis for both leucocytes and nitrites is suggestive of bacteriuria or a urinary tract infection (UTI), and if both are negative then a UTI is unlikely. If either are positive, then a further confirmatory urine culture is required [5, 6]. Samples should be processed rapidly to avoid degradation of esterases and false negative results [7]. For children under the age of two, urinalysis is less reliable for the diagnosis of UTI [6]. For infants less than one year of age, microscopic presence of moderate bacteria and >10 white cells/high powered field is more accurate in diagnosing a UTI [6]. An alkaline pH or the ingestion of beets can cause a false positive leukocyte esterase and there may be a false negative in the setting of high urinary glucose, protein or with antibiotics. There may be a false negative nitrite when the urine SG is high, with ascorbic acid ingestion (reducing agent), if the child eats insufficient fruits and vegetables to provide the nitrate substrate in the urine or if the incubation time is too short due to urinary frequency [2, 8]. The accuracy of urinary nitrites in the diagnosis of UTIs in those under age 2 years has been recently called into question; they miss UTIs in about 75% of young children [8].

Hematuria is defined as the presence of >5 red blood cells (RBCs) per high power field in centrifuged urine. The presence of RBCs can only be confirmed by microscopic evaluation of fresh urine. Reagent strips detect RBCs, myoglobin, and hemoglobin because all contain a heme moiety. As with nitrite detection, ascorbic acid can cause a false-negative due to it being a strong reducing agent. The supernatant of a centrifuged urine containing red blood cells will be clear yellow as opposed to being pink if the urine contains hemoglobin or myoglobin. The morphology of the cells can help determine their origin. The presence of dysmorphic red blood cells suggests glomerular hematuria.

Urine Microscopy

"…the ghosts of dead patients that haunt us do not ask why we did not employ the latest fad of clinical investigation. They ask us, why did you not test my urine?"—Sir Robert Grieve Hutchison (1871–1960) [9].

Microscopic evaluation of fresh urine (within 1–2 h) is extremely valuable in the evaluation of renal disease. Ideally, 10 mL of urine is centrifuged at 1500 rpm for 5 min, and about 9.5 mL is decanted off. The remainder is gently agitated; a single drop is placed on a glass slide; and a cover slip is added. The urine sediment is examined at low (×10) and high (×40) magnification for the presence of casts, cells, and crystals. Although hyaline (containing primarily uromodulin) and granular casts can be seen in normal states, cel-

lular casts are pathologic. Granular casts contain degraded cell lysosomes and other debris from degenerated renal tubular epithelial cells and typically reflect tubular injury or acute tubular necrosis. Red blood cell casts are pathognomonic of glomerular disease and white blood cell (WBC) casts can be seen with pyelonephritis or post-infectious glomerulonephritis [10].

Crystals are rarely seen in fresh urine but appear after the urine stands for a period. Uric acid, calcium oxalate, amorphous urate, cystine, tyrosine, leucine, and cholesterol crystals are usually found in acid urine. Uric acid and calcium oxalate crystal can be seen in normal and pathological conditions. Amorphous urate crystals are of no clinical significance. Cystine, tyrosine, leucine, and cholesterol crystals are always relevant. Cystine crystals (colorless and hexagonal) are present in patients with cystinuria, leucine crystals can be associated with maple syrup urine disease, methionine malabsorption syndrome and severe liver disease. Tyrosine crystals also occur in severe liver disease, tyrosinosis, and methionine malabsorption syndrome. The presence of cholesterol crystals can indicate excessive tissue breakdown, or nephritic or nephrotic syndrome [3]. Triple phosphate, calcium carbonate, ammonium biurate, amorphous phosphates and calcium phosphate crystals are usually found in alkaline urine. Calcium carbonate and amorphous phosphate are of no clinical significance [10]. Many drugs are associated with crystalluria, including acyclovir, amoxicillin, atazanavir, ciprofloxacin, methotrexate, sulfadiazine, triamterene and vitamin C [10].

The association of urinary eosinophils with acute interstitial nephritis (AIN) was first reported by Galpin et al. [11] and became widely accepted as supportive of a diagnosis of AIN. Hansel's stain replaced Wright's stain, the latter being ineffective when urine pH was <7, and the former revealing the bright red granules of eosinophils. However, eosinophiluria may be seen in a variety of conditions, including prostatitis, cystitis, and glomerulonephritis, limiting the sensitivity and positive predictive value of the test. Compared to the gold standard of kidney biopsy, eosinophiluria lacks sensitivity and specificity, and cannot distinguish AIN from acute tubular necrosis. Hence, it really has no diagnostic utility [12].

Automated microscopy that uses laminar flow digital imaging technology can classify and count cells (RBCs, WBCs and epithelial cells) and particulate matter (bacteria, yeasts and crystals) in uncentrifuged urine. Urine particle flow cytometers can quantify cells more accurately than manual urine microscopy [13].

Urinary Protein Excretion

In the normal state, most of the filtered low molecular weight (MW) proteins (MW < 40,000) are reabsorbed in the proximal tubules. Proteins of higher MW, such as albumin (MW = 60,000), are not usually filtered. Tamm Horsfall proteins are secreted by the tubular cells in the ascending thick limb of the loop of Henle and are the main protein found in normal urine.

In disease states, increased amount of protein can be found in the urine and may reflect damage in the glomerular barrier (glomerular proteinuria) or impaired tubular reabsorption (tubular proteinuria). In glomerular proteinuria, albumin, which is not usually present, is the dominant protein. β2-microglobulin, α-1-microglobulin, and retinol-binding protein are markers of tubular proteinuria.

Proteinuria can vary by age, sex, ethnicity and body mass index [14, 15]. In the first month of life, proteinuria is 4–5 times higher than in older infants, perhaps reflecting the evolving maturity of the tubules, and the 90th percentile for urinary albumin/creatinine ratio (UACR) in the neonatal period was reported as 17.5 (90% Confidence Interval 7.1–79.7) mg/mmol in one study [16]. Normative ranges for UACR at different ages are presented in Table 1 [17].

The 2012 KDIGO Clinical Practice Guideline for the evaluation and management of CKD recommends, in order of preference, a UACR, a urine protein/creatinine ratio (UPCR), reagent strip urinalysis for total protein with automated reading and, finally, a reagent strip with manual reading for assessment of proteinuria. All should ideally be done on an early morning urine speci-

men [18]. It is not usually necessary to obtain a timed urine collection. A standard urine dipstick can be used to detect increased total urine proteins and albumin-specific dipsticks are acceptable to detect microalbuminuria. Microalbuminuria refers to albumin excretion above the normal range, but below the level of detection of the standard urine dipstick. UACR or UPCR ratio should be done within 3 months of a positive dipstick to confirm albuminuria or proteinuria, respectively. Post-pubertal children with diabetes of 5 or more years' duration should have urine albumin measured by albumin-specific dipstick or UACR, and this should be performed annually [19, 20].

Urine dipstick can provide an estimate of proteinuria and is most sensitive for albumin. False positive dipstick can be the result of prolonged immersion of the reagent strip, alkaline urine (pH > 7.5), presence of pyuria, bacteriuria or mucoprotein and penicillin [21]. A false negative can occur in very dilute urine [2].

Twenty-four-hour urine collections have long been the gold standard for quantification of urine protein excretion. The adequacy of the collection is verified by quantifying the total creatinine content of the sample, which should be about 15–20 mg/kg in females and 20–25 mg/kg in males [22]. However, collection in young children often requires catheterization and is not practical. First morning urine for UPCR is generally accepted as being valid in the assessment of proteinuria in children [18]. A recent study confirmed that UPCR is positively correlated with 24-h urine protein in children, and the cutoff of UPCR <0.2 g/g corresponds to normal protein excretion and the cutoff UPCR ≥2 g/g is nephrotic-range proteinuria [23].

Twenty-four-hour urine protein excretion of <4 and >40 mg/m^2/h is normal and nephrotic-range proteinuria, respectively. Normal urinary albumin excretion is between 30–300 mg/day on a 24-h collection, 20–200 μg/min in an overnight collection and 3–30 mg/mmol on a first morning urine sample (Table 2) [24]. There can be significant diurnal variation in proteinuria in children and adolescents that is generally not considered pathological and resolves by adulthood. Orthostatic proteinuria is defined as an elevated protein excretion in the upright position, but normal excretion in the recumbent position. It can be assessed on a split 24-h urinary protein assessment [22]. Previous studies reported an incidence of 2–5% [22]; however, a study using 24-h total urinary protein excretion found a much higher incidence of 19.8% in a cohort of 91 children [25]. The original studies had used dipstick analysis [22], spot UPCR [26] or timed collections of less than 24 h [25, 27].

In 2006, Mori et al. [28] measured the UPCR in a cohort of Japanese children with urinary tract abnormalities or glomerular disorders and found that it varies according to body size and composition, reflecting muscle mass. They suggested that evaluation of UPCR should also consider body height, because as height and therefore muscle mass (denominator) increases, the ratio will decrease. A normative range for urinary protein excretion for the different sexes and as height and body surface area increases remains to be defined [28]. Kim et al. [29] proposed urine protein-to-

Table 1 Mean urinary albumin excretion, expressed as a albumin/creatinine ratio [17]

Age	Spot urine (mg/mmol)
Neonates	5.24
1–3 months	5.01
4–6 months	4.06
7–23 months	1.76
2–4 years old	1.34
3–19 years old	3

Table 2 Reference values for urinary protein excretion [19]

	24 Hour collection (mg/m^2/h)	24 Hour Collection (mg/m^2/day)	Spot urine protein/creatinine (mg/mg)	Spot urine protein/creatinine (mg/mmol)
Normal range				
6–24 months	<4	<150	<0.5	<50
>24 months		<150	<0.2	<20
Nephrotic	>40	>3 g/1.73 m^2/day	>2	>200

osmolality ratio as an alternative test to 24-h urinary protein excretion. Urinary protein-to-osmolality corrects for hydration status, can be used in children with decreased muscle mass, and has now been validated in two further pediatric populations against both spot UPCR and 24-h urinary protein excretion [30, 31]. In children, a spot urinary protein-to-osmolality ratio above 0.33 and 1.75 mg/L/mOsm/kg represents abnormal proteinuria and nephrotic-range proteinuria, respectively [31].

Standard urine dipstick tests are primarily sensitive to detect albumin. Screening for low MW protein can be done by the sulfosalicylic acid test. The addition of sulfosalicylic acid to the supernatant of centrifuged urine will cause cloudiness in the presence of any protein in the urine. A negative reagent strip test with a positive sulfosalicylic acid test is suggestive of low MW proteinuria. Urine protein electrophoresis can confirm the diagnosis. A false positive sulfosalicylic acid test can be produced by radiographic contrast, penicillin, cephalosporins, sulfonamide metabolites and high uric acid concentration [21].

Assessment of Renal Function

Glomerular Filtration

Glomerular filtration rate (GFR) is the most commonly used measure of kidney function and is used to classify various stages of CKD (Table 3) [18]. It can be quantified by measuring the clearance rate of a substance from the plasma. The substance can be endogenous or exogenous. It is often referred to as the "marker". Different markers such as inulin, creatinine, iothalamate, iohexol, ethylenediaminetetraacetic acid (EDTA) and diethylenetriamine pentaacetic acid (DTPA) are available. The ideal marker must have a stable plasma concentration, should be filtered, but not reabsorbed, secreted, synthesized or metabolized by the kidney so that the amount filtered equals the amount excreted.

Table 3 NKF-K/DIGO 2012 Classification of the stages of chronic kidney disease in children greater than 2 years of age [38]

Stage	Description	GFR (mL/min/1.73 m^2)
1	Kidney damage with normal or increased GFR	>90
2	Kidney damage with mild reduction of GFR	60–89
3a	Moderate reduction of GFR	45–59
3b	Moderate to severe decrease of GFR	30–44
4	Severe reduction of GFR	15–29
5	Kidney failure	<15

The renal clearance of the substance x (Cx) can be obtained by multiplying the urinary concentration of substance x (Ux) times the urinary flow rate in mL/min (V) divided by the plasma concentration of substance x (Px).

$$Cx = Ux * V / Px$$

Inulin

Determination of urinary inulin clearance during a continuous intravenous infusion is considered the "gold standard" method for measurement of GFR. Inulin has all the properties of an ideal marker. Inulin is inert and not synthesized or metabolized by the kidney. It is freely filtered by the glomerulus, and not secreted or reabsorbed in the tubules [32].

The measurement of urinary inulin clearance requires a constant intravenous infusion to maintain a constant level of inulin over a period of 3–4 h. After an equilibration period, timed urinary specimens and plasma are collected every 30 min and urinary and plasma inulin is measured to calculate urinary inulin clearance. The mean clearance of the 4–5 measurements determines the individual's GFR [33]. Urinary catheterization in young children is often required. To avoid this cumbersome procedure, two methods of plasma inulin clearance have been developed: the continuous infusion method and the single bolus method [34, 35]. The continuous infusion method is based on the concept that once a marker has reached steady state in the plasma and the volume of distribu-

tion is saturated, the rate of elimination of the marker will equal the rate of infusion (RI).

The clearance of the marker can then be measured [34]

$$Cx = RI\,x\,/\,Px$$

The equilibration period can take more than 12 h in certain situations. To avoid this long period, a bolus can be given prior to the infusion to reach steady state more rapidly.

After a single bolus injection, 10–12 blood samples are collected up to 240 min after injection and the inulin concentration measurements are used to construct a plasma concentration versus time curve (plasma disappearance curve). Plasma clearance of inulin can be calculated by dividing the dose by the area under the plasma concentration-time curve. This method has been shown to give accurate results in adults [36]. van Rossum et al. developed and validated sampling strategies to minimize the number of blood samples, making it more acceptable for children [37]. He concluded that two (at 90 and 240 min) to four samples (at 10, 30, 90, 240 min) allow accurate prediction of inulin clearance in pediatric patients with a non-significant bias and good imprecision (<15%) [37]. The single bolus injection method tends to overestimate GFR (average 9.7 mL/min 1.73 m^2), but the difference between the two methods becomes smaller at lower GFR (less than 50 mL/min/1.73 m^2) [37].

Although measurement of inulin clearance remains the gold standard for assessment of GFR, most laboratories cannot routinely measure inulin, which makes this test unpractical. Furthermore, simple, rapid determination of GFR is often needed in clinical practice.

The KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease recommends the use of serum creatinine and a pediatric-specific GFR estimating equation which incorporates a height term in the initial assessment of pediatric renal function [38].

Serum Creatinine

Creatinine is an amino acid derivative produced in muscle cells. Its production increases in proportion to muscle mass, it is freely filtered, and about 10% of the creatinine found in urine is secreted by the proximal tubules. Tubular secretion varies between and within individuals [39]. Creatinine is used as a measure of renal function. In the past, laboratories used different measurement methods and the lack of standardization was clinically significant. In 2006, the National Kidney Disease Education Program published recommendations to improve creatinine measurement [40]. It is now recommended that creatinine measurements in all infants and children utilize methods that minimize confounders and that are calibrated against an international standard [38].

Creatinine Clearance (Ccr)

Creatinine clearance measurement has been widely used and correlates well with inulin clearance within the normal range of GFR [41]. Creatinine has the advantage of being an endogenous marker, which precludes the need to use an injection. However, as GFR declines the percentage of secreted creatinine increases; therefore, Ccr at low GFR will significantly overestimate true GFR [42]. In order to decrease tubular secretion of creatinine and obtain a creatinine clearance more reflective of the true GFR, cimetidine can be given in patients with renal disease since cimetidine decreases tubular secretion of creatinine [43, 44]. The cimetidine protocol involves the administration of cimetidine (20 mg/kg to a maximum of 1600 mg divided twice daily for a total of five doses) prior to assessment of urinary creatinine clearance. For the 24 h prior to the test, the patients were placed on a meat-free diet. Dose adjustments in cimetidine according to renal function are advised and the complete protocol is reported [45].

Equations to Predict GFR

Schwartz [46] and Counahan [47] first developed equations to predict GFR. In the clearance formula, the numerator UCr × V is the excretion rate of creatinine; in steady state this must equal the rate of production. Since the rate of production is a function of muscle mass, Schwartz tested different variables of body size to provide the best correlation with GFR measured by creatinine clearance. The body length had the best correlation. The GFR can be estimated using the equation known as the Schwartz formula:

$$\mathrm{GFR}\left(\mathrm{mL/min/1.73\ m^2}\right) = \mathrm{K} * \mathrm{Ht/PCr}$$

where K is a constant determined by regression analysis for different ages, Ht = height in cm and PCr = plasma creatinine.

Following the standardization of creatinine measurement, the pilot study for the Chronic Kidney Disease in Children (CKiD) study showed that the Schwartz formula overestimates GFR when compared to measured iohexol [48]. This was attributed to the fact that creatinine values determined by enzymatic creatinine assays are lower than those determined by the Jaffe method [49].

Subsequently, a number of new equations have been developed and validated. Many of these new equations were developed using a "gold standard" other than inulin and may overestimate GFR. Furthermore, the precision and accuracy of each equation may not be the same at all GFR levels, depending on which populations were used for validation. As per KDIGO, currently the most robust pediatric estimated GFR (eGFR) formula derived using iohexol disappearance and creatinine measurements, which were measured centrally and calibrated and traceable to international standards, is from the CKiD study [38, 50, 51].

The most common creatinine-based formulas recommended for use in clinical practice is the updated bedside Schwartz formula also known as the CKID 2009 equation [50]

$$\mathrm{eGFR}\left(\mathrm{mL/min/1.73\ m^2}\right) = 41.3 \times \mathrm{height\ (m)\,t/Scr(mg/dL)}$$

or

$$\mathrm{eGFR}\left(\mathrm{mL/min/1.73\ m^2}\right) = 36.5 \times \mathrm{height(cm)/creatinine(\mu mol/L)}$$

The CKID 2009 equation was validated in a population consisting primarily of children aged 8–15 years of age with reduced GFR and used creatinine as the marker.

Equations based on multivariate analyses are superior to those using univariate analysis. However, in some situations, a univariate equation might be preferred. For example, since creatinine is highly dependent on muscle mass, a cystatin C based equation may be preferable in patients with reduced muscle mass.

In 2012, the CKiD study group developed an improved equation to estimate GFR by adding cystatin c and urea as filtration markers and sex as additional variables [51].

$$\mathrm{eGFR}\left(\mathrm{mL/min/1.73\ m^2}\right) = 39.8 * \left[\mathrm{ht(m)/Scr(mg/dL)}\right]^{0.456} \left[1.8/\mathrm{cystatin\,C(mg/L)}\right]^{0.418}$$
$$\left[30/\mathrm{BUN(mg/dL)}\right]^{0.079} \left[1.076^{\mathrm{male}}\right] \left[\mathrm{ht(m)}/1.4\right]^{0.179}$$

Cystatin C

Cystatin C is a low MW protein (MW = 13.36) member of the cystatin superfamily of cysteine protease inhibitors. It is produced at a stable rate by all nucleated cells. Cystatin C is freely filtered by the glomerulus and metabolized after tubular reabsorption [52]. Since it is not excreted in the urine, its clearance cannot be calculated.

Cystatin C is less influenced by age, gender and muscle mass than creatinine [53]. Levels decline from birth to one year of age then remain stable until about 50 years of age. However, cystatin C levels may be influenced by cigarette smoking, high c-reactive protein, steroid use and thyroid disorders [54–56]. One pediatric study showed that for children with CKD stage 3–5 the intrapatient coefficient of variation of cystatin C was significantly lower than serum creatinine and proposed that cystatin C is a better tool for longitudinally monitoring patients with advanced CKD [57]. Equations based on cystatin c and/or creatinine using different variables can be found in Table 4.

Some of the equations were developed using height as one of the variables whereas some are height independent to allow quick estimation of GFR when patient height is not available [58, 59]. These equations were externally validated against the gold standard single injection inulin clearance. The eGFR Pottell was superior to the eGFR-BCCH and comparable to the eGFR Schwartz [60]. Pottel developed an equation to be used in children, adolescents and young adults since none of the previous equations were validated for adolescent and young adults [61]. KDIGO suggest that measuring cystatin C based eGFR (not serum cystatin C) could be undertaken in adults with a creatinine-based eGFR of 45–59 mL/min/1.73 m^2 who do not have markers of kidney damage, such as proteinuria, in an attempt to confirm CKD. No specific recommendation for pediatrics or equation was made [38].

Since 2010, standards of measurement for cystatin C have been developed by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) to reduce variability. The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) recommends that eGFR based on cystatin C use an equation that was developed using data from measurement procedures that were standardized to the certified reference. This new IFCC-standardized assay increases measured cystatin C significantly and can thus affect the validity and reliability of GFR estimating equations [62]. The CKiD equation was one such equation initially affected by the change in standardization, but cystatin C values are now standardized to the appropriate IFCC reference. To correct older results to the new IFCC concentrations the value is multiplied by 1.17 [63].

Table 4 Equations to estimate GFR

Name	Equation to estimate GFR	Reference method used to derive
	Height dependent	
Updated Bedside Schwartz [50]	eGFR(mL/min/1.73 m^2) = 41.3[Height (m)/Scr (mg/dL)] or eGFR(mL/min/1.73 m^2) = 36.5[Height (cm)/Scr (μmol/L)]	Iohexol
Updated CKiD [51]	eGFR(mL/min/1.73 m^2) = 39.8[ht (m)/Scr (mg/dL)]$^{0.456}$[1.8/CysC (mg/L)]$^{0.418}$[30/BUN (mg/dL)]$^{0.079}$[1.076^{male}][ht (m)/1.4]$^{0.179}$	Iohexol
	Height independent	
Pottel [59]	eGFR(mL/min/1.73 m^2) = 107.3/[Scr (mg/dL)/Q] where Q is the median serum creatinine concentration for children based on age and sex Median serum creatinine (mg/dL) = 0.0270 × age + 0.2329 To express serum creatinine concentration in μmol/L, multiply by 88.4	Inulin
Modified BCCH equation [58]	eGFR(mL/min/1.73 m^2) = Inverse ln of: 8.067 + (1.034 × ln[1/SCr (μmol/L)]) + (0.305 × ln[age (years)]) + 0.064 if male	Iothalamate
Filler [223]	eGFR(mL/min/1.73 m^2) = 91.62[1/CysC]1.123	TcDTPA

Pediatric and adult equations for eGFR were developed using different datasets. The equations don't align at the transition from adolescent to adult. The CKiD equation generally underestimates GFR whereas the CKD-EPI equation, which is recommended for eGFR in adults, tends to overestimate GFR in young adults. Therefore, to improve the estimation of GFR in adolescent and younger adults, Pierce et al. modified the CKiD 2009 bedside estimation of GFR to include sex and age and used a dataset with patients 1–25 years old. The two equations, one for creatinine and one for cystatin C, are known as the CKiD U25 [64].

Bjork et al. also tried to improve the accuracy of eGFR in children and young adults [65]. They first established sex specific creatinine growth curves for children and young adults. These curves can be used to project childhood levels of serum creatinine to corresponding adult levels. Using the estimated adult creatinine, he then developed a modified CKD-EPI equation, the CKD-EPI 40, to improve precision and accuracy for both children and young adults at all measured GFR levels. This allows the use of the same equation for most patients without artificial change when transitioning from adolescent to adult care. However, the cohorts used for validation were from 4 European countries and may not be generalized to non-European populations or specific ethnic groups.

Other Methods

KDIGO suggests measuring GFR using an exogenous filtration marker when more accurate GFR will impact the treatment decisions. Since inulin is not widely available, other markers can be used.

Iohexol and Iothalamate

Iohexol is a safe nonionic low osmolar contrast agent (MW 821). It is eliminated exclusively by the kidneys, where it is filtered, but not secreted, metabolized, or reabsorbed. It has less than 2% binding to protein. Therefore, it makes it an ideal marker of GFR and a good alternative to the use of radiotracers that are not suitable for some patients and require special handling, storage and disposal. Iohexol and iothalamate have similar kinetic profiles, but iohexol has a lower allergic potential [66].

Clearance of iohexol correlates well with measured inulin clearance [48, 66–68]. Gaspari et al. [69] showed a highly significant correlation between GFR measured by the plasma clearance of iohexol (using a two-compartment open–model) and the GFR measured by urinary inulin clearance.

EDTA, DTPA Nuclear GFR

GFR can be accurately measured using a radioactive tracer such as Chromium 51 (51Cr) EDTA or technetium 99m (^{99m}Tc) DTPA in children. The most accurate method is based on the plasma disappearance curve after a single bolus injection, fitted by a double exponential curve. The clearance of the radiotracer is calculated as the injected dose divided by the area under the curve [70]. The initial "fast curve" represents the diffusion of the radiotracer in its distribution volume whereas the late slow exponential curve represents its renal clearance. The two-compartment model requires serial blood sampling to obtain an accurate plasma disappearance curve. In general, the more numerous blood samples that are acquired over time, the more accurate the calculated GFR value will be. However, to avoid excessive blood sampling, two simplified methods have been proposed for routine clinical use in children [71].

The Slope-Intercept Method

The slope-intercept method requires two blood samples acquired 2 and 4 h post injection and is based on the determination of the late exponential curve. An algorithm must be used to correct for overestimation of the clearance because this method neglects the early exponential curve. Late blood sampling (between 5 and 24 h) is recommended to improve the accuracy in patients with renal clearance below 10–15 mL/min/1.73 m^2.

The Distribution Volume Method

This method only requires one blood sample acquired at 2 h post injection. It appears to be valid for children of any age except for those with very poor renal function (GFR < 30 mL/min/1.73 m^2) [70].

One major limitation of these methods is decreased accuracy in the presence of significant edema. In such situations, the disappearance of the tracer will be influenced by its diffusion into an expanded extracellular volume, artifactually elevating the calculated GFR. Infiltration of the radiotracer at the injection site can also cause artifactual elevation of GFR.

The effective dose of radiation is approximately 0.011 mSv/examination regardless of the age of the child for Cr-EDTA and twice as high with low GFR (<10 min/1.73 m^2). It is 0.1 mSV/examination for Tc-DTPA [70].

Assessment of Tubular Function

Fluid filtered by the glomerulus (plasma ultrafiltrate) enters the proximal tubule where 60–65% of the filtrate will be reabsorbed [72]. In disorders of the proximal tubule, excessive amount of the solutes will be found in the urine. The fractional excretion of sodium and tubular reabsorption of phosphate can be used to assess the integrity of the proximal tubules. Detection of glucosuria and aminoaciduria can also be indicative of a proximal tubular disorder in certain situations.

Fractional Excretion of Sodium (FeNa)

This is one of the most used tests of tubular integrity. There is no "normal" for fractional excretion of salt. It must be interpreted in the context of each patient's sodium and volume status.

In the face of hyponatremic dehydration the appropriate response will be conservation of sodium and water. Therefore, the fractional excretion of salt will be low, usually with a FeNa <1% in children and less than 2.5% in neonates. The urinary sodium concentration will be <20 mEq/L in children and <30 mEq/L in neonates. The FeNa can be useful in distinguishing prerenal, assumed reversible acute kidney injury (AKI) as first shown in a seminal paper in 1976 where patients who recovered from their acute oliguria had a FeNa <1%, and those that didn't recover after volume resuscitation had a FeNA >3% [73]. If tubular damage has occurred, such as in acute tubular necrosis, the fractional excretion of sodium will be inappropriately elevated. The FeNa will be >2% in children and >2.5% in neonates. Urinary sodium will generally be more than 30 mEq/L.

However, FeNa is unreliable in certain situations, such as when patients are on diuretic therapy, receiving intravenous saline or in patients with salt losing tubulopathies or CKD [74, 75]. The FeNa may be substituted by FeUrea, as urea is less influenced by diuretics, because urea reabsorption is mostly dependent on passive forces [76]. A FeUrea <35% implies prerenal AKI; it is >50% if there is intrinsic AKI. High FeNa combined with FeUrea >35% has a 95% negative predictive value for intrinsic AKI [77].

The FeNa can be calculated as below:

$$\text{FeNa} = \frac{\text{U}(\text{Na}) * \text{PCr}}{\text{PNa} * \text{UCr}} * 100$$

UNa = urinary concentration of sodium
PCr = plasma creatinine
PNa = plasma sodium
UCr = urinary creatinine

Tubular Reabsorption of Phosphate (TRP)

Eighty-five to ninety-five percent of phosphate is usually reabsorbed in the proximal tubule [72]. Phosphate transport is primarily regulated by the plasma phosphate concentration and parathyroid hormone, which alter the Na^+-phosphate carrier activity.

The normal tubular reabsorption of phosphate (TRP) is greater than 85% and can be calculated:

$$\text{TRP\%} = 1 - \frac{\left(\text{UPO}_4 * \text{PCr}\right)}{\left(\text{PPO}_4 * \text{UCr}\right)} * 100$$

UPO_4 = urinary concentration of phosphate
PCr = plasma creatinine
PPO_4 = plasma concentration of phosphate
UCr = urinary concentration of creatinine

The renal tubular maximum reabsorption rate of phosphate to glomerular filtration rate (TmP/GFR) was initially described by Bijvoet using phosphate infusion [78]. Its initial use was for diagnosis of hypercalcemia, parathyroid disorders and renal handling of phosphate. Although it is no longer used for evaluation of hypercalcemia, it can still be helpful for evaluation of hypophosphatemia. A nomogram [79] and algorithm [80] were derived from the initial infusion data of Bijvoet and can be used to calculate the TmP/GFR ratio from the TRP. The algorithm is less prone to error and therefore recommended instead of the nomogram [81]. Basically, if the TRP is ≤0.86 then TmP/GFR = TRP × P_p (where P_p = plasma phosphate) and if >0.86 then TmP/GFR = 0.3 × TRP/[1 − (0.8 × TRP)] × P_p [80, 81].

Glucosuria

Filtered glucose is usually almost completely reabsorbed in the three segments of the proximal tubule. Glucose is transported across the apical membrane by secondary active transport dependent on the sodium electrochemical gradient generated by the Na–K ATPase. Two sodium-glucose transporters are found in the proximal tubule. SGLT2, in the early proximal tubule, has high capacity and low affinity for glucose and SGLT1, found in segments 2 and 3, has high affinity and low capacity [82].

The plasma glucose at which glucose reabsorption is maximal is defined as the *threshold for glucose* and the transport capacity when the threshold is reached is called the maximal tubular glucose reabsorption (TmG).

In presence of glucosuria, it is important to determine the serum glucose concentration.

The presence of isolated glucosuria with normal serum glucose concentration is usually a result of familial renal glucosuria. Mutations of *SGLT2* were first described by Santer et al. [83]. Isolated glucosuria with elevated serum glucose is suggestive of diabetes.

The antidiabetic SGLT2 inhibitors enhance both urinary glucose and urate excretion, but the mechanism behind the uricosuric effect remains to be fully elucidated and is possibly related to an indirect effect of the glucosuria on the expression of the urate transporter, URAT1 [84].

Transtubular Potassium Gradient (TTKG)

Potassium is secreted in the late distal and cortical collecting tubules in response to aldosterone. The transtubular potassium gradient is an indirect measure of the activity of the potassium secretory process in the cortical distal nephron and reflects the action of aldosterone. It is an important component of the evaluation of hyperkalemia and hypokalemia.

It can be calculated using the formula proposed by West [85].

$$\text{TTKG} = \frac{\text{UK} \div \left(\text{Uosm} / \text{Posm}\right)}{\text{Ppotassium}}$$

UK = urinary potassium concentration
Uosm = urinary osmolality
Posm = plasma osmolality

The urinary sodium concentration should exceed 25 mmol/L. This ensures sodium reabsorption is not limiting potassium secretion, and urine osmolality should exceed plasma osmolality.

The luminal potassium of the terminal cortical collecting duct is estimated by dividing the urinary potassium by the urine/plasma osmolality since the luminal potassium concentration is influenced by removal of water in the medullary segments. The serum potassium is an estimate of the peritubular potassium concentration.

TTKG appears to be a good indicator of aldosterone activity in both normal children and in children with hypoaldosteronism and pseudohypoaldosteronism. A TTKG below 4.1 in children or 4.9 in infants is indicative of a state of hypoaldosteronism or pseudohypoaldosteronism [86]. Ethier et al. define expected values of TTKG under stimuli that are known to modulate excretion of potassium. The expected value during hypokalemia induced from a low potassium diet is less than 2.5 and during acute potassium loading is greater than 10.0 [87]. It may also be useful in distinguishing aldosterone deficiency from resistance by repeating the calculation after initiation of mineralocorticoid therapy [88]. Kamel and Halperin have recently questioned the validity of the TTKG because one of the principal assumptions involved in calculating the TTKG, that the majority of osmoles in the medullary collecting ducts are not reabsorbed, is incorrect, as this is where urea recycling occurs. If more urea is reabsorbed, then the TTKG may overestimate the potassium excretion. As the amount of urea being recycled and excreted in the cortical collecting duct cannot be measured to provide a correction for the formula, the TTKG is no longer considered a valid test [89]. Instead, the urinary potassium to creatinine ratio calculated on spot urine can be used. The expected urine potassium/urine creatinine (UK/UCr) ratio in a patient with hypokalemia should be less than <1.5 mmol K/mmol creatinine, whereas the appropriate renal response to hyperkalemia would be a UK/UCr ratio >20 mmol K/mmol creatinine [89].

Aminoaciduria

In the normal state, most of the amino acids are reabsorbed in the proximal tubule. Sodium dependent cotransporters are responsible for the transport of glycine and glutamine whereas sodium independents carriers are responsible for the transport of neutral amino acids (leucine, isoleucine and phenylalanine), cystine and dibasic amino acids (ornithine, arginine and lysine). Mutation in *SLC3A1*, which encodes a protein responsible for the transport of cystine and the dibasic amino acids, is the cause of cystinuria (type I/I) [90, 91].

The cyanide–nitroprusside test is an easy way to detect urinary amino acids which contain a free sulfhydryl group or disulfide bond such as cystine, cysteine, homocystine and homocysteine and can diagnose cystinuria in the evaluation of nephrolithiasis [3]. Generalized aminoaciduria is usually associated with Fanconi syndrome.

Assessment of Acid Base Status

Total Carbon Dioxide (Total CO_2) and Bicarbonate (HCO_3^-)

Total CO_2 content of blood, plasma or serum consists of an ionized (bicarbonate and carbonate) and a non-ionized (carbonic acid) fraction. The ionized fraction includes HCO_3^-, CO_3^{2-} and carbamino compounds. The non-ionized fraction contains H_2CO_3 and physically dissolved (anhydrous) carbon dioxide. Total CO_2 measurement typically includes both of these fractions.

HCO_3^- results obtained from a blood gas analyzer is a calculated parameter. First, pH and pCO_2 are measured and then HCO_3^- is calculated using the Henderson-Hasselbalch equation:

$$\mathrm{pH} = \mathrm{pK1} + \log\left[\left(\mathrm{HCO_3^-}\right) \div \left(0.03 * \mathrm{pCO_2}\right)\right]$$

pK1 is usually equal to 6.1.

Discrepant values from calculated arterial bicarbonate and measured venous total CO_2 can be seen, especially in acutely ill pediatric patients who are prone to large fluctuations in pK1 [92].

Reference range for total CO_2 varies between 17 and 31 mEq/L depending on age.

Total CO_2 (tCO_2) and bicarbonate are reduced in the presence of acidosis. K/DOQI clinical practice guidelines for Bone metabolism and Disease in Children with CKD [93] recommends that serum level of tCO_2 be measured. Serum levels of tCO_2 should be maintained at ≥22 mmol/L in children over 2 years of age, and ≥20 mmol/L in neonates and young infants below age 2.

Maintaining the serum tCO_2 in the normal range, as suggested by K/DOQI, may also be desirable for better growth in children with CKD [94].

Serum Anion Gap (SAG)

The serum anion gap (SAG) is used in the interpretation of metabolic acidosis to determine if there are additional unmeasured anions contributing to the acidosis. It is the difference between the most abundant cations and anions measured in the blood. The serum anion gap is calculated as follows.

$$\mathrm{SAG} = \mathrm{Na}^{+} - \left[\mathrm{Cl}^{-} + \mathrm{HCO3}^{-}\right]$$

Potassium is generally not included in most references. However, if the serum K is significantly high or low, then it will alter the SAG. Reference values reported vary and depend on the method of quantification of the electrolytes by individual laboratories. A typical normal range is 8–16 with a mean of 12 ± 2 [95]. Correction for the serum albumin, a major anion, is advisable as the SAG changes by 2.5 mEq/L for every g/L change in serum albumin. Corrected SAG can be calculated by using the Figge equation as follows [96]:

$$\text{Corrected SAG} = \text{SAG} + 0.25 \times \left(\text{normal albumin} - \text{measured albumin}\left[\text{g / L}\right]\right)$$

or

$$\text{Corrected SAG} = \text{SAG} + 2.5 \times \left(\text{normal albumin} - \text{measured albumin}\left[\text{g / dL}\right]\right)$$

Not correcting for albumin, especially when it is low, may lead to an increased anion gap metabolic acidosis being missed in a significant number of cases [97].

Urine Anion Gap

The urine anion gap is normally positive (range 30–50 mmol/L) because there is excretion of unmeasured anions such as phosphate and sulfate. The urine anion gap can be used clinically as an indirect measurement of ammonium production by the distal nephron. Because ammonium is not routinely measured in most laboratories, clinicians need an index of ammonium secretion to use in the evaluation of normal anion gap metabolic acidosis. This was initially proposed by Goldstein et al. [98] and its clinical usefulness was also shown later by Batlle et al. [99]. In the setting of metabolic acidosis, ammonia production by the kidney is increased and ammonium is excreted into the urine with chloride.

If an excess of ammonium is present, the sum of sodium and potassium will be less than the chloride since ammonium is an unmeasured cation. This test presupposed that the chloride is the predominant anion in the urine balancing the positive charge in urine NH_4^+.

Therefore, the urine anion gap can be calculated by the equation:

$$\text{Urine anion gap} = \left(\mathrm{Na} + \mathrm{K}\right) - \mathrm{Cl}$$

A negative urine anion gap suggests gastrointestinal loss of bicarbonate or renal bicarbonate loss whereas a positive urine anion gap suggests the presence of impaired distal urinary acidification [99].

The urine anion gap cannot be used in volume depletion with a urine sodium concentration of less than 25 mmol/L; when there is increased excretion of unmeasured anions such as ketoacid or hippurate; or in neonates.

Urine Osmolar Gap

Halperin et al. [100] proposed the urine osmolar gap in addition to urine anion gap to ascertain

the etiology of normal or increased anion gap metabolic acidosis as well as mixed metabolic acidosis. The urine osmolar gap was defined as the difference between the measured urine osmolality and the sum of the concentration of sodium, potassium, chloride, bicarbonate, urea, and glucose. Normally this gap is 80–100 mOsmol/kg H_2O. Values >100 mOsmol/kg indicate increased urinary ammonium salts, the normal response to a metabolic acidosis [101]. A lower urine osmolar gap occurs in patients with dRTA.

Calculation of the urine osmolar gap is helpful in excluding glue sniffing, which causes a normal anion gap metabolic acidosis. A high urinary concentration of the unmeasured ions ammonium and benzoate will increase the osmolar gap, and can lead to a false exclusion of RTA as the cause of acidosis [102].

Urine-Blood pCO_2 (U-B pCO_2)

U-B pCO_2 can be used for the evaluation of normal anion gap metabolic acidosis with a positive urine net charge to differentiate between deficient ammonium production versus poor hydrogen secretion [103, 104]. The pCO_2 should be measured in alkaline urine. With adequate hydrogen secretion in the distal tubule, the hydrogen will couple with HCO_3 to form H_2CO_3 and then dissociate into CO_2 and H_20.

Kim et al. [105] evaluated the diagnostic value of the U-B pCO_2 in patients diagnosed as having H^+-ATPase defect dRTA based on reduced urinary NH_4^+ and absolute decrease in H^+ ATPase immunostaining in intercalated cells. U-B pCO_2 during sodium bicarbonate loading was less than 30 mmHg in all patients with H^+ ATPase defect dRTA.

General Biochemistry

Serum Sodium

The reference range for plasma sodium varies with age and method of measurement used. The following reference range for serum sodium is recommended by the Canadian Laboratory Initiative in Pediatric Reference Intervals (CALIPER) database group and the Australasian Association of Clinical Biochemists (AACB); birth to <7 days 132–147 mmol/L, ≥7 days to <2 years 133–145 mmol/L, ≥2 to <12 years 134–145 mmol/L, and ≥12 to adult 135–145 mmol/L [106, 107]. Serum sodium outside that range can have serious consequences.

Hyponatremia is the result of excess free water or sodium loss. The former is usually associated with expended extracellular fluid, while the latter is often seen with volume contraction. Hyponatremia with normal serum osmolality is usually the result of hyperlipidemia, hyperproteinemia, or hyperglycemia. Most laboratories now measure serum sodium with ion-specific electrodes and the measurement will not be affected by hyperlipidemia and hyperproteinemia. In the presence of hyperglycemia every 3.4 mmol/L increment in glucose will reduce serum sodium by 1 mmol/L because of water shift from the intracellular space to the extracellular space.

Hypernatremia is usually secondary to water deficit, either because of poor intake or increased water loss such as in diabetes insipidus or mellitus. Salt intoxication is a less common cause of hypernatremia.

Serum Potassium

Approximately 98% of body potassium is located intracellularly. Cell potassium concentration is about 140 mmol/L, whereas normal range for serum potassium varies between 3.2 and 6.2 mmol/L, depending on age. Reference range varies with the method used and age of the child. In infants, the upper limit of normal can be as high as 6.2. The upper limit then progressively decreases to about 5.0 to reach the "adult" level. Reference ranges vary depending on the methodology [108].

Hyperkalemia can be the result of intracellular to extracellular shift in the presence of acidosis, beta blockers, or cellular breakdown; decreased excretion in renal failure, hypoaldo-

steronism or pseudohypoaldosteronism; and, less commonly, with increased potassium intake. Pseudohyperkalemia is defined as a serum K^+ that exceeds plasma K^+ by 0.4 mmol/L on a sample processed within 1 h of venipuncture (delay results in glucose depletion, and less ATP generation, which is the energy source of the sodium-potassium pump) and maintained at room temperature (lower temperatures inhibit the pump leading to potassium leak out of cells). It can occur with prolonged tourniquet use, mechanical factors, excessive crying and respiratory alkalosis, potassium EDTA contamination (associated also with hypocalcemia), leukocytosis, erythrocytosis and thrombocytosis. During the clotting process activated platelets degranulate and release potassium [109, 110]. The benign, dominantly inherited familial pseudohyperkalemia presents as hyperkalemia when the potassium is measured at or below room temperature, but no hyperkalemia when the potassium is measured at normal body temperature [111]. It has recently been linked to mutations in an erythrocyte porphyrin transporter, *ABCB6*, on chromosome 2 [112].

Hypokalemia not associated with diarrhea or emesis is mostly seen in renal tubular disorders such as Fanconi's syndrome, Bartter's syndrome, and Gitelman's syndrome and in hyperaldosteronism.

Serum Calcium, Phosphorus, and Calcium-Phosphorus Product

Evaluation of calcium, phosphorus and calcium phosphorus product is reviewed in detail in the guidelines published by K/DOQI [18, 93]. Representative normal values for serum phosphorus, ionized calcium and total calcium are in Table 5. Pseudohypocalcemia may be seen with gadolinium based contrast agents or in thrombocytosis [113]. The serum phosphate may appear falsely low after mannitol infusion and falsely elevated with hyperbilirubinemia, hyperlipidemia, amphotericin administration or if the sample is taken from a central line that has been treated with tissue plasminogen activator [113].

In CKD, serum levels of phosphorus should be maintained at or above the age-appropriate lower limits and no higher than the age–appropriate upper limits. For children with CKD stage 5, the serum level of phosphorus should be maintained between 3.5–5.5 mg/dL (1.13–1.78 mmol/L) during adolescence and between 4–6 mg/dL (1.29–1.94 mmol/L) for children between the ages of 1–12 years.

Calcium in blood exists in three fractions: protein-bound calcium, free (ionized) calcium and calcium complexes. Total measured calcium should be corrected if serum albumin is abnormal to better reflect the ionized calcium. The following formula can be used:

Table 5 Normal values for serum phosphorus, blood ionized calcium concentrations (adapted from [93])

	Serum phosphorus			Total calcium	
Age	mg/dL	mmol/L[a]	Blood ionized calcium (mM)	mg/dL	mmol/L[b]
0–3 months	4.8–7.4	1.55–2.39	1.22–1.40	8.8–11.3	2.20–2.83
1–5 years	4.5–6.5	1.45–2.10	1.22–1.32	9.4–10.8	2.35–2.70
6–12 years	3.6–5.8	1.16–1.87	1.15–1.32	9.4–10.3	2.35–2.57
13–20 years	2.3–4.5	0.74–1.45	1.12–1.30	8.8–10.2	2.20–2.55

[a] Serum phosphorus converted from mg/dL to mmol/L using a factor of 0.3229
[b] Serum calcium converted from mg/dL to mmol/L using a factor of 0.250

ALGrawany

$$\text{Corrected calcium } (\text{mg/dL}) = \text{total calcium } (\text{mg/dL}) + 0.8 \times [4 - \text{serum albumin } (\text{g/dL})]$$

Ionized calcium is affected by pH since hydrogen ion displaces calcium from albumin. A fall of 0.1 unit in pH will cause an approximately a 0.1 mEq/L rise in the concentration of ionized calcium. As serum ionized calcium is not routinely measured at most institutions, K/DOQI guidelines are based on corrected total calcium. Levels should be maintained within normal range for the laboratory and preferably toward the lower end in CKD stage 5. The serum calcium-phosphorus product should be maintained at <55 mg^2/dL^2 (4.4 $mmol^2/L^2$) in adolescents greater than 12 years and <65 mg^2/dL^2 (5.2 $mmol^2/L^2$) in younger children [93].

Serum Albumin

Serum albumin is used to assess the nutritional status in children with or without renal disease. Although it is used as a measure of the nutritional state of an individual, it can be affected by non-nutritional factors, especially in children with CKD, such as infection, inflammation, hydration status, peritoneal and urinary losses [114]. Children with low serum albumin should be assessed for protein-energy malnutrition if not losing protein.

Serum Uric Acid

Serum uric acid varies with both age and gender [115, 116]. An elevated serum uric acid is much less common in childhood as compared to adulthood, and in contrast to adults where gout is the primary cause, hereditary disorders of purine biosynthesis account for the majority of cases in children. These include a deficiency of the enzyme hypoxanthine-guanine phosphoribosyltransferase (Lesch-Nyhan syndrome) and hereditary xanthinuria [117]. Hyperuricemia is also found in familial juvenile hyperuricemic nephropathy in association with mutations in the uromodulin gene [118]. The combination of hyperuricemia, anemia, early onset kidney failure and hypotensive or presyncopal episodes should raise suspicion of the recently described disorder associated with a mutation in the renin gene [119]. The renal cysts and diabetes syndrome caused by mutations in the gene for hepatocyte nuclear factor 1β (*HNF1β*) is also associated with hyperuricemia [120]. Patients with *HNF1β* mutations may also present with hypomagnesemia [121].

The kidney is the primary site of excretion of uric acid. The fractional excretion of filtered urate is 15–30% in children compared to 10% adults [122]. Hyperuricemia can be seen in AKI, tumor lysis syndrome, and secondary to certain drugs such as thiazide diuretics, salicylates and cyclosporine [116]. In dehydration, serum uric acid correlates significantly with weight change, and increased significantly with dehydration severity in one study [123].

There is ongoing debate and uncertainty as to whether there is a causal link, or merely an association, between hyperuricemia and hypertension or CKD progression [124, 125]. Uric acid may be a clinically useful marker in the management of essential hypertension in adolescents and young children, where there is some evidence that even in preschool children those with higher serum uric acid have higher blood pressures [126–128]. A pediatric study of over 100 patients with CKD found that 70% of children with an eGFR <60 mL/min/1.73 m^2 had uric acid levels above the normal range [129]. A subsequent analysis including over 600 children and adolescents from North America found that those with hyperuricemia progressed faster to CKD [130].

Drugs that lower serum uric acid include allopurinol and rasburicase. In addition, the angiotensin receptor blocker losartan is uricosuric [131], and could be beneficial in hypertensive patients with hyperuricemia [125]. Allopurinol can increase xanthine and hypoxanthine levels, leading to xanthinuria and xanthine stones. It is also associated with severe dermatological reactions in children.

Urinary Calcium

Measurement of calcium excretion should be part of the evaluation of patients with hematuria, nephrocalcinosis and renal stones and can often be useful in the assessment of children with frequency, dysuria, urgency and recurrent UTI [132]. Urinary calcium excretion varies with age, being highest in infancy and reaching its nadir during puberty.

Hypercalciuria is usually defined as a urinary calcium excretion of more than 4 mg/kg/day based on the study of Ghazali and Barratt [133]. However, several authors have studied urinary calcium excretion and published reference ranges in their study population [134–138].

A spot urinary calcium/creatinine ratio (usually collected on the second morning fasting urine specimen) correlates well with 24-h calcium excretion, especially for children with normal muscle mass. They can be used clinically; however, they are more likely to be affected than a 24-h collection by factors such as recent dietary calcium intake [139]. Sodium, protein, phosphorus, potassium, and glucose intake can influence calcium excretion. There is no apparent seasonal variation of urinary calcium/creatinine ratio in children as seen in adults [140]. A 24-h urine collection for calcium assessment is typically stored in acidified bottles containing hydrochloric acid to prevent the crystallization of calcium oxalate although this may not be necessary [141]. For those who have reduced muscle mass, a urinary calcium/osmolality ratio predicts hypercalciuria with better sensitivity and specificity than a urine calcium/creatinine ratio [142, 143].

Urinary Sodium

Measurement of urinary sodium excretion should be assessed in children with hypercalciuria. Polito et al. found that urinary sodium excretion and 24-h urinary sodium/potassium ratio (U Na/K) was higher in children with hypercalciuria [144]. Twenty-four-hour urinary sodium can also be used to assess dietary sodium intake in adults on a low sodium diet for the treatment of hypertension where a target 24 h intake of 50–100 mmol/day is recommended [145].

Urinary Magnesium

Magnesium is a known stone inhibitor as it forms complexes with oxalate and reduces supersaturation. Thirty-nine percent of children with calcium oxalate stones in one series had hypomagnesuria defined as magnesium excretion less than 1.2 mg/kg/24 h [146]. Urinary reference limits for Mg/Cr can be found in Table 6.

Urinary Citrate

Citrate inhibits calcium-oxalate and calcium-phosphate crystal nucleation, growth and aggregation. In normal circumstances, citrate is freely filtered at the glomerulus with a 65–90% reabsorption rate. Systemic acidosis, potassium depletion, starvation and acetazolamide therapy decrease urinary citrate. Citrate excretion is age and sex related. Mean molar excretion of citrate is higher in infants than in older children, and in infants is higher in females than males (Table 7) [147].

Table 6 Urinary reference limits for urinary magnesium/creatinine (adapted from [224])

	Urinary Mg/Cr	
	mol/mol (mg/mg)	
Age in year	5th Percentile	95th Percentile
1/12–1	0.4 (0.10)	2.2 (0.48)
1–2	0.4 (0.09)	1.7 (0.37)
2–3	0.3 (0.07)	1.6 (0.34)
3–5	0.3 (0.07)	1.3 (0.29)
5–7	0.3 (0.06)	1.0 (0.21)
7–10	0.3 (0.05)	0.9 (0.18)
10–14	0.2 (0.05)	0.7 (0.15)
14–17	0.2 (0.05)	0.6 (0.13)

Table 7 Mean molar citrate/creatinine ratio based on [147]

Urinary citrate/creatinine	Girls	Boys
Infants	1.9	0.63
Childhood	0.27	0.33
Adolescence	0.32	0.28

Citrate excretion of more than 1.6 mmol/1.73 m^2 in girls and more than 1.9 mmol/1.73 m^2 in boys is considered normal [148].

Hypocitraturia, either alone or in association with hypercalciuria, is an important risk factor for nephrolithiasis in children [149, 150]. In one study, hypocitraturia defined as citrate excretion <320 mg/1.73 m^2/24 h [1.66 mmol/1.73 m^2/24 h] was observed in 60.6% of children with calcium oxalate stones [146]. A study in a stone-forming adult population suggests that urinary calcium to citrate ratio >0.25 mg/mg is predictive of lithogenesis [151].

Urinary calcium-to-citrate ratio >0.326 mg/mg has been found in children to predict stone formers in a random urine sample [152]. Furthermore, urinary calcium-to-citrate ratio calculated on 24-h collection can also distinguish between solitary and recurrent calcium stone forming children. The mean urinary calcium-to-citrate was 0.41 mg/mg in those with a single stone episode and 0.64 mg/mg in recurrent stone formers as compared to the mean of 0.33 mg/mg seen in normal children without stones [153]. Hypocitraturia is also recognized as a major risk factor for nephrocalcinosis in very low birth weight infants [154] and after kidney transplantation [155].

Urinary Oxalate

Urinary oxalate excretion is significantly increased in primary hyperoxaluria type I (PH I), PH II and PH III and in secondary hyperoxaluria. In PH I, there is excessive endogenous production of oxalate caused by a deficiency of hepatic alanine:glyoxylate amino transferase (AGT), which catalyzes the peroxisomal conversion of glyoxylate to glycine and in PH II, a deficiency of cytosolic glyoxylate reductase/hydroxypyruvate reductase (GRHPR), an enzyme that catalyzes the reduction of glyoxylate and hydroxypyruvate as well as the dehydrogenation of glycerate [156, 157]. PH III is due to a defect in a hepatocyte specific mitochondrial enzyme, 4-hydroxy-2-oxoglutarate aldolase (HOGA) [158].

The secondary forms are due to increased intestinal absorption of oxalate due to malabsorptive states or impaired vitamin status. Reference values for oxalate/creatinine can be found in Table 8.

Patients suspected of having abnormalities in oxalate metabolism should have more extensive studies, including measurement of oxalate, glycolate and L-glycerate and in some cases liver biopsy to assess the activity of AGT and GRHPR.

Elevated oxalate and glycolate is associated with PH1; however, normal glycolate is found in 25% of subject with PHI. Elevated urinary oxalate and L-glycerate is the typical finding of hyperoxaluria type II, but likewise elevated L-glycerate is not always present. Genetic analysis is now considered the gold standard for diagnosis and liver biopsy, to measure intrahepatic enzyme levels, is generally reserved for patients in whom no mutation can be found [156, 157].

Table 8 Urinary reference limits for calcium/creatinine, oxalate/creatinine, urate/creatinine [224, 225]

	Urinary calcium/creatinine		Urinary oxalate/creatinine		Urinary urate/creatinine	
	mol/mol (mg/mg)[a]		mol/mol (mg/mg)[a]		mol/mol (mg/mg)[a]	
Age	5th	95th	5th	95th	5th	95th
1–6 months	0.09 (0.03)	2.2 (0.81)	0.07 (0.0560)	0.22 (0.175)	0.80 (1/189)	1.60 (2.378)
6 months–1 year	0.09 (0.03)	2.2 (0.81)	0.06 (0.0480)	0.17 (0.139)	0.70 (1.040)	1.50 (2.299)
1–2	0.07 (0.03)	1.5 (0.500)	0.05 (0.04)	0.13 (0.103)	0.50 (0.743)	1.40 (2.080)
2–3	0.06 (0.02)	1.4 (0.41)	0.04 (0.032)	0.10 (0.080)	0.47 (0.698)	1.30 (1.932)
3–5	0.05 (0.02)	1.1 (0.30)	0.03 (0.024)	0.08 (0.064)	0/40 (0.594)	1.10 (1.635)
5–7	0.04 (0.01)	0.8 (0.25)	0.03 (0.024)	0.07 (0.056)	0.30 (0.446)	0.80 (1.189)
7–10	0.04 (0.01)	0.7 (0.24)	0.02 (0.016)	0.06 (0.048)	0.26 (0.386)	0.56 (0.832)
10–14	0.04 (0.01)	0.7 (0.24)	0.02 (0.016)	0.06 (0.048)	0.20 (0.297)	0.44 (0.654)
14–17	0.04 (0.01)	0.7 (0.24)	0.02 (0.016)	0.06 (0.048)	0.20 (0.297)	0.40 (0.594)

[a]Conversions have been performed with higher precision, then rounded for this presentation [224, 225]

Urinary Uric Acid

Increased urinary uric acid excretion can present with microscopic hematuria, abdominal and/or flank pain, dysuria, gravel and macroscopic hematuria. About half of patients with hyperuricosuria (HU) will have microlithiasis on ultrasonography [159]. HU may be defined by urine uric acid concentration corrected for creatinine clearance >0.53 mg/dL/GFR [160]. The excretion varies with age, being highest in infants. However, a simpler estimate of urinary urate excretion can be calculated from the urine urate/creatinine ratio. Reference values for urine urate/creatinine can be found in Table 8.

Assessment of the Renin-Angiotensin-Aldosterone System

Renin is a proteolytic enzyme predominantly formed and stored in the juxtaglomerular cells of the kidney. Renal hypoperfusion and increased sympathetic activity are the major physiologic stimuli to renin secretion [72]. When released in the circulation, renin cleaves angiotensinogen to produce a decapeptide angiotensin I. Angiotensin I is then converted to an octapeptide, angiotensin II, by the angiotensin I-converting enzyme. Angiotensin II is a potent vasoconstrictor and promotes salt and water retention. The converting enzyme is mainly located in the lung, but angiotensin II can be synthesized at a variety of sites, including the kidney, luminal membrane of vascular endothelial cells, adrenal gland and brain. Angiotensin II promotes renal salt and water reabsorption by stimulation of sodium reabsorption in the early proximal tubule and by indirectly activating aldosterone biosynthesis in the zona glomerulosa of the adrenal cortex.

Measurement of plasma renin activity may not reflect the tissue activity of the local renin-angiotensin system.

Assessment of the renin-angiotensin-aldosterone system may be required in the evaluation of hypokalemia/hyperkalemia, adrenal insufficiency, and hypertension, see Table 9.

Renin

Renin release is dependent on renal tubular sodium concentration, renal perfusion pressure and beta-adrenergic vascular tone. The enzymatic activity of renin can be measured. It is expressed as the amount of angiotensin I generated per unit of time and is expressed in pmol or ng of generated angiotensin I per mL of plasma.

The "normal values" for plasma renin activity (PRA) are highly dependent on sodium intake, time of day, posture, age and methodology [161]. PRA varies inversely with age in infants and children. Reference values derived from measurement of PRA in 79 children age 1 month to 15 years in supine position were published in 1975 [162].

PRA can be useful in the management of hypertension to distinguish between a volume dependent hypertension, where renin is suppressed, and hypertension mediated by excess

Table 9 Assessment of the renin-angiotensin-aldosterone system

	PRA	PAC	PAC/PRA	BP	Potassium
Primary hyperaldosteronism	Decrease	Increase	Very high >20–50	High	Low
GRA	Decrease	Increase	High	High	Normal or low
Renin-secreting tumor	Increase	Increase		High	Low
Bartter' syndrome	Increased	Increased		Normal	Low
Renovascular disease	Increased	Increased	<10		
Apparent mineralocorticoid excess, Cushing, licorice ingestion	Low	Low		High	Low

PRA plasma renin activity, *PAC* plasma aldosterone concentration, *BP* blood pressure, *GRA* glucocorticoid remediable hypertension

renin secretion. A value less than 0.65 ng/mL/h suggests renin suppression, while a value above 6.5 ng/mL/h implies excess renin. This can help guide appropriate therapy [163].

During renal angiography, renal vein renin sampling may be done to predict feasibility of correcting hypertension or to identify which kidney contributes to the hypertension. A ratio of the renal vein renin from the diseased kidney (R) to the renal vein renin from the normal or less diseased contralateral kidney (RC) above 1.5 (R/RC >1.5) is considered significant, and there is greater probability that blood pressure will improve after surgery. A RC/infrarenal inferior vena cava ratio less than 1.3 further supports the presence of a lesion that may respond to repair. Segmental veins within a kidney may also be sampled [164–168].

Aldosterone

Aldosterone is synthesized in the zona glomerulosa of the adrenal gland. It regulates electrolyte excretion and intravascular volume, mainly through its effects on the distal tubules and cortical collecting ducts of the kidneys, where it acts to increase sodium reabsorption and potassium excretion [169].

Aldosterone is measured by radioimmunoassay. Like renin, it is dependent on sodium intake, posture and time of the day. Serum aldosterone concentration is highest at the time of awakening and lowest shortly after sleep onset. Hyperaldosteronism should be sought for in children with hypertension, hypokalemia and metabolic alkalosis [170].

Plasma Aldosterone Concentration (PAC) to Plasma Renin Activity Ratio

The PAC/PRA ratio is a screening test for hyperaldosteronism prior to confirmation with a suppression test. Patients should not be taking aldosterone receptor antagonists, ACE inhibitors and angiotensin receptor blockers for 3–6 weeks, and hypokalemia needs to be corrected prior to the test. Any medication that stimulates renin production, such as diuretics, may lead to a false negative result. False positives can be expected with the reduced renin and increased salt and water retention of renal impairment. The patient needs to be in the seated position for 10–15 min after ambulating for at least 2 h, and midmorning is considered the best time to perform the test [171]. The mean normal value is 4–10 compared to more than 30–50 in patients with primary hyperaldosteronism [172].

Endocrine Testing Relevant to Nephrology

ADH/Copeptin

Arginine vasopressin (AVP) or antidiuretic hormone (ADH) is one of the key hormones in the regulation of salt and water balance. The measurement of ADH can be helpful in the diagnosis and work-up of sodium disorders. However, due to its structural instability, very short plasma half-life, and long laboratory processing time, its use in clinical practice is limited. Copeptin is a well-established ADH surrogate. It is secreted in an equimolar amount to ADH and can be easily measured in plasma or serum. The distribution of copeptin levels in pediatric cohorts indicate a range between 2.4 and 8.6 pmol/L [173].

The distribution of plasma copeptin in percentiles in a population of children without disturbance of the AVP system can be found in a study published in 2021 [174]. Finally, measuring copeptin after infusion of 3% saline to induce hypernatremia of at least 150 mmol/L (hypertonic saline infusion test) may have greater diagnostic accuracy for diabetes insipidus (plasma copeptin <4.9 pmol/L) and primary polydipsia (plasma copeptin >4.9 pmol/L) than the indirect water-deprivation test [175].

Biochemical Testing for the Diagnosis of Pheochromocytoma

Typical testing of patients suspected of having pheochromocytoma includes measurement of catecholamines and metanephrines in the urine and plasma. Plasma and urinary metanephrines are recommended as they are more reliable, with the best negative predictive value. This is because metanephrines are produced continuously by the pheochromocytoma, whereas catecholamines are only intermittently secreted. Plasma metanephrines are most often measured using high-performance liquid chromatography coupled with either electrochemical detection or mass spectrometry. The sensitivity of plasma free metanephrines to diagnose pheochromocytoma is 97% and has a specificity of between 80 and 100%. Twenty-four-hour measurement of urinary fractionated metanephrines has similar sensitivities and specificities. Plasma metanephrines should ideally be obtained when the patient is fasting and has been in the supine position for at least 30 min to avoid sympathoadrenal activation. False positives due to interference with the analytical methods can be seen with acetaminophen, mesalamine and sulfasalazine. Alternatively, there may be false positives caused by drugs that affect metabolism or secretion, of catecholamines, such as caffeine, cocaine, amphetamines, ephedrine, venlafaxine (serotonin-noradrenaline reuptake inhibitor), tricyclic antidepressants, dihydropyridine calcium channel blockers, doxazocin and phenoxybenzamine [176].

Complement Pathway Assessment

The complement system consists of at least thirty plasma membrane proteins that provide an innate defense against microbes and an adjunct or complement to humoral immunity [177, 178]. The complement system is divided into three major pathways: classical, lectin and alternative. The lectin pathway is activated by the binding of lectin (which has a similar structure to C1q) to sugar residues on the surface of a pathogen and the alternative pathway which is an amplification loop for C3 activation is activated by polysaccharide antigens, aggregated IgA, injured cells or endotoxins. The classical pathway is activated by the binding of C1q to the Fc portion of antibody. The alternative pathway is a constitutively active and amplifiable system. Plasma factor H and factor I are important fluid phase complement regulators of the alternative pathway, while CD46 (membrane cofactor protein), CD55 (decay accelerating factor) and CD59 (protectin) are the principal surface bound regulators. Each of these three pathways lead to the deposition of an activated C3 fragment (C3b), inducing the final steps of the complement cascade, which include opsonisation, phagocytosis, induction of inflammation, and formation of the membrane attack complex (MAC) and cytolysis [179]. A fourth pathway by which thrombin can directly activate C5 and thus the terminal complement cascade was also identified and links the complement and coagulation cascades [180]. However, the significance of this pathway in the pathogenesis of disease is still unclear. There is an evolving spectrum of kidney diseases now recognized as being either complement-mediated or having complement dysregulation as part of their pathogenesis, and these will be discussed in a later chapter.

Evaluation of the complement system, or *complement analysis*, plays an integral part in the diagnosis of various infectious, inflammatory and autoimmune diseases as well as many glomerular diseases caused by either overactivity, or loss of regulation, of the complement system. Much progress has been made not just in the understanding of the role of complement in the pathogenesis of these diseases, but also in the quality and standardization of the methods of complement analysis [179, 181]. Concentrations of C3, C4 and C1q can be quantified by immunological methods. Glomerular disease associated with activation of the classical pathway will typically have a low C4 and C3, whereas disease associated with activation of the alternative pathway will have a low C3 and normal C4. Serial assessment of complement proteins can also be helpful in monitoring disease activity in immune complex mediated disease such as lupus. C4 is less likely to normalize because one or more C4 null genes

are common in SLE; therefore, patients in remission can continue to have low C4. However, C3 is sensitive to change in disease activity [182]. The normal range for C3 varies considerably between laboratories [182]; therefore, no normal values are provided in this chapter.

There are functional assays that assess the total hemolytic activity of the entire classical (CH50) and alternative (AH50) pathways [181]. All nine components (C1–C9) are required to have a normal CH50, a test which in the past assessed the ability of the patient's serum to lyse sheep erythrocytes optimally sensitized with rabbit antibody, but now typically uses a synthetic liposome coated with antibody instead of erythrocytes [181]. A suppressed CH50 suggests a deficiency or consumption of one or more component, or complement activation and consumption of complement proteins. The CH50 is useful to diagnose hypocomplementemic states (for example congenital C2 deficiency) that would be missed if only C3 and C4 were measured.

AH50 is a functional assay of the alternative pathway. The assay typically used erythrocytes from rabbit, guinea pig or chicken, and the classical pathway is kept inactive by the addition of a calcium chelator (EGTA). Suppressed AH50 suggests a deficiency or consumption of one or more components of the alternative pathway. Depressed or absent AH50 activity, in the presence of a normal CH50, suggests a deficiency in an alternative pathway component (such as C3, C5, C6, C7, C8, C9, factor D, factor B, properdin or the regulators factor H or factor I). When the AH50 is normal, but the CH50 is absent, then there might be a classic pathway deficiency such as C1q, C2 or C4, or the presence of a C1 inhibitor. When both CH50 and AH50 are depressed this reflects either a late component deficiency or a setting of complement consumption and one should measure C3, C5–C9 as well as levels of factor H and I [183].

When a complement-mediated disease is suspected, more in-depth analysis of the complement system can be performed. The International Complement society provides guidelines for modern complement analysis [179]. There are a number of steps in order to complete a thorough analysis of the complement system including (1) functional assays of global complement pathway function, with the CH50, AH50 and lectin pathway (assessed by semiquantitative enzyme-linked immunosorbent assay [ELISA]), (2) analysis of individual complement components/proteins, such as C3, C4 and C1q, (3) analysis of key complement regulators such as CFH, CFI and C1-INH, (4) detection of complement activation or split products such as C3dg, C3a, Bb, sC5b-9, typically measured by ELISA in the serum, urine or tissues, (5) measuring auto-antibodies to various complement components, to the C3 convertase (C3 nephritic factor), or to the alternative pathway regulator CFH (anti-CFH) and (6) genetic analysis for mutations in C3, CFB, CFH, CFI, CD46/MCP, now linked to various diseases including atypical hemolytic uremic syndrome, membranoproliferative glomerulonephritis and antibody-mediated rejection.

The measurement of complement split products such as C3a, C3d, C4d or Bb and the soluble form of the membrane attack complex (sC5b-9) have allowed more sensitivity and accuracy in defining whether it is the classical, alternative, or terminal common pathway that is involved, and monitoring the degree of complement activation. Measuring these split products, and in particular sC5b-9, can be useful to ensure complete complement inactivation by complement inhibitors such as eculizumab [181].

Laboratory Assessment of Various Glomerulopathies

Antineutrophil Cytoplasmic Antibodies (ANCA)

Antineutrophil cytoplasmic antibodies (ANCA) are IgG autoantibodies directed against constituents of primary granules of neutrophil and monocyte lysosomes. They were first described in 1982 in patients with pauci-immune glomerulonephritis [184]. Indirect immunofluorescence (IIF) and ELISA are the most widely used techniques to detect ANCA. By IIF, two major immunostaining patterns can be seen: the dif-

fuse granular cytoplasmic pattern with central accentuation known as C-ANCA and the perinuclear pattern which is defined as perinuclear fluorescence with nuclear extension known as P-ANCA. Diffuse flat cytoplasmic staining without interlobular accentuation can also be seen and is termed atypical C-ANCA. Atypical ANCA includes all other neutrophil-specific or monocyte-specific IIF reactivity, most commonly a combination of cytoplasmic and perinuclear fluorescence. Proteinase 3 and myeloperoxidase are two antigenic targets associated with vasculitis. The cytoplasmic pattern usually suggests the presence of serum proteinase 3 ANCA (PR3-ANCA), whereas the perinuclear pattern with nuclear extension is usually associated with myeloperoxidase ANCA (MPO-ANCA). Occasionally, antinuclear antibodies can give a false positive P-ANCA and this can be avoided by fixing the neutrophils with formalin rather than ethanol [185]. ELISA is used in addition to prove the presence of myeloperoxidase and proteinase 3 ANCA [186]. In order to enhance specificity, both IIF and ELISA have been recommended.

More novel assays for ANCA confirmation include capture ELISAs, high sensitivity or anchor ELISAs and automated immunoassays, the latter able to provide a result in under an hour, have become more widespread [187]. There are now several commercially available antigen-specific immunoassays that detect antibodies (IgG) to either MPO or PR3. These include first, second (monoclonal antibody capture-based) and third generation (peptide anchor-based) ELISA as well as fluorescent-enzyme immunoassays, chemiluminescent, or multiplex bead assays. These assays have excellent comparative performance with area under the curve (AUC) ranging from 0.92 to 0.98, better than those for IIF in recent studies [188, 189]. Current guidelines now recommend using a high-quality antigen-specific immunoassay to screen for MPO- or PR3-ANCA given that the turnaround time is faster and the cost is lower [190].

Antibodies to several azurophilic granule proteins (lactoferrin, elastase, cathepsin G, bactericidal permeability inhibitor, catalase, lysozyme and more) can cause a P-ANCA staining pattern. The ELISA will determine the specific antibody responsible. Only anti-PR3 or anti-MPO antibodies, however, are clinically relevant [191].

ANCA measurement should only be done for patients who are strongly suspected of having vasculitis. Clinical indications for ANCA testing can be found in the International Consensus Statement on Testing and Reporting of Antineutrophil Cytoplasmic Antibodies [186]. Compliance with guidelines for ANCA testing would decrease the number of false positives, which may lead to misdiagnosis and potentially harmful treatments [192].

Granulomatosis with polyangiitis (GPA), microscopic polyangiitis, Churg-Strauss syndrome, renal limited vasculitis and drug induced ANCA-associated vasculitis are associated with positive ANCA. C-ANCA anti-PR3 ANCA suggests a diagnosis of GPA whereas P-ANCA anti-MPO associates more with MPA and Churg Strauss syndrome. ANCA are positive in 70–95% of patients with GPA and MPA, but only about 40% of patients with a diagnosis of Churg Strauss syndrome are positive [191].

ANCA can also be positive in non-vasculitic disease such as anti-glomerular basement membrane disease, inflammatory bowel disease, neoplasia, and other autoimmune disorders. Both PR3 and MPO ANCA may be found with infectious conditions such as tuberculosis, leprosy, HIV and subacute bacterial endocarditis. Various drugs can also be associated with ANCA positivity. These include propylthiouracil, hydralazine, penicillin, sulfonamides, quinolones, thiazides, and allopurinol [193].

Correlation with disease activity was first recognized by van der Woude et al. in 1985 [194]. Although the diagnostic value of ANCA is not disputed, the utility of measuring serial ANCA to monitor disease course, response to treatment or relapse prediction has overall been disappointing [191, 195]. A patient in clinical remission whose titers are rising, have returned to positivity or that remain persistently positive should be followed very closely as they may be at increased risk for relapse [190]. A rise in ANCA may be predictive of an imminent relapse, but this is not universal and may be limited to certain subgroups

of patients, though this remains to be clarified. MPO-ANCA carries worse renal and adult patient survival [196, 197]. Relapse is more common with PR3-ANCA, especially if there is persistence of PR3 positivity after induction therapy [198–200].

Some progress in terms of the so called ANCA negative vasculitides has been made recently with the discovery of pathogenic versus naturally occurring MPO epitopes, previously undetectable by conventional testing [201]. In the future, epitope specificities may prove useful in distinguishing varying disease characteristics in ANCA-associated vasculitides [202].

Kain et al. detected antibodies against human lysosome-associated membrane protein-2 (LAMP-2), a protein localized to the same neutrophil granules as PR3 and MPO, in over 90% of patients with ANCA-associated vasculitis and glomerulonephritis [203]. These autoantibodies may only be present early post diagnosis and disappear with the initiation of immunosuppressive therapies and disease remission; however, their relevance as a pathological or serological marker is yet to be determined [204–206]. LAMP-2 ANCA titers have been detected by ELISA and typically coincide with MPO- or PR3-ANCA positivity in a cohort of children with systemic vasculitis, but this is just preliminary data that needs validation [207].

Antinuclear Antibodies (ANA) and Anti-doubled Stranded DNA (Anti-dsDNA)

ANAs are autoantibodies directed against chromatin and its individual components, including dsDNA, histones and some ribonucleoproteins [208]. Although ANAs are frequently found in children without a rheumatic disease [209], they have been associated with several systemic autoimmune diseases, including systemic lupus erythematosus (SLE), scleroderma, mixed connective tissue disease, polymyositis, dermatomyositis, rheumatoid arthritis, Sjögren's syndrome, drug induced lupus, discoid lupus, and pauciarticular juvenile chronic arthritis. They are also occasionally seen in autoimmune disease of the thyroid, liver and lungs as well as chronic infectious disease such as mononucleosis, hepatitis C infection, subacute bacterial endocarditis, tuberculosis and HIV, and some lymphoproliferative disorders. They do not always signify disease and 13.8% of the population over age 12 have detectable ANAs, with ANA positivity increasing with age and more common in females [210].

Different types of ANAs are known and classified based on their target antigens. Antibodies can be directed against dsDNA, individual nuclear histones, nuclear proteins, and RNA-protein complexes. As some of these antibodies are more specific for a particular disease, they are helpful tests for diagnosis. They are also used for monitoring disease activity. A positive ANA may also precede disease onset [211].

In most laboratories, ANAs are measured by an indirect immunofluorescence assay using a human epithelial cell tumor line (Hep2 cells) as the antigenic substrate. Different staining patterns can be seen, reflecting the presence of antibodies to one or a combination of nuclear antigens. The patterns are neither sensitive nor specific for a single disease. In general, a homogenous or chromosomal pattern is more likely in healthy individuals and in those with SLE, whereas a speckled or extrachromosomal pattern implies antibodies against extractable nuclear antigens (ENA) such as Smith antigen. A nucleolar pattern may suggest systemic sclerosis. It is important to emphasize that anti-dsDNA antibodies associated with SLE may also present a speckled or nucleolar pattern [212]. These different immunofluorescent staining patterns may correlate with disease manifestations in SLE. For instance, proliferative lupus nephritis is more commonly associated with a homogenous pattern and organ damage may be less likely with the speckled pattern in SLE [213]. A nuclear, coarse speckled, rather than a dense fine speckled pattern on immunofluorescence, may signify an autoimmune rheumatic disease [214]. This will need further validation before being employed in more widespread clinical practice [215].

The titer of ANAs can be helpful clinically. A negative ANA makes a diagnosis of SLE or mixed

connective tissue disease very unlikely. ANAs in the sera of a normal healthy childhood population using Hep-2 cells as substrate is reported as 6% [216], and 16% at screening dilutions of 1:20 [217]. In healthy individuals, ANA titers above 1:40 are found in 32%, above 1:80 in 13% and above 1:320 in 3% [218].

The presence of a titer >1:640 should raise the suspicion of an autoimmune disease. If no diagnosis is made, the patient should be followed closely. Lower titers with no clinical sign or symptoms of disease are much less worrisome. In one study, no rheumatic disease was diagnosed in patients with ANA titers <1:160. Unless there is a high pretest likelihood of a rheumatic disease, a positive ANA has a very low positive predictive value [219].

Anti-dsDNA are relatively specific for SLE and fluctuate with disease activity [220].

Anti-nucleosome antibody is the earliest marker for the diagnosis of SLE. It is also a superior marker of lupus nephritis [208]. Among those with SLE, the prevalence of anti-nucleosome antibodies was higher in those with renal disease (58%) compared to those without nephritis (29%) [221]. Antibodies to the Smith antigen, which is a nuclear non-histone protein, are very specific for SLE but insensitive.

In SLE, autoantibodies to the complement component C1q are strongly associated with proliferative lupus nephritis, correlate with disease severity, can herald the onset of nephritis and be used to monitor response to therapy [220, 222]. Anti-dsDNA are also strongly associated with nephritis in SLE [220].

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3 Imaging and Radiological Interventions in the Pediatric Urinary Tract

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Introduction

Imaging plays an important role in the diagnosis and monitoring of many diseases of the kidney and urinary tract in children [1–4]. Both congenital and acquired diseases of the urinary tract are imaged using a variety of modalities, and in many cases it is the imaging study that offers a diagnosis or at least narrows what may begin as a lengthy differential diagnosis. Radiography, excretory urography, contrast fluoroscopy, ultrasonography (US), computed tomography (CT), magnetic resonance imaging (MRI) and nuclear scintigraphy have all been used to assess the urinary tract, each with its own relative strengths and weaknesses. In some cases, two or more complementary modalities will be necessary to narrow the differential diagnosis. It is important not only to know the most appropriate modality for the investigation of a patient, but also to understand the risks and benefits associated with the various modalities. In this chapter, we provide an overview of these imaging modalities and their associated risks and benefits and present examples of their application in the evaluation of children with kidney and urinary tract abnormalities.

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Imaging-Associated Risks

Ionizing Radiation

Several of the modalities used in urinary tract imaging employ ionizing radiation. It is well known that exposure to radiation has deleterious effects, including a strong association between exposure to radiation (particularly at doses reached in CT) and subsequent development of malignancies [5, 6].

The use of medical imaging that exposes patients to ionizing radiation has been increasing. Medical radiation, on average, accounts for the same radiation dose as background radiation in the United States [7]. The use of ionizing radiation is of particular concern in children due to their increased susceptibility to its negative effects. Compared to adult patients, pediatric

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patients have a higher lifetime radiation risk and higher relative dose for a given radiation exposure [5]. To decrease risks to children, it is important to minimize use of ionizing radiation when possible and employ radiation dose reduction strategies applicable to imaging modalities.

Fluoroscopy is an important imaging modality in the evaluation of pediatric genitourinary diseases, as voiding cystourethrography (VCUG) and retrograde urethrography provide necessary anatomic and functional information. A wide variation of radiation doses has been observed in pediatric fluoroscopy [8]. Technical considerations to reduce dose during imaging acquisition include proper collimation and position of patient close to the imaging receptor [9]. Further strategies include the removal of anti-scatter grids for smaller pediatric patients and use of grids for larger pediatric patients [10]. Fluoroscopy units can also be adjusted to optimize settings for pediatric patients, including the automatic brightness control [10]. A pulsed frame rate rather than continuous fluoroscopy can be used without sacrificing imaging quality with considerably less radiation exposure [11].

CT is used for the evaluation of pediatric stone disease and genitourinary trauma and is often the imaging modality of choice in the emergent setting. While CT represents 17% of radiologic procedures in the United States, it accounts for almost 50% of medical radiation [12], highlighting the importance of CT dose optimization. Dose mitigation strategies are of particular importance in the CT evaluation of genitourinary diseases, as the effective doses for pediatric CT of the abdomen and pelvis are the highest when compared to other scans such as head, chest, and spine [13]. Size-based protocols are essential for the reduction of pediatric radiation dose [14]. These protocols factor the patient size into the parameters used to acquire CT images, such as the tube voltage and tube current. Use of a lower tube voltage can cause a substantial dose reduction, and automated tube voltage selection systems can optimize the tube voltage and corresponding tube current based on the scout image [12]. Iterative reconstruction is a method of data processing that refines the raw data and reduces noise, allowing decrease in the radiation dose necessary to acquire diagnostic quality images [12].

When available, dual energy CT (DECT) can also provide further dose reduction when multiphase contrast examinations are necessary. DECT is performed by scanning a patient with two x-ray tubes operating at different energies, one at a low energy and one at a high energy. DECT can be acquired with a dose comparable to conventional single source CT, but the image acquisition at different energies enables differentiation of structures based on atomic number rather than beam attenuation [15]. This data allows the virtual creation of an unenhanced phase, allowing a multiphasic examination without additional radiation dose. Applications of DECT for CT urography have been explored, demonstrating sufficient image quality with radiation dose reduction [16].

Intravenous Contrast Agents

Risks associated with the administration of intravenous (IV) contrast agents include contrast-induced nephropathy and adverse contrast reactions [17, 18]. The risk of post-contrast acute kidney injury (PC-AKI), defined as acute kidney injury (AKI) occurring within 48 h of IV administration of iodinated contrast media, has been controversial in recent decades. Observational studies designed to evaluate the association between iodinated CT contrast administration and PC-AKI have had conflicting results, likely due to differences in institutional contrast-enhanced CT protocols, variances in strategies for propensity matching, and discrepant subgroup sample sizes with different baseline kidney function [19–24]. Despite recent contributions to the pediatric literature by McDonald et al. [23] and Gilligan et al. [24], knowledge of the risk of PC-AKI in children remains limited.

Nephrotoxicity associated with gadolinium-based contrast agents for MRI has also been described, though the overall consensus is that the risk is low when used at approved doses, even in patients with kidney dysfunction [25, 26].

Another potential risk of IV contrast administration is nephrogenic systemic fibrosis (NSF) [27, 28], which has been described with the use

of an older generation of gadolinium-based contrast agents in patients with kidney failure. This rare though significant side effect has limited gadolinium use in patients with advanced chronic kidney disease (CKD) [26]. Nevertheless, the risk of NSF is thought to be lower with newer macrocyclic gadolinium-based agents (e.g. gadobutrol, gadoteridol, gadoterate) [29]. There may also be a risk of gadolinium deposition throughout the body as studies have proven deposition in the bone, brain, and skin but further study is required to determine the clinical significance, if any [30–32]. Rarely, severe reactions to gadolinium-based contrast agents can occur [33].

Potential risks associated with US contrast agents are discussed in the subsequent section, "Contrast-Enhanced Ultrasonography."

Sedation and Anesthesia

Due to the scan times associated with some imaging modalities, some children will require sedation or general anesthesia, and these risks must also be considered when choosing an imaging modality [34, 35]. In some cases, techniques such as "feed and wrap" can be used to obtain high quality images in infants for specific studies such as functional MR urography [36].

Ultrasonography

US is arguably the most important part of the pediatric imaging armamentarium. Its main strengths are that it does not use ionizing radiation and sedation is very rarely required.

The most common indications for US of the kidneys and urinary tract include: urinary tract infection (UTI) [37–39], follow-up of antenatally diagnosed hydronephrosis, evaluation of a palpable mass, assessment for vascular abnormalities (including renal artery stenosis), assessment of medical kidney diseases, screening of patients at known risk to develop kidney neoplasms (for example Beckwith-Wiedemann syndrome and other cancer predisposition syndromes) [40], and assessment for possible urinary obstruction. US can also assess other findings noted on antenatal imaging such as kidney agenesis, ectopia, dysplasia or mass.

The US examination can be tailored in many ways to suit the patient and clinical situation. A patient who is upset or frightened can be scanned lying next to a parent or in the arms of a parent, which can alleviate some anxiety. Coupled with a calm and reassuring environment and various distractions (e.g. toys, music, videos, computer tablets), this setting often allows for the performance of a satisfactory diagnostic study. The need for sedation is extremely rare but may be considered on a case-by-case basis.

The patient can be scanned in various positions (supine, prone, decubitus) depending on the scenario. In fact, altering position can at times be helpful particularly in determining if a structure such as a calculus is mobile. In some situations, the US examination can be repeated after an intervention has been performed in order to determine whether it was successful or resulted in a complication. One can study the urinary tract before or after voiding, after placement of a bladder catheter, ureteral stent or nephrostomy catheter, and throughout a kidney biopsy procedure. These repeated examinations can be done without concern for the effects of radiation.

In general, the smaller body habitus of children allows for excellent US imaging of the kidneys and urinary tract. However, US of the urinary system can be suboptimal in some larger teenagers or obese children. Scanning of the kidneys is performed mainly with curved array transducers for assessment of kidney length and to examine the status of the kidney parenchyma, pelvicalyceal system, ureters, and bladder. These images can be supplemented with those obtained with a high-resolution linear transducer, which offers superior spatial resolution but is limited in penetration depth. For that reason, high-resolution US is particularly well suited to neonates, infants and younger children. In older children, the distance between the transducer and the kidneys may preclude this type of higher resolution examination.

The kidneys are ovoid organs that typically lie in the retroperitoneal renal fossae, although they can be ectopic. Their lengths can be measured and compared with published nomograms [41–43] (Fig. 3.1). Growth of the kidneys can be followed on serial examinations; however, it is important to keep in mind that kidney lengths can

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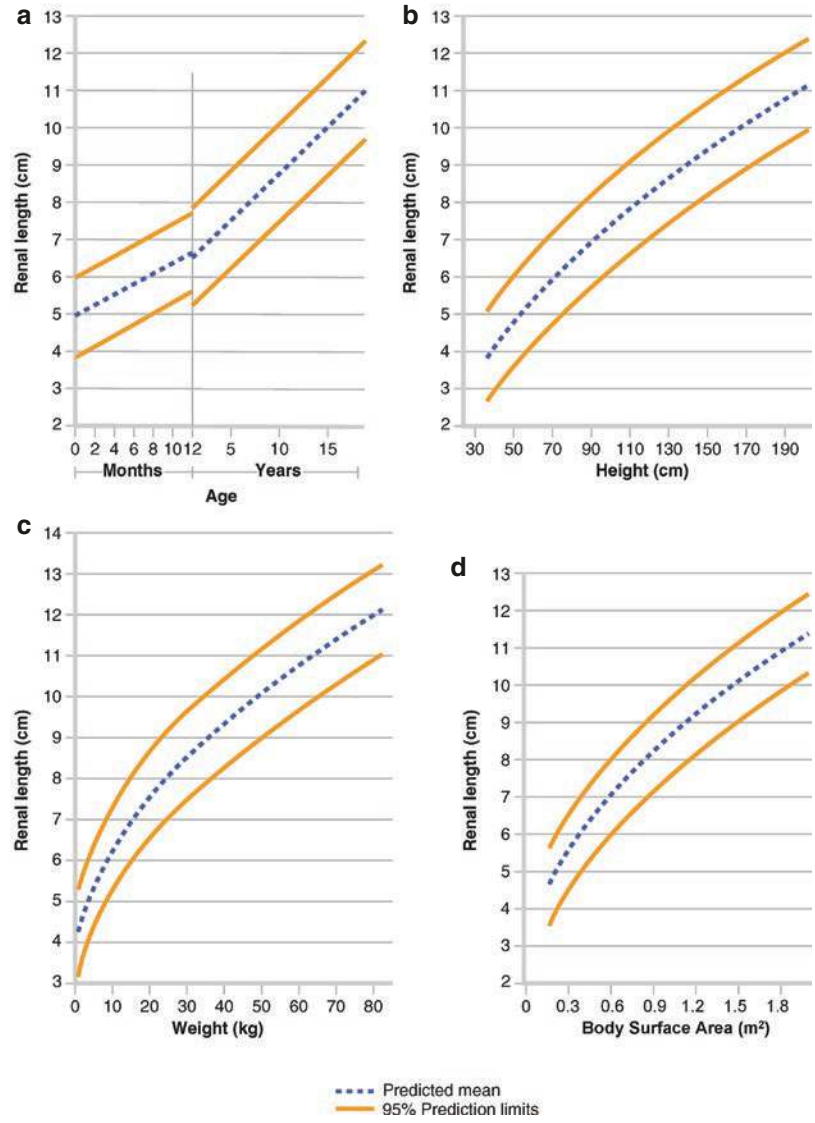


Fig. 3.1 Nomograms for kidney size. Nomograms delineate the predicted mean and 95% prediction limits of kidney length as a function of (**a**) age, (**b**) height, (**c**) weight, and (**d**) total body surface area

occasionally be over- or under-measured depending on the circumstances of the examination. Retardation in kidney growth can be a sign of ongoing insult such as vesicoureteral reflux [44].

Greyscale US

In healthy children there is a difference in echogenicity between normal kidney cortex and the medullary pyramids, with the former more echogenic and the latter more hypoechoic (Fig. 3.2). This difference is termed *corticomedullary differentiation.* The echogenicity of the kidney cortex can be compared to an internal and adjacent

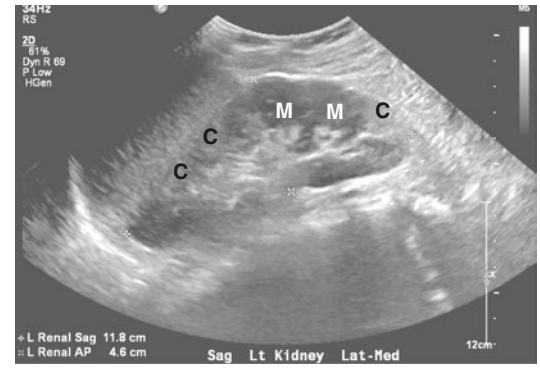


Fig. 3.2 US of normal kidney. Greyscale longitudinal US image of a 12-year-old child showing normal left kidney with relatively more echogenic cortex (C) and hypoechoic medulla (M)

standard—that is, the right kidney is compared to the liver, and the left kidney to the spleen. One must, however, ensure that this reference organ (the liver or spleen) is normal. The pattern of normal kidney echogenicity varies in childhood. In the neonate, the kidney cortex can be isoechoic or even hyperechoic compared to the liver and the renal pyramids are often profoundly hypoechoic, which can make corticomedullary differentiation quite marked [45] (Fig. 3.3a). By the time the child is several months of age the kidney cortex should be hypoechoic compared to the echogenicity of the liver [45, 46]. Any alteration in cortical echogenicity after that age suggests intrinsic kidney disease. The medullary pyramids, particularly in the neonate, can be so hypoechoic that they can be mistaken for a dilated collecting system. There are exceptions to the hypoechogenicity of the renal pyramids, the majority of which relate to disease states such as medullary nephrocalcinosis. The most common exception, however, seen in many neonates may be the transient increase in pyramidal echogenicity, which has been attributed to precipitation of Tamm Horsfall proteins [47]. In addition, there may be lobulation of the kidney outline, especially in neonates. This should not be confused with scarring. The notching of normal lobulation tends to be seen in the portion of the cortex between pyramids (Fig. 3.3b), whereas focal scarring tends to occur in portions of the cortex directly overlying the pyramid.

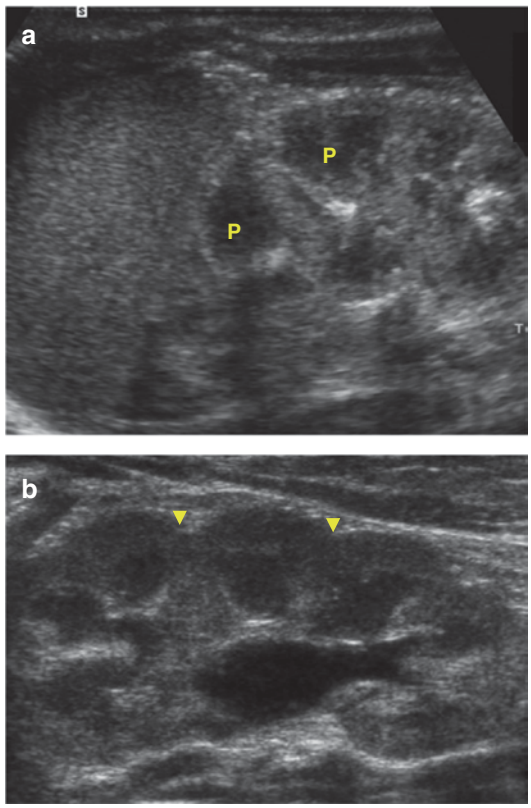


Fig. 3.3 US of normal neonatal kidney. (**a**) Greyscale longitudinal US image of a neonatal kidney showing hypoechoic pyramids (P) and hyperechoic cortex when compared to the liver with prominent corticomedullary differentiation. (**b**) Neonatal kidney showing "lobulations" (arrowheads) between pyramids

The degree of kidney collecting system dilation can be assessed both qualitatively and quantitatively. Measurement of pelvic dilation can be assessed at the level of the kidney hilum—or just beyond it in the case of an extrarenal pelvis. Classification of urinary tract dilation (UTD) is discussed in more detail later in this chapter in the section "Applications of Diagnostic and Interventional Radiology Techniques: Neonatal Diseases." A full bladder can exaggerate the degree of dilation. It is therefore prudent to assess the pelvic diameter after voiding if the bladder is overdistended. If the ureter is dilated, its diameter can be assessed along its course, although it can be visualized most reliably proximally and distally (Fig. 3.4). Overlying bowel gas often obscures the midportion of the ureter. The thickness of the wall of the intrarenal collecting system, ureter or bladder can also be assessed. Thickening of the urothelium anywhere along the urinary tract can be associated with, though is not pathognomonic for, infection or inflammation. Urolithiasis can be diagnosed as an echogenic focus with distal acoustic shadowing [48] and/or with twinkle artifact on color Doppler. The degree of obstruction caused by a calculus can also be assessed with US.

Color Doppler

Color Doppler and pulsed Doppler interrogation of the kidneys can be used to assess vascularity of the kidneys. The study can assess the

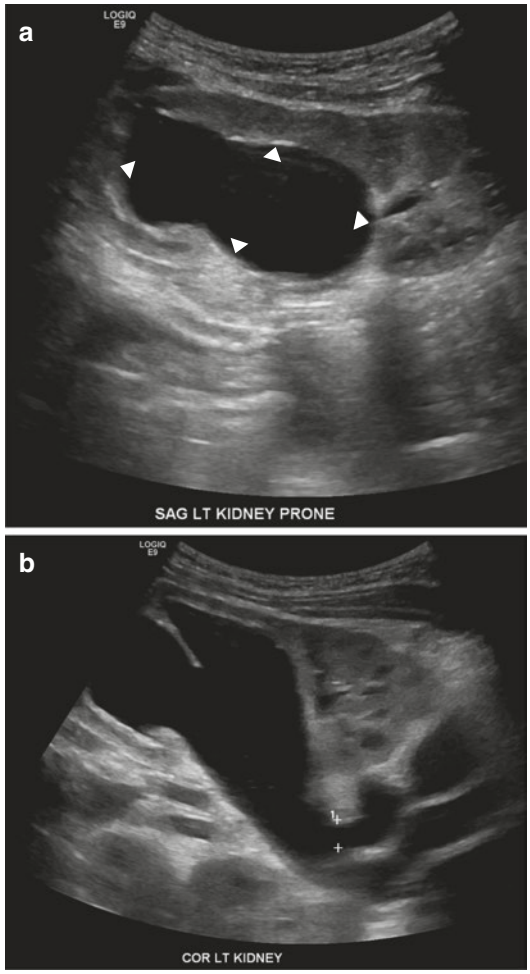


Fig. 3.4 US of urinary tract dilation and ureteral dilation. (**a**) Greyscale longitudinal US image of a 1-year-old child with congenital hydronephrosis of the left kidney showing marked dilatation of the upper pole collecting system (arrowheads) and thinning of the parenchyma with a relatively normal lower pole. The upper pole pelvis and calyces are markedly dilated. (**b**) The ureter is markedly dilated and tortuous with a proximal diameter of 0.53 cm (cross marks)

vessels from the ostia of the main renal arteries and veins through the arcuate vessels in the kidney parenchyma. Indications for Doppler evaluation include suspicion of renal arterial or venous thrombosis [49], arterial stenosis [50], trauma [51], infection [52], acute tubular necrosis, and transplant rejection [53]– though the role in the evaluation of rejection remains controversial [54].

Contrast-Enhanced Ultrasonography

Contrast-enhanced US (CEUS) is an innovative imaging modality that utilizes non-nephrotoxic microbubbles as an IV US contrast agent (UCA). UCAs consist of a gas core with lipid monolayer stabilizing shell no bigger than a red-blood cell. UCAs are currently approved by the United States Food and Drug Administration for IV use in cardiac and hepatic US, and for intravesical use in the evaluation of vesicoureteral reflux (VUR) in children. Despite being "off-label" in the United States, its kidney applications have grown in recent years, including evaluation of kidney trauma, masses and cysts, as well as to guide procedures [55–59]. CEUS is more sensitive in detecting perfusion abnormalities than Doppler (Fig. 3.5), as CEUS enhances the vascular echo signal through the use of a purely intravascular contrast agent, rather than detecting the Doppler shift, which is highly dependent on many technical factors such as speed of acquisition, angle of imaging, depth of imaging, and frequency [60]. CEUS in clinical practice provides dynamic information about an organ, such as microvascular perfusion, potential quantification of blood flow, and perfusion characteristics of masses and cystic lesions [61–63]. For example, inflammatory processes of the kidneys such as acute pyelonephritis and glomerulonephritis will show hyper-enhancement whereas scarred kidneys present with focal areas of hypo-enhancement. In the evaluation of lesions, cysts show no enhancement while solid lesions do. Moreover, malignant lesions usually enhance avidly and "wash out" because of their nearly complete arterial blood supply and abnormal arteriovenous shunts throughout. In kidney transplantation, CEUS has been able to detect early features of acute allograft dysfunction in the setting of acute tubular necrosis, rejection, cortical necrosis and other vascular complications such as local areas of hypoperfusion (Fig. 3.5) [64–66].

CEUS can also provide real-time images for guidance of interventional procedures, such as percutaneous drainage and biopsy, by providing

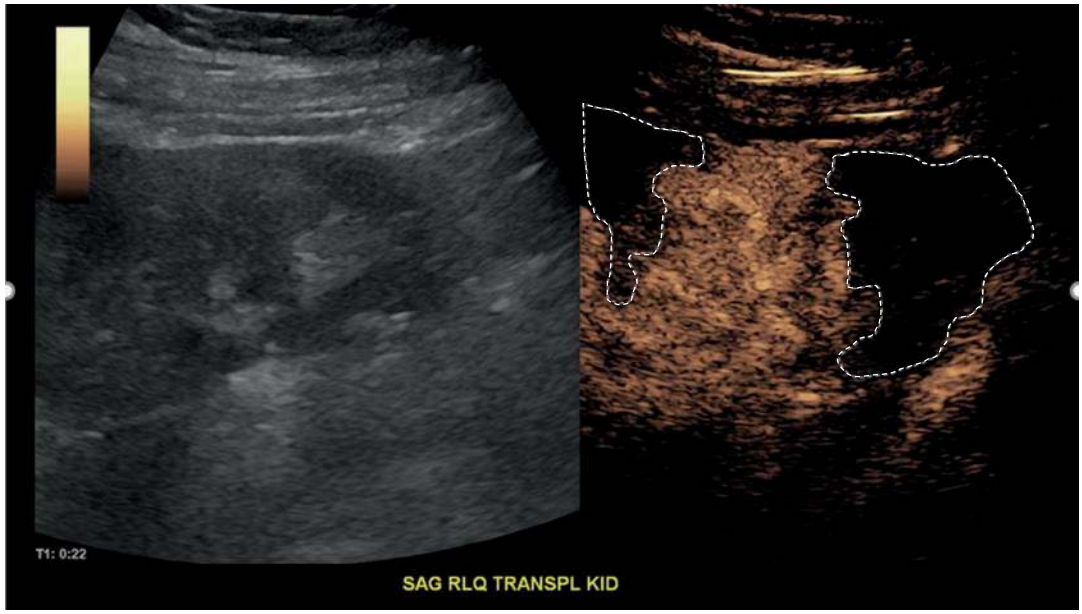


Fig. 3.5 Contrast enhanced US (CEUS) of kidney transplant. CEUS of a transplant kidney in an adolescent recipient showing lack of perfusion in the lower one third of the kidney along with a wedge-shaped perfusion defect in the anterior aspect of the upper-pole (dashed lines), highly suggestive of kidney infarction

evidence of communication between cavities or flow from the placed catheter into the region of interest. It can also be used during percutaneous nephrostomy and thermal ablation of tumors [56]. CEUS can also allow bedside evaluation of organ perfusion and lymphatic leakage without the need for ionizing radiation [67].

Post-acquisition quantification of perfusion or relative enhancement are, to date, a work in progress with no standardization but with promising preliminary results [68, 69]. Additionally, given that most US machines that have the capability to perform CEUS also perform conventional US, one can simultaneously evaluate images with both modalities.

Contrast-Enhanced Voiding Urosonography (ceVUS)

A special application of CEUS is contrast-enhanced voiding urosonography (ceVUS), an US-based alternative to evaluate for VUR or urethral pathology [70, 71]. CeVUS, like fluoroscopic VCUG, evaluates the urinary tract with contrast administration via a bladder catheter, but uses an US scanner for evaluation. Benefits of ceVUS include a more child-friendly imaging environment and lack of ionizing radiation. The detection of microbubbles within a ureter or kidney collecting system indicates VUR, which is graded similarly to VCUG [70, 72, 73]. ceVUS has been shown to be more sensitive in detecting VUR, with a higher grade of reflux in up to two thirds of patients, and as good for the evaluation of the urethra when compared to VCUG (Fig. 3.6) [74, 75]. CeVUS can also be used in the evaluation of posterior urethral valves (PUV) and urethral trauma [72, 76]. Moreover, in therapeutics, intraoperative applicability of ceVUS has improved the success rate of minimally invasive sub-trigonal injection procedure (Deflux®) for resolution of VUR by approximately 20% [77].

US Contrast Safety

US contrast agents have a good safety profile for intravesical use. The most common side effects relate to the urethral catheterization, with no known side effects from the actual US contrast agent [70].

Over two decades of experience with IV US contrast administration in thousands of pediatric

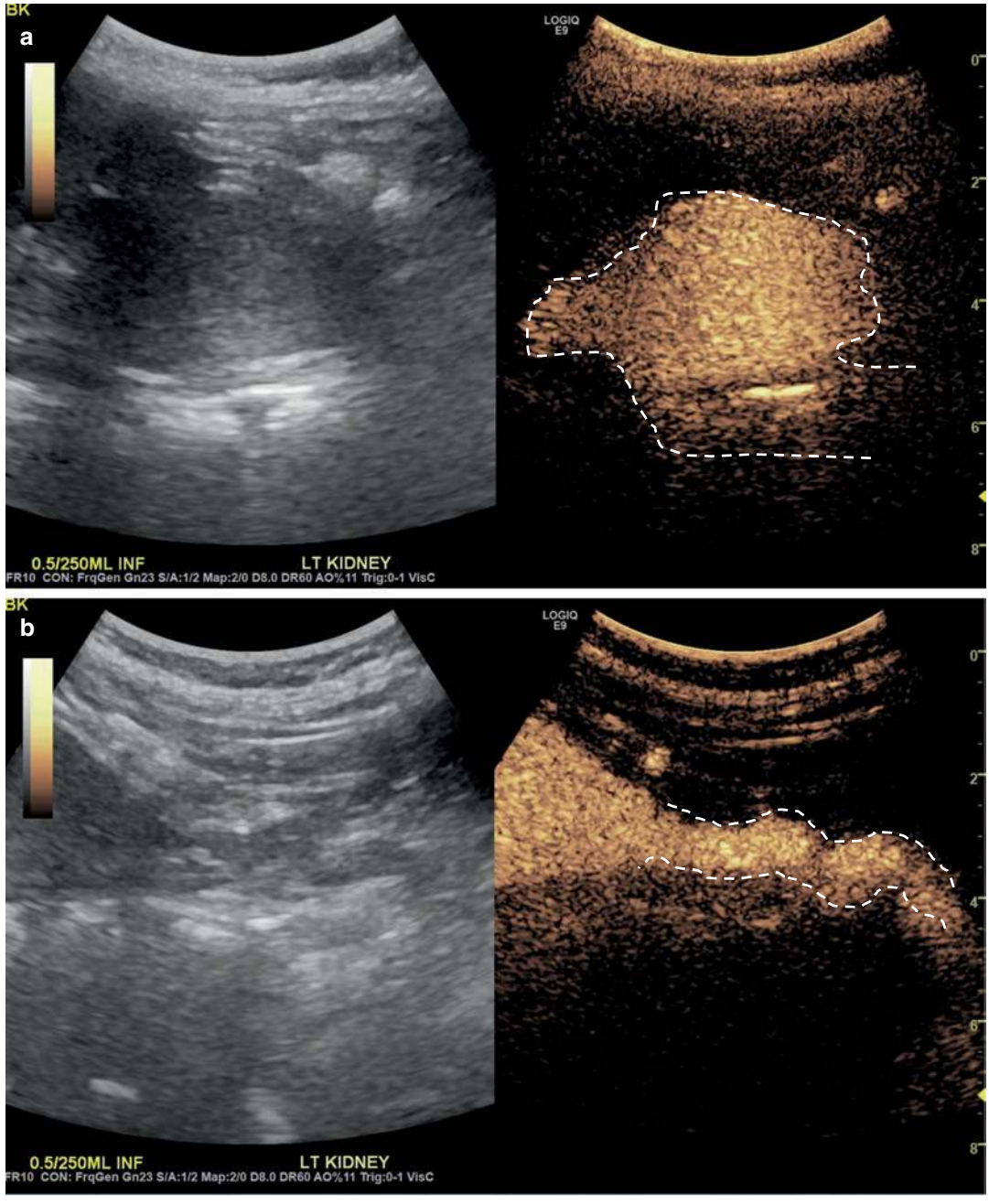


Fig. 3.6 Contrast enhanced voiding US (ceVUS). (**a**) CeVUS of a 4-year-old child shows grade 4 reflux to the left kidney. The renal pelvis and calyces are dilated (dashed lines). (**b**) The left ureter is moderately dilated and tortuous (dashed lines)

patients in Europe, Asia, and most recently in the United States, has shown a good track record of safety. Specifically, in over 1900 reported IV US contrast exams in children, two children had severe allergic reactions that were successfully treated [78–81]. Other reactions reported in children include tachypnea, unusual taste, mild itching and hives, and other minor reactions that did not require treatment [78–83]. Nausea and headache are the most common reactions associated with the administration of the commercially available US contrast agents Lumason® (approved in the United States) [84] and SonoVue (approved in Europe and Asia) [85].

Voiding Cystourethrography

VCUG has traditionally been the study of choice for diagnosing VUR and assessing the anatomy of the bladder and urethra. Indications include UTI [39], antenatally or postnatally diagnosed hydronephrosis, and suspected PUV, among others. At most institutions, sedation is not administered. In our experience, the examination can be performed without sedation in the vast majority of children, given proper explanation and reassurance.

A catheter is placed via the urethra into the bladder using aseptic technique; alternatively, a suprapubic catheter or appendicovesicostomy (Mitrofanoff) can be used if present. The bladder is filled with water soluble contrast under the pressure of gravity until pressure within the bladder induces micturition. The amount of contrast used will vary according to the patient's age and bladder capacity. If a child is unable to void on their own, the bladder can be drained via the catheter in situ. If the child is reticent or unable to void, warm water applied to the perineum can induce voiding. Some children will not void on the fluoroscopy table despite a variety of maneuvers. In these cases, the micturition phase of the study is not possible and the sensitivity of the study to detect reflux is diminished. In some institutions an image is taken after the child has been allowed to void in the toilet. At some institutions a single cycle of filling and voiding is performed. At others, two or three cycles are the routine [86]. This latter method, termed *cyclic VCUG*, has demonstrated greater sensitivity in detecting reflux, but results in a higher radiation dose compared to the single cycle method. In all cases, care is given to minimizing the dose of ionizing radiation [87].

Exact views obtained will vary between institutions, but all will include images of the bladder that will allow for assessment of its wall characteristics and detection of structural abnormalities such as diverticula, ureteroceles or urachal abnormalities. These images should demonstrate whether there is any reflux into the ureters. Images of the urethra will be obtained during voiding, either with the catheter in place or after its removal depending on the practice of the institution and the individual radiologist. At our institution an image of the urethra is obtained with the catheter in place as well as after its removal, thus ensuring an image of the urethra in cases in which the child stops voiding just as the catheter is removed. An image of the renal fossae will assess for any reflux to the level of the kidneys, characterize the collecting system anatomy (duplex or not) and assign a grade to that reflux [88]. The system of the International Reflux Study in Children classifies reflux into five grades. Reflux into the ureter alone is classified as Grade I. When contrast fills the intrarenal collecting system but without dilation, it is classified as Grade II. Grades III–V demonstrate progressive dilation of the ureter, pelvis and calyces [88] (Fig. 3.7).

Complications related to VCUG are similar to those encountered in any catheterization of the bladder, with infection and trauma being the most common. At our institution, we do not administer prophylactic antibiotics unless there is a clinical indication for procedure related prophylaxis. If the examination is positive and the patient is not on long-term antibiotic prophylaxis, a prompt communication of the results to the referring clinician is appropriate. One can also encounter urinary retention post-procedure.

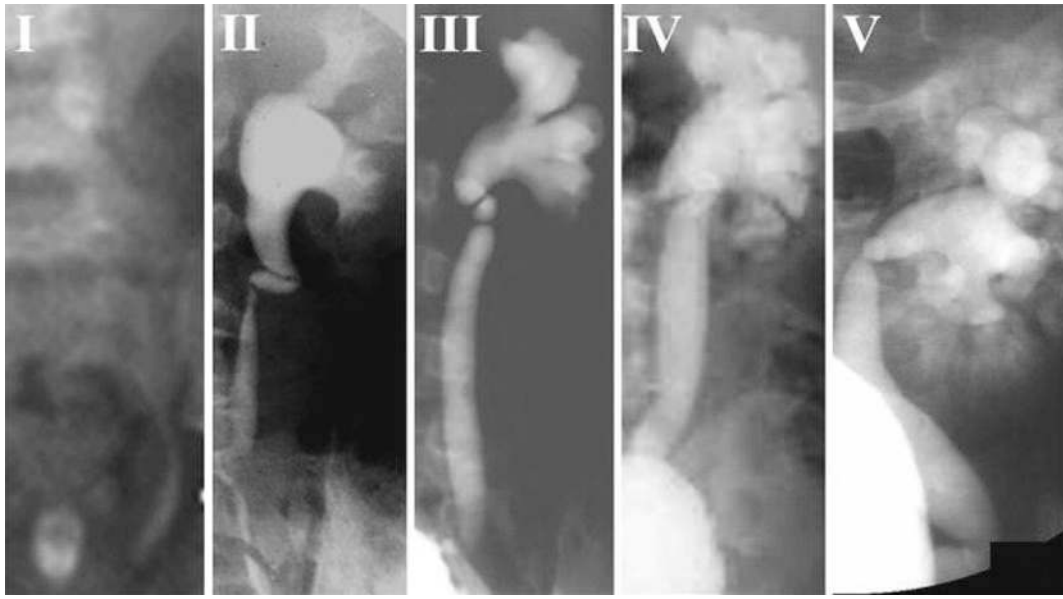


Fig. 3.7 Voiding cystourethrogram (VCUG). International Grading System of vesicoureteral reflux, illustrating VCUG grades I–V

Nuclear Medicine

Nuclear medicine is a modality that comprises a variety of examinations for evaluating the pediatric urinary tract. Nuclear medicine techniques differ from other imaging modalities in that they focus on function rather than detailed anatomic structure. As a result, nuclear imaging plays an important complementary role to other modalities, particularly to the structural evaluation obtained with US.

The physical principles of how scintigraphic images are generated also differ from those of other imaging modalities. Rather than transmitting x-rays through the patient as is done with fluoroscopy, radiography and CT, nuclear medicine introduces a radioactive tracer into the patient's body. An Anger camera is then positioned adjacent to the patient, and images are created by detecting the gamma-rays emitted from the patient's own body. In nuclear urinary tract imaging, depending on the specific examination being performed, the radiopharmaceutical can be injected IV to be extracted by the kidneys, or can be instilled via catheter into the bladder. Radiation doses in nuclear medicine examinations of the urinary tract are lower than those encountered in CT and lower or comparable to those in fluoroscopy.

Most pediatric patients do not require sedation, as they are either cooperative in lying still on the scintigraphy imaging table or are infants small enough to be safely restrained with swaddling. However, if a child is anticipated to have difficulty lying still for at least 30 min, sedation can be considered. Rarely, general anesthesia is necessary to perform a successful examination.

Urinary tract imaging comprises over half of the examinations performed in a typical pediatric nuclear medicine department. The most common clinical indications for performing nuclear kidney imaging examinations include UTI, ante- or post-natally detected hydronephrosis, vesicoureteral reflux, suspected urinary obstruction, and suspected impairment of kidney function.

Overview of Radiopharmaceuticals

Technetium-99m (99mTc) is the radionuclide (i.e. gamma-emitting isotope) that is used to label most radiopharmaceuticals in urinary tract imaging. It emits a 140 keV gamma-ray and has a physical half-life of 6 h.

Technetium-99m pertechnetate is the base form of 99mTc that is obtained from a portable generator unit found in any nuclear medicine radiopharmacy. 99mTc-pertechnetate can be used to radiolabel other pharmaceuticals using commercially available labeling kits. Other radiopharmaceuticals routinely used in nuclear urinary tract imaging are described in Table 3.1.

Table 3.1 Radiopharmaceuticals routinely used in nuclear urinary tract imaging

	Use	Mechanism of action	Imaging analysis
Glomerular filtration agents			
DTPA			
99mTc-diethylenetriaminepentaacetic acid	• Calculate GFR	Measuring the rate of DTPA extraction from plasma by serial blood sampling provides an accurate estimate of GFR. Approximately 90% of DTPA is filtered by the kidneys into the urine within 4 h after IV injection [126].	Kidney imaging can be performed using 99mTc-DTPA, providing additional information on excretion and drainage, as well as the ability to plot dynamic renogram time-activity curves.
EDTA			
51Cr-ethylenediaminetetraacetate	• Calculate GFR (standard GFR agent used in Europe)	Due to better radioisotope binding to the tracer, 51Cr-EDTA produces slightly higher values for GFR than 99mTc-DTPA. However, this difference is small (5% or less) and is not considered to be clinically relevant [284].	Kidney imaging is not performed with 51Cr-EDTA as it does not emit gamma-rays of suitable energy levels for imaging.
Tubular secretion agents			
MAG3			
99mTc-mercaptoacetyltriglycine	• Agent of choice for functional kidney imaging • Split kidney function • Detect obstruction • Evaluate kidney transplant allografts	MAG3 is cleared predominantly (95%) by the kidney tubules [126]. MAG3 clearance is proportional to effective kidney plasma flow.	Better image quality as the extraction fraction of MAG3 is more than twice that of DTPA, resulting in a much higher target-to-background ratio.
Kidney tubular fixation agents (cortical agents)			
DMSA			
99mTc-dimercaptosuccinic acid	• Agent of choice to detect cortical scarring	Binds to sulfhydryl groups of proximal kidney tubules after filtration [126]. Only 10% is excreted into the urine during the first several hours after IV injection.	Produces excellent high-resolution images of the kidney cortex without interference from urinary activity. Is the preferred cortical imaging agent.
GH			
99mTc-glucoheptonate	• Early imaging evaluates kidney perfusion, urinary excretion and drainage • Late imaging at 1–2 h visualizes kidney cortex	Cleared by the kidneys through both tubular secretion and glomerular filtration, with 10–15% remaining bound to the kidney tubules at 1 h after injection.	99mTc-GH has less cortical binding affinity than 99mTc-DMSA.

Direct Radionuclide Cystogram (DRC)

Direct radionuclide cystography (DRC) has good sensitivity to detect VUR, but provides very little anatomic detail for detecting bladder and urethral abnormalities [89, 90]. DRC is performed in a similar manner to VCUG, except the bladder is filled with a 99mTc-based radiopharmaceutical and an Anger camera is used for imaging, often for several cycles of filling and voiding. DRC can be used as a complementary modality to VCUG [89–91]. Typically, VCUG is the primary modality to evaluate for VUR in patients with febrile UTI or hydronephrosis [92]. Subsequently, DRC may be used as a follow-up examination to determine if VUR has resolved or is persistent, including post-operative evaluation after ureteral reimplantation surgery or minimally invasive sub-trigonal injection procedure (Deflux®). DRC can also be performed as a primary screening examination to detect reflux in asymptomatic patients with a small kidney or solitary kidney, or who have a family history of VUR (first degree relative, i.e. parent or sibling).

Indirect Radionuclide Cystogram

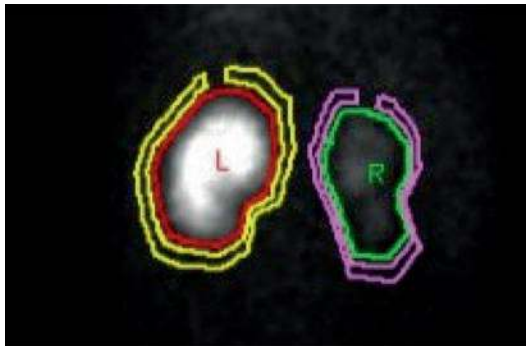
An alternative test for the detection of VUR reflux is the indirect radionuclide cystogram (IRC) [93–102]. This examination should be reserved for children >3 years of age [103] in whom bladder catheterization is impossible. IRC is performed by injecting 99mTc-mercaptuacetyltriglycine (MAG3) IV, then acquiring continuous dynamic images of the kidney and bladder during bladder filling and voiding. The patient must be able to remain still during imaging and to void on command after bladder filling. Regions of interest are drawn over the intrarenal collecting systems and the ureters, and time-activity curves are plotted. A sudden increase in activity in the renal pelvis and ureter indicates the presence of VUR.

Direct vs. Indirect Radionuclide Cystogram

There is ongoing debate regarding whether direct vs. indirect radionuclide cystography is preferable for detecting VUR. In theory, IRC is a better mimic of physiologic slow antegrade bladder filling, whereas DRC involves rapid retrograde bladder filling via a catheter, which some believe induces artificial reflux. Others assert that this higher sensitivity of DRC (up to 95%) [104] is an advantage, and may be a better comparator to prior VCUG results due to the same method of bladder filling. Patients with abnormal kidney function may have insufficient excretion of radiotracer during IRC, resulting in lower sensitivity ranging between 32% and 81% [94, 97, 104–106]. In practice, there is a high rate of failure of IRC due to the inability of children to remain still while voiding or the inability to void at all during image acquisition [107]. In the case of a negative IRC examination, a subsequent DRC or VCUG may be needed to confidently exclude vesicoureteral reflux [103].

Kidney Cortical Scan

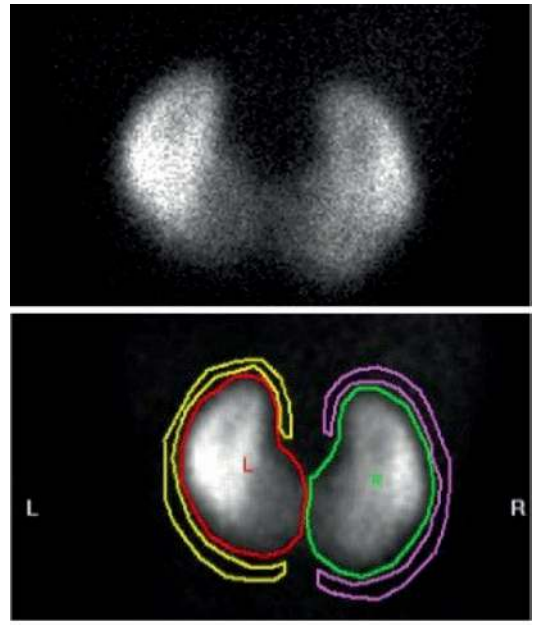
Cortical scintigraphy with 99mTc-dimercaptosuccinic acid (DMSA) is a highly sensitive examination for the detection of both acute lesions (i.e. pyelonephritis) and late sequelae (i.e. permanent parenchymal scarring) in children with UTI. Acute lesions of pyelonephritis can take up to 6 months to resolve scintigraphically. Therefore, permanent scarring can only be reported when the DMSA scan is performed at least 6 months after the acute infection. If less than 6 months have elapsed since the acute infection, any cortical defects should be interpreted as either resolving pyelonephritis or potential scar. Therefore, kidney cortical scintigraphy should only be performed within 6 months of an acute infection if there is an acute need to document kidney involvement. Otherwise, a repeat scan will likely be needed later to exclude



RELATIVE UPTAKE

	Left	Right
Upper [%]		
Lower [%]		
% Diff/Total Vol	92	8
Counts/Total Vol	34212	2834
Counts/Unit Vol	22.8	2.8
% Diff/Unit Vol	89	11

Fig. 3.8 DMSA scan. DMSA scan in a 33-month-old boy with a history of posterior urethral valves and pyelonephritis showed small right kidney with significantly decreased uptake and irregular contour consistent with scarred right kidney and decreased activity in the lower pole of the left kidney suggestive of possible pyelonephritis vs. scar in the left lower pole



RELATIVE UPTAKE

	Left	Right
Upper [%]		
Lower [%]		
% Diff/Total Vol	55	45
Counts/Total Vol	108964	89807
Counts/Unit Vol	30.6	24.0
% Diff/Unit Vol	56	44

Fig. 3.9 DMSA scan. DMSA scan in a patient with horseshoe kidney showing normal cortical activity in upper portions of both kidneys with relatively less activity in the lower portions and the isthmus. Differential function was 55% for the left moiety and 45% for the right

permanent scarring [39, 103, 108–110]. When requesting a DMSA scan, it is helpful for the referring physician to note the date of the most recent UTI.

Kidney scarring tends to occur at the upper and lower poles due to the round-shaped orifices of the compound papillae at these locations. The simple papillae at the mid-poles have slit-like orifices that are less prone to reflux of infected urine. Kidney cortical defects are reported as unilateral or bilateral, single or multiple, small or large, with or without loss of parenchymal volume. Permanent scarring tends to cause loss of parenchymal volume (Fig. 3.8), whereas acute infection does not. If present, a dilated renal pelvis can also be visualized. DMSA cortical scintigraphy is more sensitive than IV pyelography and US for the detection of both acute lesions and permanent scarring [52, 109, 111, 112].

Other causes of cortical defects on DMSA scan include kidney cysts and masses. Normal variations in appearance of the kidney cortex can include indentation by the adjacent spleen, fetal lobulation, columns of Bertin, duplex kidney, and malrotated kidney. Kidney cortical scans are often useful in confirming the diagnoses of horseshoe kidney, ectopic kidney, or cross-fused renal ectopia when US is equivocal (Fig. 3.9).

Images are acquired 2–3 h after injection of 99mTc-DMSA. Planar images are acquired in the posterior, and right and left posterior oblique positions. In infants, additional pinhole images may be acquired that offer higher spatial resolution. In older, sufficiently cooperative children, additional single photon emission computed

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tomography (SPECT) images may be acquired to improve spatial resolution [113–117]. SPECT iterative reconstruction algorithms can further improve spatial resolution, or maintain spatial resolution with a lower administered dose of radiopharmaceutical [118]. However, the clinical significance of any additional small cortical defects detected by these higher-resolution methods is unclear [119–125].

Functional Kidney Imaging and Renography

Functional kidney imaging uses dynamic image acquisition to evaluate kidney perfusion, uptake, excretion, and drainage of radiotracer by the urinary system. Renography refers to the process of plotting the radiotracer activity in the urinary system as a function of time, resulting in renogram (time-activity) curves. A large amount of information can be acquired with functional kidney imaging. For example, abnormal perfusion can suggest arterial stenosis or occlusion; delayed uptake and excretion of radiotracer suggest parenchymal disease; and poor drainage of radiotracer into the bladder can suggest obstructive uropathy or over-compliance of the collecting system. Functional kidney imaging can be custom-tailored for specific clinical problems. For example, a diuretic challenge can be administered to more sensitively evaluate for urinary obstruction (see below).

Although 99mTc-DTPA is widely used for functional kidney imaging, 99mTc-MAG3 is preferred due to its higher extraction fraction and better target-to-background ratio. This advantage is particularly important in patients with impaired kidney function or urinary obstruction, and in very young patients with immature kidney function.

Immediately after the injection of radiotracer, imaging of kidney perfusion can be performed. The patient lies supine with the camera positioned posteriorly. Radiotracer activity should reach the kidneys about 1 s after the tracer bolus in the abdominal aorta passes the renal arteries; there should be symmetric perfusion of the kidneys [126]. Over the next 20–30 min, imaging of kidney function is performed. Maximal parenchymal activity is seen normally at 3–5 min after injection (T_{max}) [126]. Urinary activity in the renal pelvis is typically seen by 2–4 min after injection (calyceal transit time); however, there is no widespread consensus on what constitutes a normal calyceal transit time [127]. There should be prompt drainage of tracer into the bladder, with less than half of the activity at T_{max} remaining in the renal pelvis by 8–12 min after injection ($T_{1/2}$) [126].

Renogram curves are generated by plotting the activity within regions of interest drawn around each kidney. The renogram is a graphic representation of the uptake, excretion, and drainage phases of renal function, and the curves for each kidney should be reasonably symmetric. Patients should be well-hydrated, preferably with IV fluids, when functional kidney imaging is performed, as dehydration will result in an abnormal renogram with globally delayed function and slow drainage.

Diuretic Renogram

In the setting of urinary collecting system dilation not due to VUR, the possibility of urinary tract obstruction must be considered. Diuretic renography performed with furosemide is useful in determining the presence of a high-grade obstruction at the ureteropelvic junction (UPJ) or the ureterovesical junction (UVJ). Diuretic renography is commonly used to evaluate the results of surgery in patients who have undergone pyeloplasty for ureteropelvic junction obstruction.

Diuretic renography is performed as described above for dynamic kidney imaging, with the additional step of administering IV hydration and furosemide to cause maximal urine flow through the collecting system. The dose of furosemide is usually 1 mg/kg, with a maximum dose of 40 mg [128]. The timing of the furosemide administration varies among institutions, as several diuretic

protocols have been described, validated, and debated in the literature [103, 129, 130]. The most commonly used protocols are: "F+20" (furosemide is given 20 min after radiotracer if normal spontaneous drainage has not occurred [131]; this protocol is endorsed by the American Society of Fetal Urology); "F−15" (furosemide is injected first, followed 15 min later by radiotracer; this protocol is the widely-used European standard) [128]; and "F0" (radiotracer and furosemide are injected immediately following one another) [132, 133]. Dynamic images are acquired from the time of radiotracer injection for approximately 20 min. In the case of the F+20 protocol, an additional 20 min of imaging is performed after injection of furosemide.

Bladder catheterization is not always necessary but should be performed in patients who are not toilet-trained, or who have known hydroureter, VUR, bladder dysfunction, or PUV. In this subset of patients, back pressure from urine in the bladder may cause a false-positive result.

In the absence of urinary obstruction, there is rapid drainage of radiotracer from the renal pelvis into the bladder to a minimal residual by 20 min. In quantitative terms, a drainage half-time, $T_{1/2}$, of less than 10 min usually means the absence of obstruction.

In an obstructed system, the drainage of radiotracer from the collecting system will be slow. In this case, a $T_{1/2}$ of greater than 20 min indicates obstruction (Fig. 3.10). When $T_{1/2}$ ranges between 10 and 20 min, this is usually considered an equivocal result, and a follow-up examination will typically be performed to see if the drainage remains unchanged, normalizes, or becomes frankly obstructed.

When UPJ obstruction is suspected, the above drainage parameters are used when analyzing a region of interest drawn around the renal pelvis. These values can also be applied to the ureter and to a region of interest combining the ureter and renal pelvis when UVJ obstruction is suspected.

If a large amount of radiotracer remains in the renal pelvis and/or ureter at the end of dynamic imaging, the patient can be positioned upright to void if possible. A final static image can then be acquired to see if the postural/gravitational effect caused additional drainage [103].

Pitfalls are common in the interpretation of diuretic renography. Poor kidney function from prolonged, severe obstruction can result in poor accumulation of radiotracer in the collecting system, making the renogram difficult or impossible to interpret. A very dilated, overly-compliant, but non-obstructed collecting system may have a prolonged $T_{1/2}$ because the capacious collecting system easily accommodates a large urine volume [128, 130]. This "reservoir effect" can be observed in the setting of primary megaureter, and in patients who have undergone successful pyeloplasty for UPJ obstruction.

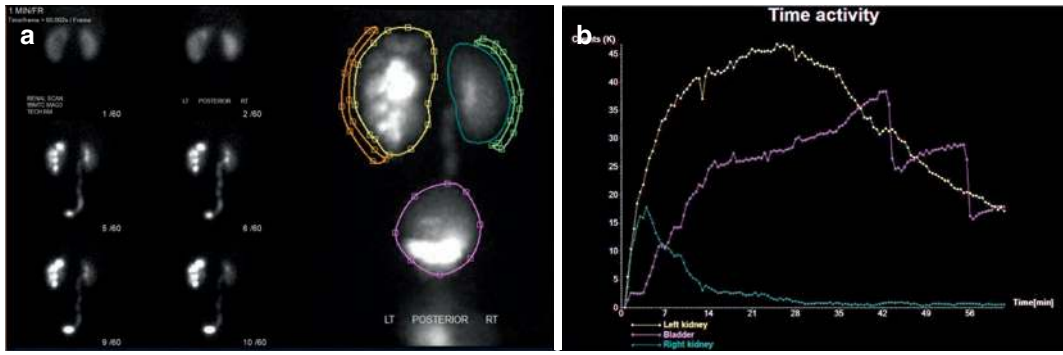

Fig. 3.10 MAG3 diuretic renogram. (**a**) MAG3 renogram of a 27-month-old girl with a history of congenital hydronephrosis shows prompt blood flow to both kidneys. (**b**) Time-activity curves shows significant urine stagnation on the left side at 25 min (white curve), which responds poorly to furosemide administration with a prolonged half-clearance time. Normal clearance of radiotracer of right kidney (blue curve)

Computed Tomography

CT is often the imaging modality of choice for evaluation of blunt or penetrating genitourinary trauma and may be used in the evaluation of stone disease or neoplasm. The indication for imaging determines the CT protocol and the need for the administration of IV contrast.

In the evaluation of trauma, IV contrast should be administered unless there is a contraindication. Obtaining a CT in the arterial phase, when contrast opacifies the arterial structures, can evaluate for arterial injury such as dissection or pseudoaneurysm, as well as active arterial hemorrhage. A delayed phase CT, obtained approximately 10 min after contrast administration when the contrast has been excreted into the collecting system, can be obtained if there is a suspicion for a collecting system injury, and will show extravasation from the collecting system when injured. A dedicated cystogram, or retrograde filling of the urinary bladder with contrast after placement of a catheter, can be performed if there is concern for bladder injury, as routine CT of the abdomen and pelvis is not sensitive for the detection of bladder injury [134].

Multiphasic CT evaluation with a single contrast bolus may offer increased clinical certainty to detect vascular extravasation (Fig. 3.11), devascularization and urinary leak, but results in increased radiation dose because multiple CT scans must be obtained at different time points corresponding with the structures that are opacified by the contrast [135, 136]. Double and triple split bolus techniques can opacify multiple structures during a single CT acquisition, reducing radiation dose [137–139].

Although rarely the initial imaging modality in the work-up of urinary tract disease, CT contributes significantly to the imaging of children with suspected urinary tract disorders. Indications include neoplasia [140], trauma [141, 142], severe infections [143] and occasionally complex questions regarding anatomy [144] (although MRI often would be the preferred modality). Though US is the mainstay of imaging urolithiasis in children, CT can be useful in cases that on US are equivocal or non-diagnostic.

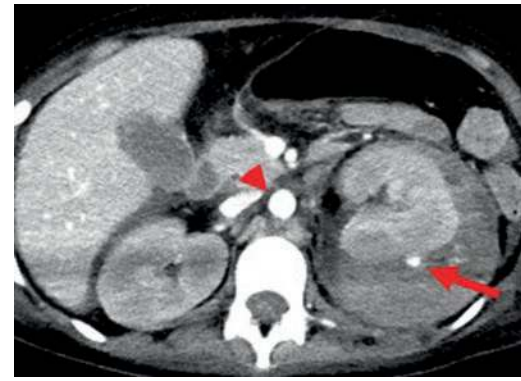

Fig. 3.11 Contrast enhanced CT of vascular extravasation. Arterial extravasation from the kidney on CT in a 14-year-old female following kidney biopsy. Contrast-enhanced axial CT image obtained in the arterial phase demonstrates opacification of the aorta (arrowhead) and remainder of the arterial system. There is arterial enhancement external to the left kidney (arrow) within a large perinephric hematoma, consistent with active arterial extravasation

CT allows for cross sectional imaging of the urinary tract and has the ability to reconstruct images in any plane for analysis. CT also provides excellent resolution of the urinary tract structures. The addition of IV contrast to the CT imaging allows for even greater accuracy in the detection of disease. Newer generations of CT technology provide higher spatial and temporal resolution and importantly can be done in many instances without sedation or general anesthesia, which may be required for MRI.

On unenhanced scans, the kidneys demonstrate similar attenuation to the normal liver or spleen. They are surrounded by a variable amount of retroperitoneal fat depending on the age and health status of the child. Administration of contrast results in a reliable pattern of enhancement beginning in the renal cortex, followed by enhancement of the renal pyramids, and later by opacification of the pelvicalyceal system, ureters and bladder.

The ability of CT to differentiate between tissues of various densities allows for the detection of hydronephrosis, calcifications (Fig. 3.12) and diseases extending into the perinephric fat even without the administration of IV contrast. With the addition of IV contrast, however, one can detect individual lesions of the kidney paren-

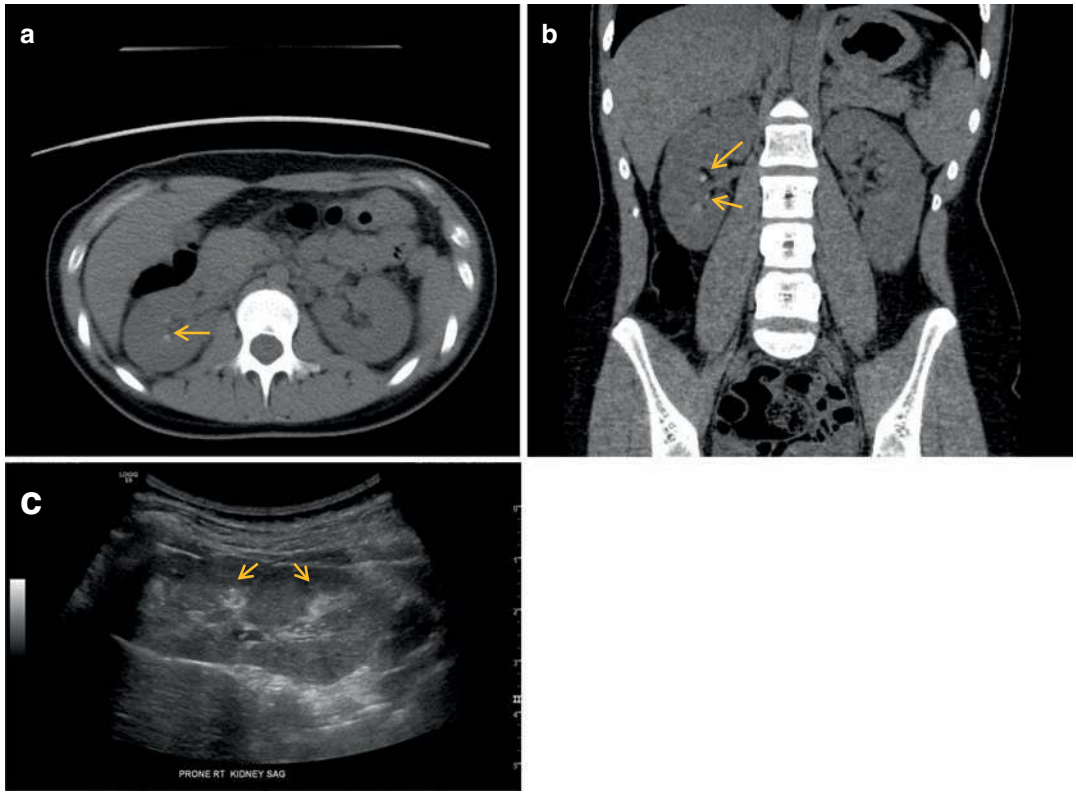

Fig. 3.12 Nephrocalcinosis. (**a**) Axial and (**b**) coronal planes of non-contrast CT of 17-year-old child showing variable hyperattenuation in multiple medullary papilla (arrows), which gradually decreases in density more proximally along the expected course of the medullary tubules. (**c**) Greyscale US shows diffuse increased echogenicity of the medullary pyramids. This is most suggestive of medullary calcifications (nephrocalcinosis)

chyma, such as cysts, tumors or nephroblastomatosis; focal areas of diminished enhancement, such as foci of pyelonephritis (Fig. 3.13) or contusion/laceration (Fig. 3.14); and global abnormalities of enhancement, such as is evident in renal artery stenosis (RAS) or thrombosis.

When ordering a CT examination, potential risks, including radiation exposure and possible contrast-induced nephropathy, must be considered and balanced with potential benefits such as avoidance of sedation or anesthesia.

Magnetic Resonance Imaging

MRI, like US, is uniquely suited to the imaging of children due to avoidance of ionizing radiation. Although radiofrequency energy is

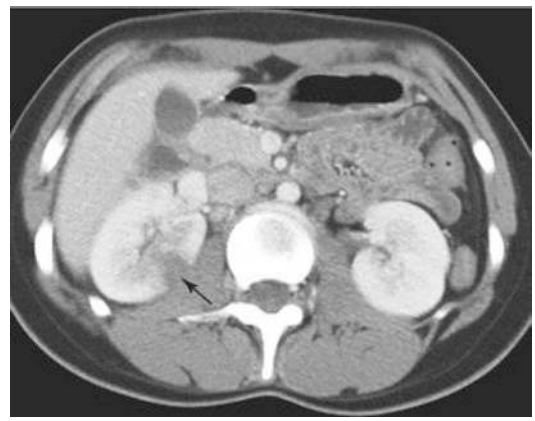

Fig. 3.13 Contrast enhanced CT for pyelonephritis. Contrast-enhanced CT at the level of the kidneys demonstrates an area in the posteromedial aspect of the right kidney with diminished enhancement (arrow), consistent with the clinical suspicion of pyelonephritis

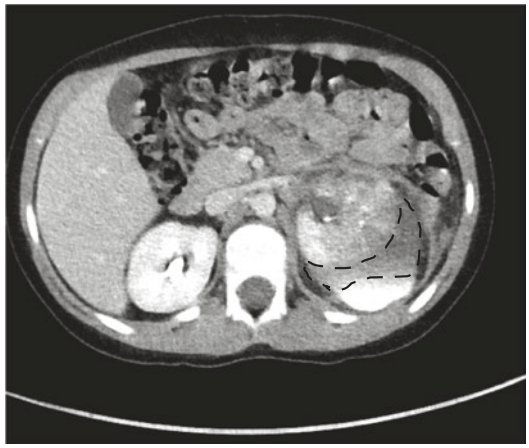

Fig. 3.14 Contrast enhanced CT of kidney laceration. Contrast-enhanced CT at the level of the kidneys demonstrates an area of diminished enhancement in the inferior aspect of the left kidney (dashed line), consistent with a laceration bisecting the lower pole of the kidney

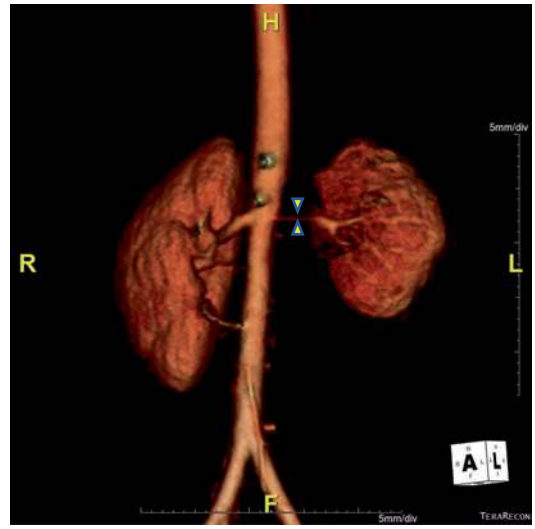

Fig. 3.15 MR angiography (MRA) of renal artery stenosis. MRA in a patient with hypertension showing stenosis in the left main renal artery (arrow heads)

imparted, MRI has not been shown to have the deleterious potential of CT. For that reason, MRI is often preferred over CT for children. However, longer scan times with MRI may necessitate sedation or general anesthesia in patients who are not able to lie still, such as children younger than 6 years and in those with development delay or claustrophobia [145]. In addition, access to an MR scanner remains limited in some regions of the world.

The superior tissue characterization of MRI makes it a powerful tool in assessing diseases of the urinary tract. Administration of gadolinium-based IV contrast can provide improved visualization but may be associated with risks such as NSF as discussed previously. For patients in whom gadolinium contrast is contraindicated, MRI without contrast can still provide detailed structural imaging of the urinary system.

MRI is particularly well suited in assessing neoplasms and tumor-like conditions of the kidneys [146, 147] including nephroblastomatosis [147]. MRI can help to characterize lesions, for example by demonstrating necrosis and hemorrhage in lesions such as Wilms tumor or renal cell carcinoma, or areas of fat in angiomyolipomas [148, 149]. Calcifications, however, are not as reliably seen with MRI as with CT.

As in adults, MRI can be used to assess the renal arteries and veins in children. Bland (non-tumor) thrombosis can readily be demonstrated, as can tumor extension into the vessels [150, 151]. MR angiography (MRA) can be used to assess for RAS in patients with hypertension [152–155] (Fig. 3.15). However, MRA may not be sensitive enough to definitively exclude RAS even in adults [156], and is additionally challenging in children due to their smaller vasculature. MRI can also be used in the assessment of infection [157–159] and trauma [160, 161]. Research is ongoing in adults and children into the application of additional advanced MR sequences in the kidney, such as diffusion-weighted MRI, diffusion tensor imaging, arterial spin labeling, and MR elastography [162–165].

Magnetic Resonance Urography

Magnetic resonance urography (MRU) is an advanced imaging technique that provides detailed anatomic information without ionizing radiation. The optimal visualization of the collecting system on MRU allows differentiation of complex genitourinary anatomy and evaluation

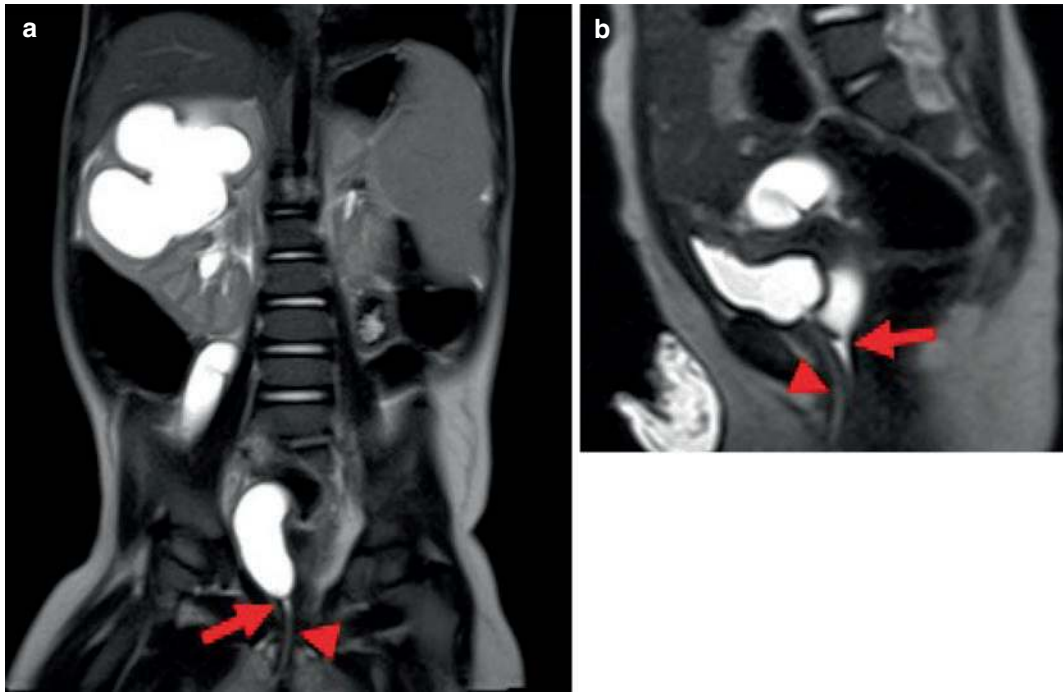

Fig. 3.16 MR urography (MRU) of ectopic ureter. Ectopic ureteral insertion on MRU in a 2-year-old with urinary tract infections. (**a**) Coronal and (**b**) sagittal T2-weighted images demonstrate a right-sided duplicated collecting system, with a markedly dilated upper pole system ectopically inserting into the urethra (arrows). A Foley catheter is in place (arrowheads), which demarcates the position of the urethra

of the course and insertion of the ureters (Fig. 3.16). This anatomic detail is provided by heavily T2-weighted, high spatial resolution two-dimensional and three-dimensional images [166]. With optimization of the imaging protocol, scan times with MRU can be reduced to 30 min [167].

Multiple technical factors of MRU must be considered and planned. Patients receive IV hydration and furosemide prior to imaging to distend the urinary tract and improve visualization [166]. A Foley catheter is often placed, allowing patients to tolerate the examination without the discomfort of needing to void. If evaluation of the bladder is necessary, the Foley catheter can be clamped during the examination to distend the bladder and allow better assessment. Patient positioning is also an important consideration, as patients with a dilated collecting system may need to be placed in a prone position to facilitate drainage of the system [167].

Functional information including differential kidney function, cortical transit time, calyceal transit time, and renal transit time can also be discerned with functional MRU (fMRU). Functional information coupled with delineation of anatomy is invaluable for treatment decisions and surgical planning [166]. If fMRU is desired, patients cannot have a contraindication to gadolinium contrast, as contrast enhancement and excretion provides the functional assessment.

MRU may require sedation in children younger than 6–10 years of age [166]. In infants, MRU without sedation is feasible using the "feed and wrap" technique [36]. In unsedated patients, motion robust sequences including radial volumetric interpolated breath-hold examination (VIBE) and periodically rotated overlapping parallel lines with enhanced reconstruction (PROPELLER) can be used. Respiratory or navigator gating can also be used to further improve image quality.

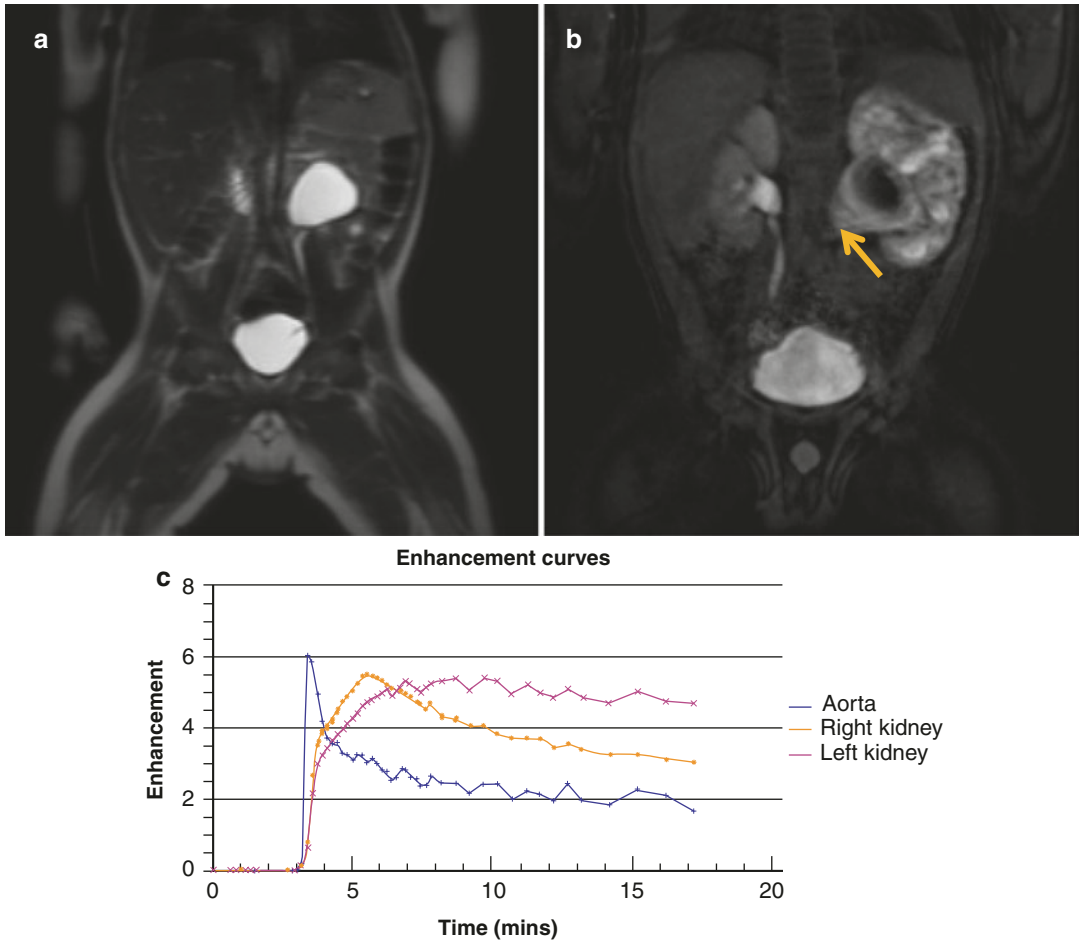

Fig. 3.17 Functional MR urography (fMRU). An 8-month-old boy with left ureteropelvic junction (UPJ) obstruction. (**a**) Coronal T2-weighted and (**b**) post-contrast VIBE images show left hydronephrosis with transition at the level of left UPJ (arrow). (**c**) Signal intensity versus time curves demonstrate asymmetric perfusion and excretion of contrast agent. The curve of the right kidney (blue line) is normal, and the left kidney (green line) reveals a dense delayed nephrogram and delayed excretion

MRU can be used to distinguish a capacious non-obstructed system from a UPJ obstruction. A UPJ obstruction should demonstrate delayed renal transit time correlating with the obstructed system (Fig. 3.17). MRU can confirm that the level of the obstruction is at the UPJ rather than at the level of the ureter, as seen with ureteral strictures. Additionally, arterial and venous phase postcontrast images can evaluate for crossing vessels as the etiology of the UPJ obstruction [166], a necessary step in surgical planning. MRU can also be used for postoperative reevaluation for UPJ obstruction following pyeloplasty or vessel reimplantation [168].

Radiography

Radiography is the oldest modality used in the evaluation of urinary tract disease, but its utility is limited. The normal urinary tract is not sufficiently different in tissue density compared to the surrounding abdominal and pelvic structures to be properly evaluated using radiography alone. There may, however, be cases in which there is sufficient retroperitoneal fat to outline the kidneys on plain radiographs and even assess their relative sizes. A kidney mass or severely hydronephrotic kidney might be detected by the presence of a soft tissue mass, calcification or fat, and

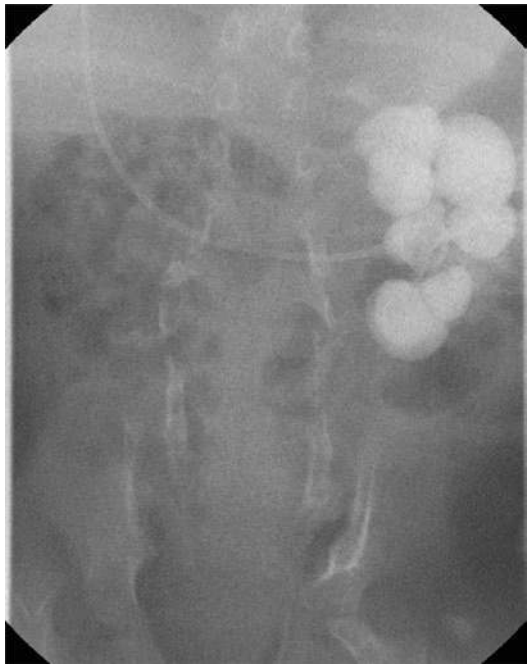

Fig. 3.18 Radiograph of staghorn calculus. Abdomen posteroanterior radiograph showing a radio-opaque staghorn calculus occupying the pelvis and collecting system of the left kidney

displacement of adjacent structures. A full bladder can also be seen as a midline structure in the pelvis, which will occasionally displace bowel loops out of the pelvis.

Calculi in the urinary collecting system can at times be seen on radiography depending on their composition [169–171] (Fig. 3.18). Nephrocalcinosis may also be detected depending on the degree of involvement [172]. Radiography is the mainstay of imaging for bone changes associated with advanced CKD, such as renal osteodystrophy [173].

Radiographs can also be beneficial in determining the correct positioning of various drainage catheters and stents. Most catheters and stents are sufficiently radio-opaque to be visible on radiographs (Fig. 3.19).

Overall, however, the role of radiography has largely been supplanted by the cross-sectional imaging modalities (US, CT and MRI) and by nuclear medicine.

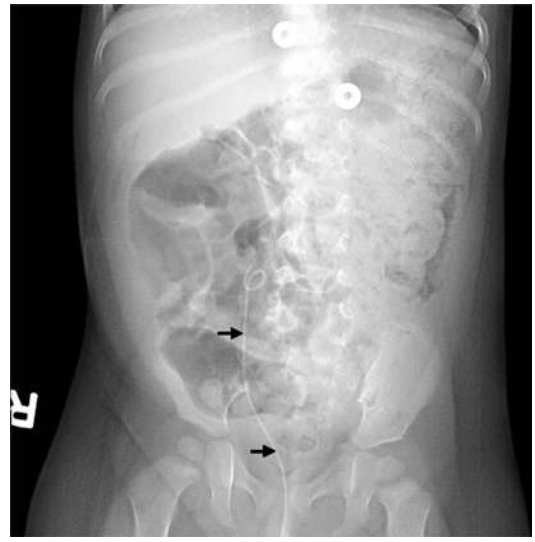

Fig. 3.19 Radiograph of ureteral stent. Abdomen posteroanterior radiograph showing right ureteral stent (arrows), which appears radiopaque

Excretory Urography

Excretory urography (IV pyelography) relies on the administration of IV contrast to enhance the urinary tract relative to the remainder of the abdominal tissues [174–177]. Excretory urography was historically used to delineate and characterize the anatomy of the urinary tract, but has now largely been supplanted by other imaging modalities such as US, CT, MRI and nuclear medicine [178–181]. Current applications for excretory urography can include evaluation for ectopic ureters and diagnosis of urothelial disorders and papillary necrosis [182–184].

Retrograde Urethrography

Though retrograde urethrography is rarely performed, it remains useful in the evaluation of suspected acute trauma or stricture to the male urethra, [185] and both congenital and acquired urethral abnormalities [186–188] (Fig. 3.20). The examination involves placement of a balloon tipped catheter into the distal urethra and careful

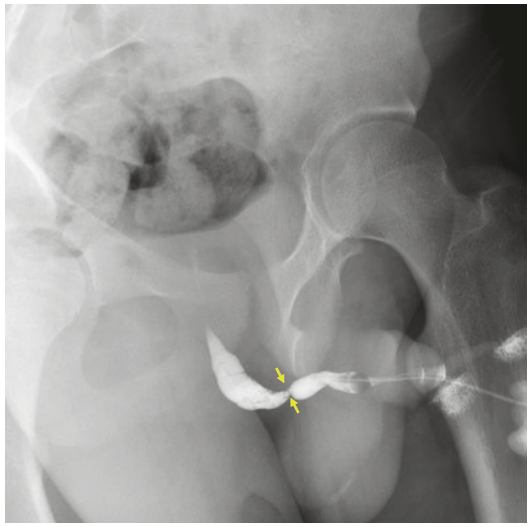

Fig. 3.20 Retrograde urethrogram. Retrograde urethrogram showing bulbar urethral stricture (arrows)

inflation of a balloon in the fossa navicularis. Images of the urethra are taken in an oblique projection during a hand injection of water-soluble contrast. Due to the presence of the external sphincter, the posterior urethra is usually not optimally assessed as part of this study but rather can be imaged during voiding after filling the bladder directly.

Interventional Radiology

Minimally invasive image-guided techniques are increasingly available and integrated into the diagnosis and treatment of pediatric kidney disease. Imaging innovations such as improved US imaging resolution and low radiation dose fluoroscopy and angiography allow for detailed evaluation and guidance in even the smallest patients. The development of pediatric interventional radiology (IR) as a subspecialty has resulted in innovative techniques specifically designed for application in children. Finally, innovative design has resulted in a greater selection of pediatric specific or applicable devices, allowing for application of adult techniques that were previously unavailable in children because of the technical limitations of devices designed for larger patients.

Patient Evaluation and Preparation

Pediatric patients frequently require deep procedural sedation or general anesthesia for minimally invasive image guided procedures, creating greater complexity for the performing proceduralist and health care system. Therefore, many pediatric institutions have adopted care teams with an additional care provider (critical care physician, nurse anesthetist, anesthesiologist) to monitor and manage the patient's sedation/anesthesia during genitourinary interventions, allowing the interventional radiologist to focus solely on the procedural goals. Patients must be screened for anesthetic risks and triaged appropriately to a team that can manage acute sedation or airway complications.

Procedure-specific evaluation includes review of the relevant presentation, medical history, goals of therapy, and allergies. Complete pediatric-specific guidelines for pre-procedure labs and anticoagulation protocols are not currently available from the Society of Pediatric Interventional Radiology, but pediatric literature increasingly incorporates guidelines published by the Society of Interventional Radiology (SIR). Pediatric interventional radiologists may use the SIR adult recommendations for procedural preparation or can develop institution-specific guidelines and protocols [189, 190]. Because of the robust blood supply of the kidneys, most interventions are considered at high risk for bleeding, and therefore anticipation of bleeding and/or the need for transfusion should be considered [189].

Facilities and Equipment

Proper equipment management and sterile technique are critical to reduce infectious complications. The portability of US, which has robust applications and favorable anatomic detail in the pediatric population, allows for the potential of bedside procedures. However, this increased portability should be used deliberately, as the IR suite frequently offers easier access and space to perform patient resuscitation in emergencies and adjunctive imaging modalities that can prevent complications and shorten procedures.

Types of Interventions

Pediatric renal interventions are often divided into two large subsets, vascular and non-vascular, and will be described in more detail in the next section, "Applications of Diagnostic and Interventional Radiology Techniques" Vascular interventions involve trans-arterial access to the renal arteries via the umbilical, femoral, brachial or radial artery. This technique can be utilized to treat renovascular hypertension, kidney hemorrhage in the setting of trauma, and benign kidney tumors using angioplasty, stenting or embolization. Non-vascular interventions frequently involve direct access to the collecting system of the kidney, ureter or bladder to address infection or obstruction. US guidance has become the standard of care for kidney biopsy and will be discussed specifically in a later chapter [191].

Applications of Diagnostic and Interventional Radiology Techniques

Neonatal Diseases

The increase in antenatal imaging (US and to a much lesser degree MRI) and prenatal detection of urinary tract abnormalities has resulted in a commensurate increase in postnatal imaging in the work-up of antenatal findings. The most common indications for postnatal imaging are follow-up of antenatally diagnosed UTD and renal ectopia, agenesis or dysplasia. US is the most common initial study to determine whether two kidneys are present, their location and the status of the parenchyma and collecting system. An increase in parenchymal echogenicity, loss of corticomedullary differentiation, parenchymal loss or scarring, and cyst formation can be signs of underlying kidney disease.

In many institutions, the initial postnatal US will be done after the first 48 h of life to avoid missing hydronephrosis during the relative dehydrated state of the newborn period and the non-distended state of the collecting system. The UTD classification system, described in further detail below, is used to quantify the degree of dilation. The region of dilatation helps to localize a urinary tract obstruction. Dilatation of only the pelvicalyceal system suggests UPJ obstruction [192] (Fig. 3.21); if the ureter is also dilated, UVJ obstruction may be present [193]; if the bladder is distended and perhaps trabeculated, the obstruction may involve the bladder outlet or urethra (Fig. 3.22) [194]. Any degree of pelvicalyceal and/or ureteral dilatation can also be due to VUR.

The work-up of a dilated collecting system may vary among institutions, but in general focuses on screening for VUR, obstruction, and other congenital anomalies of the kidneys and urinary tract (CAKUT). VCUG can be used to assess for VUR or urethral obstruction due to PUV or stenosis. If VCUG does not reveal VUR or PUV, nuclear medicine diuretic renography can assess for any degree and level of urinary obstruction.

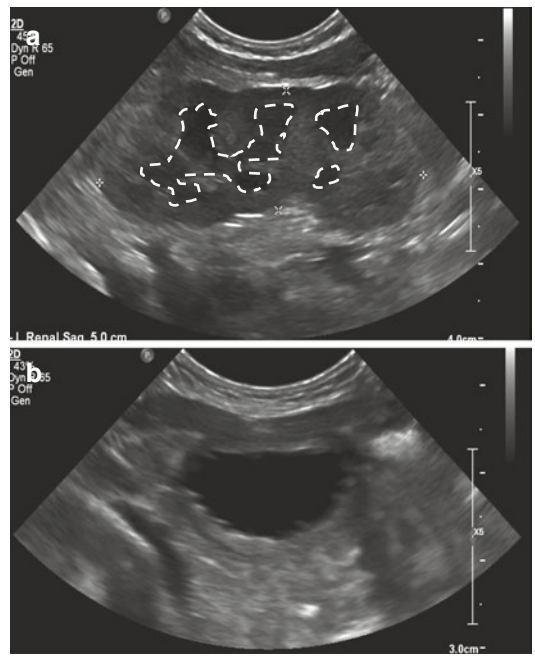

Fig. 3.21 US of urinary tract dilation (UTD) due to ureteropelvic junction (UPJ) obstruction. UTD due to UPJ obstruction in a 2-month-old male. (**a**) Longitudinal greyscale US image of the left kidney demonstrates central and peripheral calyceal dilation (dashed line). There was no abnormality of the kidney parenchyma or (**b**) the bladder

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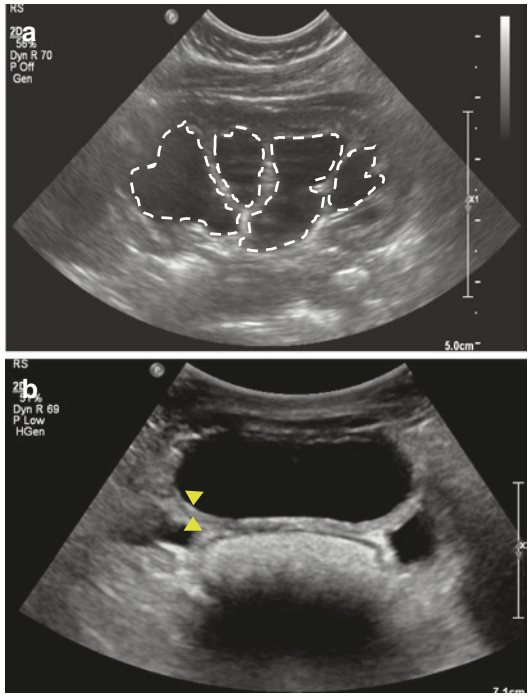

Fig. 3.22 US of urinary tract dilation (UTD) due to bladder outlet obstruction. UTD due to bladder outlet obstruction in a 2-month-old male. (**a**) Longitudinal greyscale US image of the left kidney demonstrates marked central and peripheral calyceal dilation (dashed line) with minimal renal parenchyma. (**b**) The urinary bladder is markedly distended with thickened bladder wall (arrow heads)

Urinary Tract Dilation Classification System

The UTD classification system is a multidisciplinary consensus intended to standardize the terminology used to describe and quantify the degree of pre- and postnatal UTD and to propose a management scheme based on risk stratification. The UTD classification system is based on the US findings of anterior-posterior renal pelvic diameter (APRPD), calyceal dilation, kidney parenchymal thickness, kidney parenchymal appearance, bladder abnormalities, and ureteral abnormalities [195].

Prenatally detected UTD is stratified into low risk (UTD A1) or increased risk (UTD A2–3) based on gestational age and US findings. Low risk patients have an APRPD of 4 to <7 mm at 16–27 weeks gestation and 7 to ≤10 mm at ≥28 weeks gestation, with central or no calyceal dilation. An increased APRPD of ≥7 mm at 16–27 weeks gestation or ≥10 mm at 28 weeks gestation is classified as increased risk. Additional findings such as peripheral calyceal dilation, parenchymal thinning, abnormalities of the parenchymal appearance, bladder abnormalities, ureteral abnormalities, and unexplained oligohydramnios classify patients as increased risk, with the classification based on the most concerning finding present.

As previously mentioned, initial postnatal US is typically performed greater than 48 h after birth to avoid underestimation of UTD due to dehydration. Patients that may require evaluation prior to 48 h after birth include those with oligohydramnios, urethral obstruction, bilateral high-grade dilation, or if there are concerns about patient compliance with postnatal evaluation. Low risk (UTD P1) patients have an APRPD of 10 to <15 mm and central calyceal dilation. APRPD of ≥15 mm, peripheral calyceal dilation, or ureteral abnormalities increase patient risk to the intermediate (UTD P2) category, whereas abnormal kidney parenchymal appearance or thickness and bladder abnormalities increase patient risk to the high (UTD P3) category.

The UTD classification system has been associated with clinical outcomes, with higher risk scores being associated with genitourinary diagnoses and need for surgical intervention [196].

Other Congenital Abnormalities of the Kidneys and Urinary Tract

Many other types of CAKUT can be diagnosed with antenatal or neonatal imaging. Multicystic dysplastic kidney (MCDK) is characterized by multiple non-communicating cysts with no identifiable normal kidney parenchyma [197, 198]. It is thought to arise either due to prenatal renal pelvic ureteral atresia or abnormal interaction between the ureteric bud and metanephric blastema [198]. Demonstration of the lack of communication between the cysts differentiates this process from pelvicalyceal dilation. Imaging can also demonstrate agenesis, ectopia, and horseshoe or cross-fused kidneys. Again, US is the preferred initial imaging modality, although in some cases the findings are discovered inciden-

tally on other modalities (including DMSA renal cortical scintigraphy).

The presence of a duplicated collecting system can be inferred on US when the renal sinus echo complex is interrupted by a band of renal cortical tissue, though it can be difficult to distinguish this pattern from a prominent column of Bertin [199]. The presence of a duplex collecting system may be associated with obstruction (usually of the upper moiety) and/or reflux (usually of the lower moiety) according to the Weiger-Meyer rule [200]. This rule states that in a duplicated collecting system, the upper moiety is drained by a ureter, which inserts ectopically and the lower moiety is drained by a ureter that inserts orthotopically, with the former often obstructed by a ureterocele and the latter demonstrating VUR. These entities (obstruction and VUR) can coexist in the same patient.

Ureteroceles can be found using almost any modality but are most commonly diagnosed on US or VCUG [201]. Ureteroceles can occur with ectopic ureters or at the normal ureterovesical junction (termed orthotopic or simple ureteroceles), and like ectopic ureteroceles can be obstructive or non-obstructive [202].

Neonatal Renovascular Conditions

Conditions affecting the renal vasculature in the neonate, including thrombosis of the renal arteries or veins, can be assessed with Doppler US imaging of the vessels and kidneys. Associated thrombosis of the aorta and inferior vena cava (IVC) can also be assessed. If a central catheter is present, which can predispose a patient to thrombosis, its position can best be assessed with abdominal radiographs, although the catheters can also be seen with US. If a thrombus has occurred, recanalization can be assessed sonographically after anticoagulation or thrombolysis. Occasionally, residual thrombus can calcify and be visible as a linear hyperechoic structure, either along the vessel wall in major vessels or within the renal parenchyma in small vessels. Follow-up kidney US can also assess for any long-term sequelae of thrombosis, such as atrophy, abnormal parenchymal echogenicity or cyst formation.

Interventional Radiology in the Neonate

In most cases, neonatal UTD is managed surgically (ablation of PUV, ureteral reimplantation, pyeloplasty) or conservatively. Occasionally, urgent decompression of the upper urinary tract is necessary to prevent further injury to the kidney and allow postponement of definitive surgical intervention in the presence of significant comorbidities or prematurity [203].

Percutaneous nephrostomy (PCN) allows for rapid decompression of the upper urinary tract and is broadly used to treat urinary obstruction and infection in adults and children. The procedural success rate is consistently high (> 95%) in multiple case series in both neonates and larger children [203–207]. PCN is performed by placing the patient in the prone or semi-prone position and localizing the kidney using US. Periprocedural antibiotics are used routinely for infected systems and are recommended for all cases by SIR Quality Improvement Guidelines [190], although there is practice variation in antibiotic use for non-infected systems [206]. In cases with collecting system dilation, a "single stick" technique is employed to place a hollow tip or sheath needle into the collecting system via a peripheral calyx. Positioning within the collecting system is confirmed with a combination of US needle tip localization, return of urine via the needle hub, and injection of agitated saline, US contrast or iodinated contrast. Once peripheral access is confirmed, a guide wire is placed into the collecting system and coiled or advanced into the ureter or bladder. The tissues are then dilated over the wire and a pigtail drainage catheter is placed into the collecting system and secured (Fig. 3.23). In nondilated systems, a "two stick" technique may be used to access the central collecting system with a hollow tip needle to allow for distension and easier access to a peripheral calyx without endangering the central vasculature. An elegant modified technique for PCN in neonates has been described by Koral et al. that accommodates for their unique anatomy and tissue density [208]. Major complications are best avoided by using US guidance, avoiding central puncture of the renal collecting system, and using

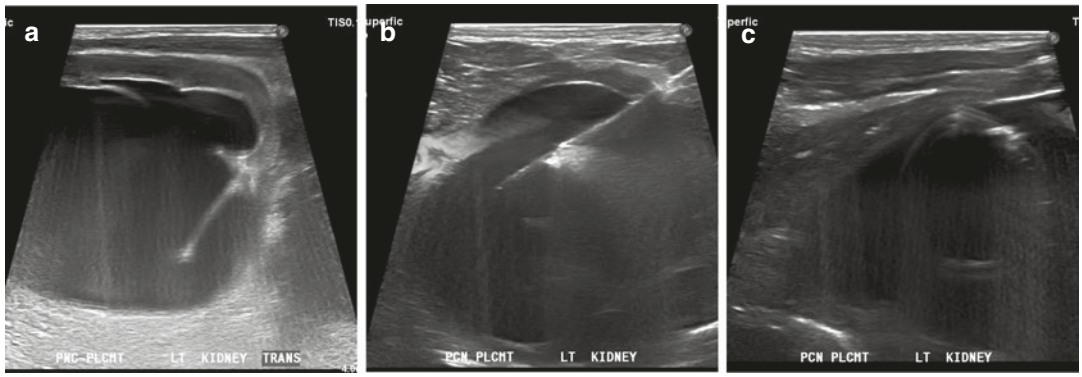

Fig. 3.23 Percutaneous nephrostomy (PCN). PCN placement in a 22-day old infant due to bilateral hydronephrosis and left sided urinoma with worsening abdominal distension despite bladder decompression. (**a**) Greyscale US showing a markedly dilated left kidney collecting system with parenchymal thinning. (**b**) The collecting system was accessed using a 21-gauge micro-puncture needle (**c**) Subsequently a 5 French Dawson-Mueller catheter was placed into the collecting system with US guidance

caution during tissue dilation with a supportive guide wire. In the immediate post-placement period, hemorrhage can occur due to central entry into the collecting system or inadvertent creation of an arterial fistula. Injury to the collecting system or ureter can occur due to needle or wire perforation during wire positioning or dilation, resulting in a urinoma. Sepsis is a feared complication in the setting of an infected collecting system. Appropriate antibiotic coverage and minimizing collecting system distension and manipulation during initial treatment of the infected system is recommended to reduce the risk of sepsis. Overall, the risk of major complication is the pediatric populations ranges from 0% to 9% [205, 209].

Retrograde placement of ureteral stents is less common in neonates than in older patients, as the size and rigidity of commercially available stents creates technical challenges.

Urinary Tract Infections and Vesicoureteral Reflux

The clinical management of UTI and VUR will be discussed more fully in later chapters. Multiple imaging modalities can contribute to the evaluation and management of UTI and VUR, including US, VCUG, ceVUS, and nuclear medicine. Imaging can help to identify urologic abnormalities such as obstruction or VUR, guide management strategies (e.g. need for antibiotic prophylaxis or surgical intervention such as ureteral reimplantation), and can aid in characterizing the extent of infection and potential complications (e.g. pyelonephritis, abscess, and kidney scarring). Clinical practice guidelines for imaging in pediatric UTI vary somewhat between international organizations [39, 210–212] but there are several unifying themes: (1) defining criteria for obtaining imaging based on risk factors for underlying urologic abnormalities (based on factors such as age, number of UTIs, and severity of symptoms); (2) using kidney and bladder US as the initial imaging method to assess for anatomic abnormalities; (3) evaluating for VUR in selected patients; and (4) using additional modalities such as DMSA based on various clinical criteria.

Kidney and bladder US can identify UTD that could suggest obstruction or reflux, evaluate bladder anatomy (e.g. wall thickness, bladder emptying [213]), and detect signs of either acute infection or sequelae of previous infection (e.g. scarring or global volume loss). Acute pyelonephritis can be associated with urothelial thickening [214], kidney enlargement [215], alterations in parenchymal echogenicity [216], or increased echogenicity of the renal sinus [217]. Focal bac-

terial nephritis (acute lobar nephronia) can resemble a kidney mass [218]. Echogenic debris in the collecting system can suggest, but is not pathognomonic for, infection. In patients with fungal UTI, particularly those who are immunosuppressed or have indwelling catheters, US can be used to assess for kidney parenchymal infection and the presence of fungal balls of the collecting systems.

Evaluation for VUR and urethral abnormalities such as PUV is most commonly performed using pulsed fluoroscopic VCUG. However, ceVUS is increasingly being used in some centers. Both VCUG and ceVUS can assess for the presence of VUR, whether it occurs during filling or voiding, and its severity in the five-grade system as described previously. Grading of VUR is similar between VCUG and ceVUS [73]. VCUG and ceVUS can also be used to assess for other anatomic abnormalities, including duplicated collected system, calyceal diverticula and PUV.

DMSA kidney cortical scintigraphy can be used to detect pyelonephritis and scars, both of which will appear as photopenic or "cold" areas. The distinction between acute pyelonephritis and scarring therefore depends on clinical signs and symptoms, as well as comparison with prior DMSA if available. If cortical defects persist beyond 6 months after clinical resolution of the acute infection, they are considered to be permanent scars that can predispose to CKD and hypertension [219].

Although contrast-enhanced CT (CECT) is the preferred imaging modality for acute pyelonephritis is adults [220], it is generally reserved for selected situations in children to minimize radiation exposure (for example, children with equivocal US findings at centers where MRI is unavailable, or when the risk of sedation for MRI is considered greater than that of radiation and IV contrast). CECT findings in acute pyelonephritis can include kidney enlargement and parenchymal hypodensities that can be wedge-shaped, linear, or patchy [220] (Fig. 3.13). The "striated nephrogram" is considered a classic finding of acute pyelonephritis, but can also be seen in other conditions such as renal vein thrombosis or ureteral obstruction [221].

MRI provides excellent anatomic definition of the kidneys without exposure to ionizing radiation. Contrast-enhanced MRI is generally considered to be the reference imaging technique for children with suspected acute pyelonephritis, but newer diffusion-weighted imaging sequences also appear to have comparable performance without the need for gadolinium contrast [222].

Interventional Radiology Techniques in UTI

Infection in the obstructed renal collecting system is considered a medical emergency and an indication for urgent decompression by stenting or percutaneous therapy. The need for minimally invasive decompression is less clear in infection occurring from ascending bacteria, direct spread from an adjacent source or hematogenous disseminated infection of the non-obstructed system.

Intraparenchymal kidney abscess and perinephric abscess are frequently grouped together in treatment algorithms. In general, collections smaller than 3 cm may be managed effectively with medical therapy alone, but percutaneous US-guided drainage may be advised in larger collections, immunocompromised or critically ill patients, or failure to improve despite appropriate antibiotics [223, 224] (Fig. 3.24).

Pyonephrosis refers to purulent material within the collecting system, and historically has been an indication for percutaneous drainage because of the risk of severe urosepsis with any extraneous manipulation. When collecting system decompression is indicated, US-drain placement, similar to PCN placement, is the technique of choice.

Neoplasm

Most kidney neoplasms in children present as a large palpable mass detected by the parent or physician. The initial imaging modality is most often US, which can suggest the renal origin of an abdominal mass by demonstrating extension of kidney tissue around the mass—the so-

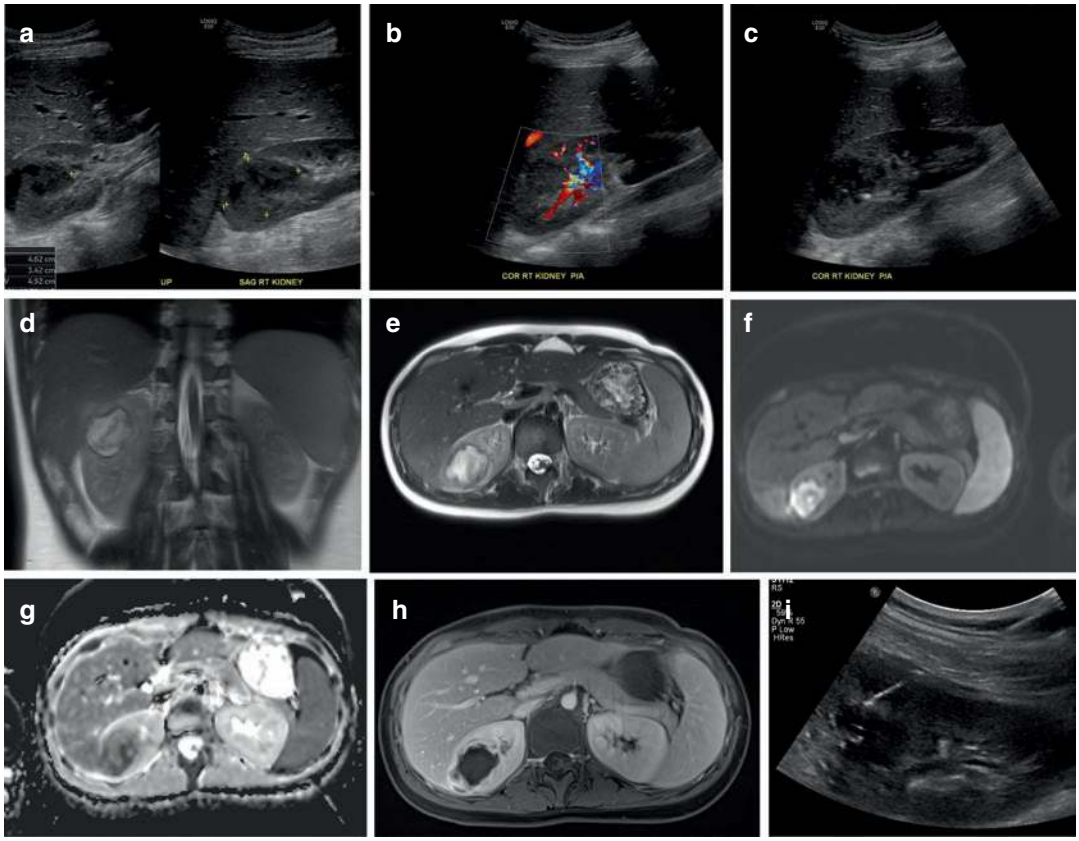

Fig. 3.24 Kidney abscess. 17-year-old female with acute pyelonephritis and increasing right flank pain despite appropriate antibiotic therapy. (**a–c**) US images with a heterogenous hypovascular expansile lesion of the right upper pole, with distortion of the adjacent vasculature and color Doppler signal. (**d**, **e**) MRI shows a T2 hyperintense collection with (**f**) restricted diffusion (**g**) confirmed with ADC map, with (**h**) no enhancement on post-contrast imaging (**h**). The collection was aspirated under US guidance (**i**)

called "claw sign" (Fig. 3.25). US can differentiate solid from cystic or necrotic areas, demonstrate if hemorrhage or calcification is present, and determine whether the tumor involves the renal vein or IVC or extends into the heart. Once an US confirms the presence of a tumor, CT or MRI can better assess the size, extent, involvement of adjacent structures, and spread, and are particularly useful for Wilms tumor staging [225–227]. Although CT and MRI are relatively equivalent in assessing the primary kidney mass, CT is the modality of choice to assess for lung metastases. If the tumor has a propensity to metastasize elsewhere, then appropriate imaging modalities (CT or MRI of the brain, bone scan, etc.) can be performed.

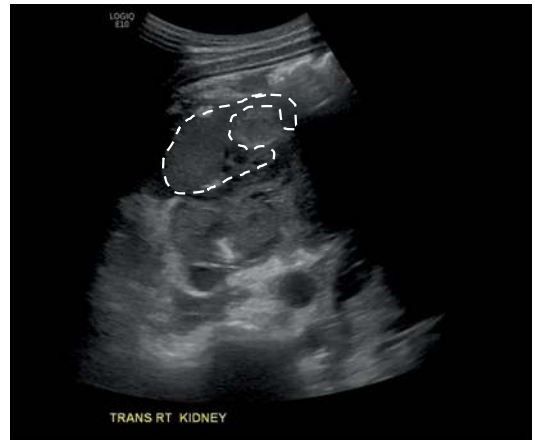

Fig. 3.25 US of kidney mass with "claw sign". Greyscale US showing transverse plane of right kidney with normal kidney tissue surrounding a mass ("claw sign," dashed outline)

Interventional Radiology in Pediatric Kidney Tumors

Interventional techniques can be applied in the management of pediatric kidney tumors in various situations. Percutaneous biopsy of Wilms tumor has been controversial since its use has been attributed to upstaging tumor classification in some staging systems [228] and it may not be necessary in patients with classical imaging presentation. Therefore, image guided diagnosis should be discussed with a pediatric oncologist before proceeding with a biopsy (Fig. 3.26). The technique for a targeted mass biopsy is similar to that of a non-targeted renal biopsy, although localization of a representative sample is critical. Adjunctive techniques, including image fusion and CEUS, can be helpful for targeting smaller lesions or obtaining viable tissue for pathologic analysis [229].

Rarely, a malignant kidney tumor can present with acute hemorrhage. Similar to kidney trauma, angiography and embolization can be useful either independently or in conjunction with surgery to stabilize patients and reduce blood loss [230].

Careful differentiation of kidney tumors from adrenal masses is critical prior to performing a biopsy. Tissue requirements for characterizing neuroblastoma are more extensive than other masses, necessitating larger coring needles and a greater number of samples for gene sequencing. Adrenal lesions, such as pheochromocytoma, can have catecholamine activity, and may result in hypertensive crisis if instrumented without alpha blockade [231].

The most common benign neoplasm of the kidney is angiomyolipoma (AML). In children, AML is seen almost exclusively in tuberous sclerosis complex (Fig. 3.27a, b), rather than as an independent lesion as seen in the middle-aged female population. These hamartomatous lesions are at risk for hemorrhage regardless of size, with increased risk in larger lesions or in the presence of intranidal aneurysm [232, 233]. Multiple lesions can be treated medically with mammalian target of rapamycin (mTOR) inhibitors, although embolization and partial nephrectomy have long been critical components of therapy. Embolization involves arterial access, renal angiography and microcatheter selection of the tumor vascularity of the AML (Fig. 3.27c, d) [233–235]. Liquid (dehydrated alcohol), glue (n-BCA) or particulates (PVA or Embospheres@) are administered until vascular stasis is achieved, resulting in tumor necrosis and lesion shrinkage over subsequent months [236].

Ablation therapy is a newer method of treating kidney tumors and has generally been accepted to treat small malignancies while preserving kidney parenchyma. In general, a probe is placed percutaneously into the target lesion and energy is imparted to the surrounding tissue to incur target

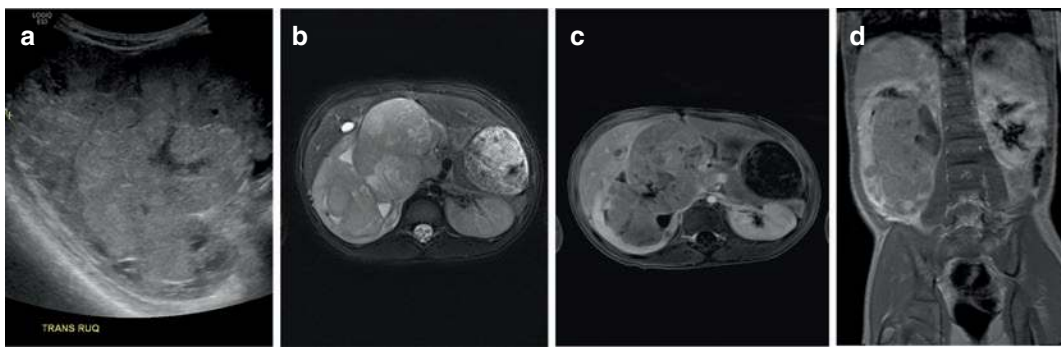

Fig. 3.26 Kidney mass. 6-year-old female with right abdominal mass. (**a**) Greyscale US demonstrates a heterogenous vascular mass expanding and distorting the contour of the right kidney. (**b**) T2, (**c**) post-contrast axial T1, (**d**) coronal MRI of the abdomen confirms a lesion arising from the right kidney. Staging evaluation confirmed metastatic spread to the lungs, therefore percutaneous biopsy was obtained for tissue diagnosis to guide presurgical chemotherapy

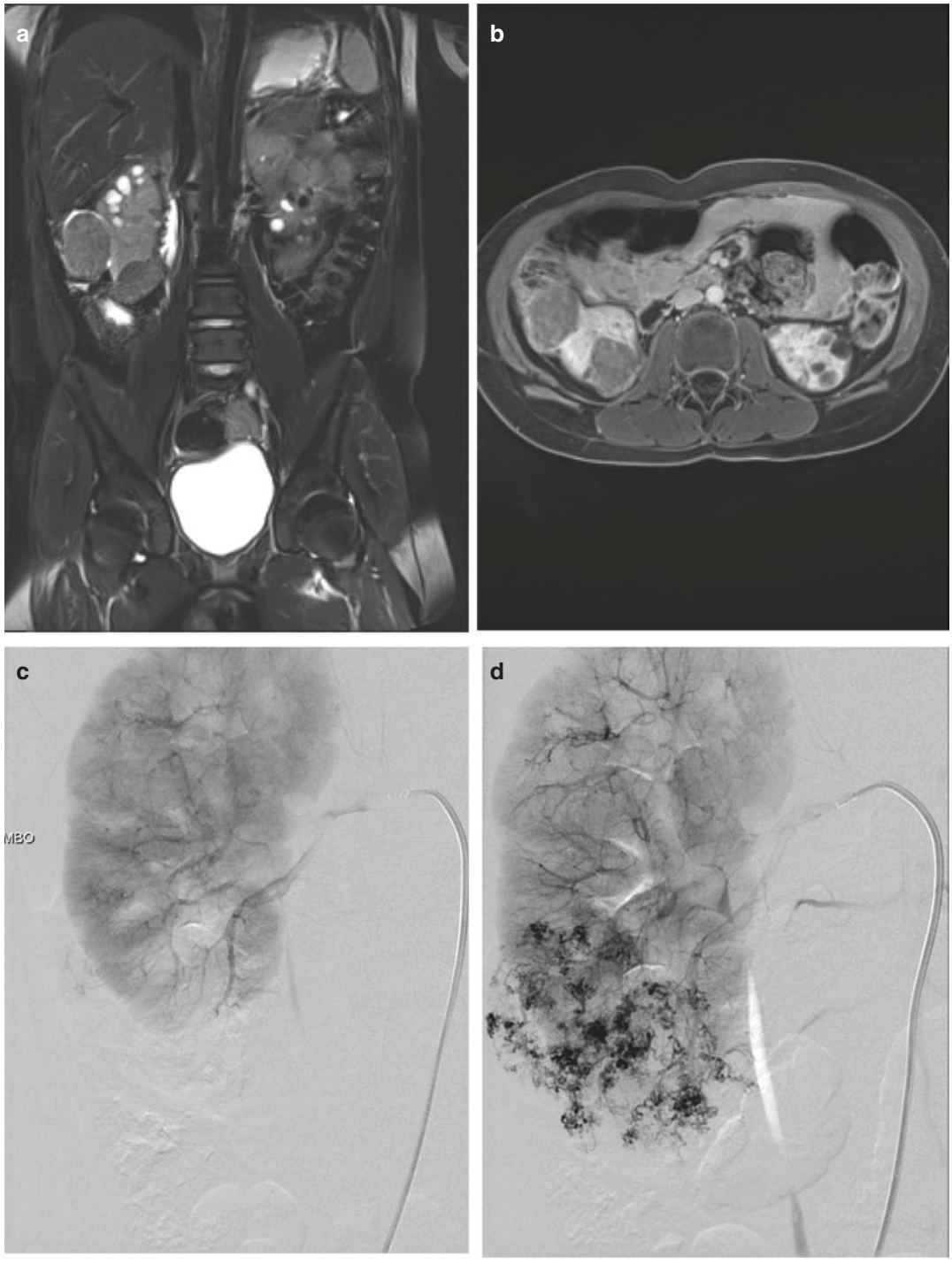

Fig. 3.27 Kidney angiomyolipoma. (**a**) Coronal T2 and (**b**) axial post contrast T1 abdominal MRI in a 12-year-old with tuberous sclerosis demonstrating interval growth of two pedunculated lesions from the lower pole of the right kidney. (**c**) Catheter directed angiography confirms angiomyolipoma with characteristic "cork-screw" vasculature. (**d**) Following particle embolization, the abnormal vasculature is no longer present and adjacent kidney parenchyma is preserved

cell injury and apoptosis. Currently, cryotherapy is commonly used in the GU system [237, 238], although radiofrequency ablation and microwave are also reported as effective for treatment of small kidney tumors. The primary limiting factors are proximity to critical structures or vascularity that can result in heat or cold sump, preventing target lesions reaching threshold damage.

Trauma

Due to the relatively large size of the kidneys compared to body size, decreased protection from the rib cage, and increased pliability of Gerota's fascia and capsule, there is increased frequency of kidney injury following blunt abdominal trauma in children and adolescents [239].

Imaging of trauma to the urinary tract has been studied extensively and remains a topic of debate [240]. In the acute setting, the imaging evaluation of the injured child is determined by the extent and type of injury as well as the practice of the particular institution [141, 241]. In some institutions, evaluation of trauma to the abdomen begins with abdominal US to assess for free fluid and obvious visceral injuries [242, 243]. At other institutions, CT is the modality of choice in the initial assessment [92, 142, 240]. The decision as to which modality is used will depend on the clinical situation. US has a high sensitivity in detecting intraperitoneal fluid; however, in the setting of trauma, the presence of fluid is not an absolute indication for surgery. Moreover, there can be injury to the urinary tract without the presence of free fluid [244]. CT, on the other hand, can accurately assess for the presence of free fluid and assess the solid and hollow abdominal viscera.

US imaging is effective for evaluating the kidneys and can be useful in triaging patients (FAST exam), in lower acuity patients, and to monitor patients following initial characterization of injuries [135]. US is not considered the primary study for trauma because of the lower sensitivity for kidney contusions and lacerations, operator dependence, length of the study, and patient discomfort in the acutely injured patient. The adjunctive use of US contrast shows promise for increasing the sensitivity for parenchymal and vascular injury, but is not yet widely utilized and does not overcome the drawbacks of length of study and operator dependence [136].

US can depict a kidney laceration or contusion as a focal area of abnormal echotexture. The area can be hypoechoic, isoechoic or hyperechoic to the remainder of the kidney depending on the contents of the area and the stage of the evolution of the injury. US can also characterize the quality and amount of perinephric fluid (blood, urine or both) and follow the appearance to assess whether the collection is diminishing, remaining stable or increasing in size. Doppler US of the kidneys can assess both for areas of kidney parenchymal ischemia due to vascular interruption and for arterial or venous thrombosis and pseudoaneurysm formation. Renal vascular injury can also be visualized with nuclear medicine functional renal imaging, with non-perfused regions of the kidney appearing as cold defects.

CT is often the modality of choice in the evaluation of abdominal trauma, since most pediatric kidney injuries are related to blunt trauma and are associated with additional organ injuries [245]. Imaging is indicated in the setting of clinical suspicion based on mechanism or hematuria on presentation. As described previously in the section "Computed Tomography," IV contrast is generally used in the evaluation of trauma, and multiphase imaging can offer increased sensitivity to detect vascular disruptions or urinary leak. The appearance of the kidneys, particularly their patterns of enhancement on CT, can allow diagnosis of kidney contusions and lacerations. Areas devoid of enhancement, particularly if they are regional, suggest infarction. Perinephric and/or periureteral fluid can also be assessed. Delayed imaging may show disruption of the collecting system if dense contrast is seen outside of the collecting system (Fig. 3.28). CT cystography has also been used to assess for injuries to the urinary bladder and urethra.

In the past IV urography was used extensively in the evaluation of urinary tract trauma but is

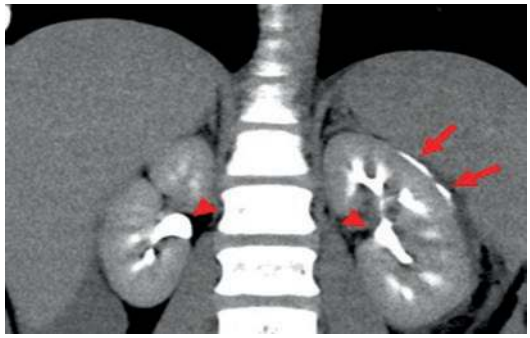

Fig. 3.28 Contrast enhanced CT (CECT) for trauma. CECT of collecting system injury in a 13-year-old following a snowboarding accident. CECT image obtained in the delayed phase shows opacification of the collecting system (arrowheads). There is contrast accumulation outside of the collecting system of the left kidney (arrows), compatible with collecting system injury

seldom used today. Fluoroscopic studies (retrograde urethrography and cystography) are, however, still used extensively in the imaging of bladder and urethral injury.

Interventional Radiology in Kidney Trauma

The American Association for the Surgery of Trauma (AAST) Organ Injury Scale (or the European Classification System) classifies kidney injury on a scale from grade I to V based on the depth and extent of injury, involvement of vascular structures, and urinary tract injury (Table 3.2). Most pediatric kidney injuries fall in grade I, II or III, and are frequently managed conservatively (Figs. 3.29 and 3.30) [246, 247]. Analysis of trauma registries and additional case series have described increased rates of surgical or interventional management within grades IV and V (Fig. 3.31) [246–248].

The goal of kidney trauma management is first to preserve life and second to preserve kidney integrity and function. The confined retroperitoneal space allows for tamponade of most hemorrhage when the facial plains remain intact. Initial patient instability frequently dictates the need for immediate operative intervention. However, delayed decompression of hemorrhage into the kidney collecting system or into the peritoneal space can result in refractory anemia and the need for persistent transfusion in the initially stable or stabilized patient. Patients requiring >3 transfusions in the initial 24–48 h should be deemed unstable, and angiographic intervention or surgery should be considered. Injury to the collecting system and ureter is a critical component of the trauma evaluation, as urine leak and urinoma can result in morbidity. Grade I–III injuries as well as most grade IV injuries can be successfully managed conservatively or with percutaneous or endoscopic therapy [249, 250].

When available, image guided intervention can successfully manage refractory hemorrhage while preserving kidney parenchyma. Trauma registries have demonstrated a high rate of nephrectomy when early surgery is performed for hemorrhage in the pediatric population [248]. Embolization can be performed rapidly and has been shown to be effective in salvaging

Table 3.2 American Association for the Surgery of Trauma (AAST) kidney injury scale (2018 revision)

AAST grade	Imaging criteria
I	Subcapsular hematoma and/or parenchymal contusion without laceration
II	Perirenal hematoma confined to Gerota's fascia Parenchymal laceration ≤1 cm in depth without urinary extravasation
III	Parenchymal laceration >1 cm without urinary extravasation or collecting system rupture Any injury in the presence of a kidney vascular injury or active bleeding contained within Gerota's fascia
IV	Parenchymal laceration extending into urinary collecting system with urinary extravasation Renal pelvis laceration and/or complete ureteropelvic disruption Segmental renal vein or artery injury Active bleeding beyond Gerota's fascia into the retroperitoneum or peritoneum Segmental or complete kidney infarction(s) due to vessel thrombosis without active bleeding
V	Main renal artery or vein laceration or avulsion of hilum Devascularized kidney with active bleeding Shattered kidney with loss of identifiable parenchymal renal anatomy

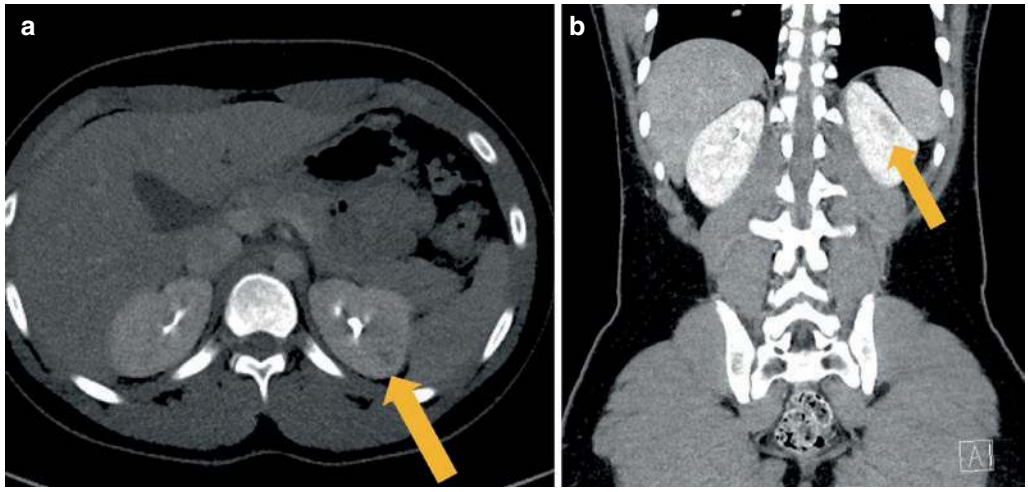

Fig. 3.29 Grade I left kidney parenchymal contusion on contrast enhanced CT. (**a**) Axial and (**b**) coronal contrast enhanced CT showing small focal hypoattenuating area (arrows) in the left interpolar region suggestive of grade I kidney contusion

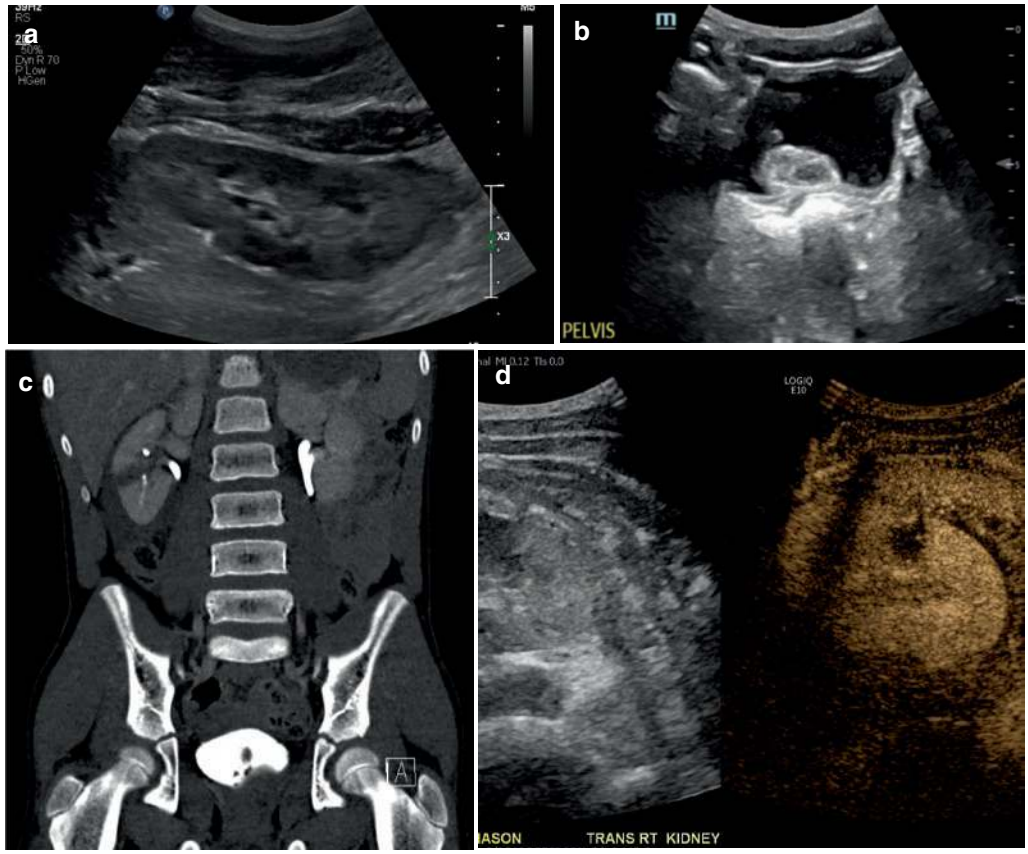

Fig. 3.30 Grade III right kidney injury. (**a**) Greyscale US of the right kidney shows increased lower pole echogenicity and a band of hypoechoic tissue at the juncture between the interpolar region and lower pole. (**b**) Bladder US provides visual confirmation of hematuria with a blood clot. (**c**, **d**) Contrast enhanced CT and US more accurately depict the extent of this Grade III right kidney injury

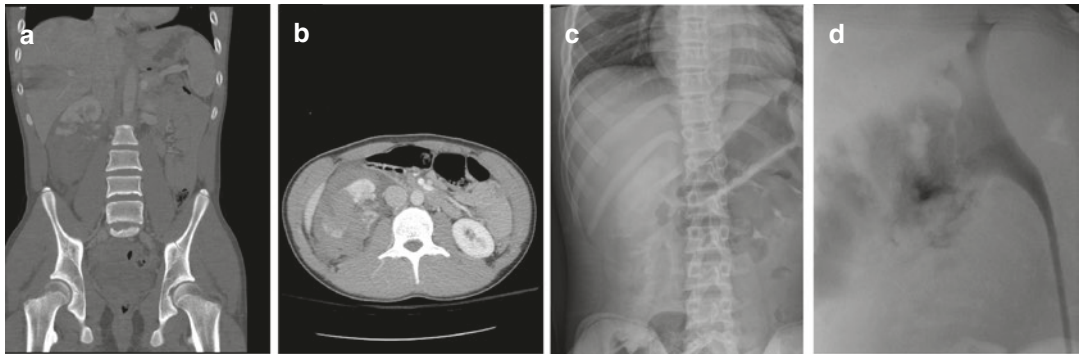

Fig. 3.31 Grade IV right kidney injury. Adolescent male following sports injury to the right flank. (**a**, **b**) Coronal and axial contrast enhanced CT angiography show a complex right kidney laceration involving the collecting system. Excretory phase imaging was not obtained. (**c**) Abdominal radiograph shows persistent contrast in the right kidney parenchyma with amorphous density in the perinephric tissues. (**d**) Retrograde ureterogram confirms urine leakage from the right kidney collecting system that was managed with placement of an endoscopic ureteral stent

the kidney without nephrectomy [245]. Embolization can be performed with a variety of agents, although temporary agents (such as Gelfoam®) and coils are the most common. A secondary procedure may be required if bleeding persists and has been shown to be beneficial for kidney salvage according to national trauma data bank [251].

Renovascular Hypertension

Imaging plays a critical role in identifying and managing renin-mediated renovascular hypertension. Renovascular hypertension can be described by stenosis location, extent and syndromic or non-syndromic association. Renovascular hypertension, once thought to be a rare cause of hypertension in children, has received growing attention in the past decade and is reported as a cause of hypertension in 10% of children with hypertension [252].

RAS can be unilateral or bilateral, involving the main, main branch or segmental renal arteries. Additionally, renal perfusion can be affected by upstream short or long segment stenosis of the lower thoracic or abdominal aorta. This distribution of disease, named middle aortic syndrome (MAS), can be isolated or can occur in combination with renal artery involvement.

Commonly identified causes for renovascular hypertension and MAS in children include fibromuscular dysplasia (FMD), vasculitis, neurofibromatosis type I, and Williams syndrome. However, the underlying cause is often difficult to confirm because of the overlapping imaging appearances, and the presumed diagnosis is made based on a combination of clinical and imaging features. For instance, "burned out" or senescent vasculitis can have imaging features identical to FMD. Additionally, the classically described "beaded string" appearance of FMD in adults is uncommon in children. In the young patient, the angiographic appearance of FMD is commonly a short segment uniform stenosis secondary to the involvement of both the intima and media within this age group.

Non-invasive imaging is critical for evaluating renovascular hypertension and determining the need for further intervention. US is the mainstay as it offers moderately high-resolution images with a combination of anatomic features and vascular flow assessment (Fig. 3.32) [253]. Differences in kidney lengths of >1 cm in the presence of hypertension may suggest main RAS and deserve additional evaluation. Established criteria for diagnosing RAS include direct visualization of stenosis, *parvus et tardus* wave form [acceleration time (time from onset of systole to peak systolic velocity) of >70 ms], main renal

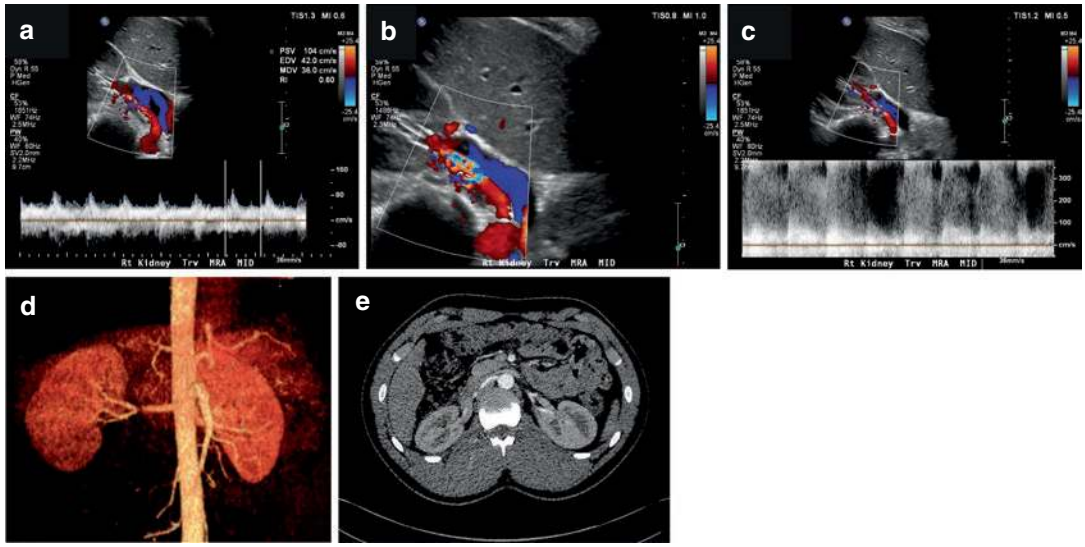

Fig. 3.32 Doppler US and CT angiography (CTA) of renal arteries. 16-year-old male with newly detected hypertension without hereditary risk factors. (**a–c**) Doppler US shows increased flow velocities in the mid- to distal right renal artery with aliasing on Doppler tracing and velocities in excess of 180 cm/s. (**d**, **e**) Contrast enhanced CTA confirms a short segment stenosis of the right renal artery, just proximal to a bifurcation into the upper and lower main branch renal arteries

Table 3.3 US criteria for renovascular hypertension (adapted from Dillman et al. [254])

US characteristic	Criteria suggesting renovascular hypertension
Kidney size	Asymmetric kidney lengths with difference of >1 cm
Doppler waveform	*Parvus et tardus* waveform Acceleration time of >70 ms
Main renal artery peak systolic velocity (PSV)	>180–200 cm/s
Renal-aortic (velocity) ratio (RAR)	>2.3–3.5
Renal-intrarenal (velocity) ratio (RIR)	>5

artery peak systolic velocity (PSV) of >180–200 m/s, main renal artery to aortic PSV ratio (RAR) of >2.3–3.5, or main renal artery to intrarenal artery PSV ratio (RIR) of >5 (Table 3.3) [254, 255]. Unfortunately, the sensitivity for RAS on Doppler US is low at 63–73% [256, 257].

CT angiography (CTA) (Fig. 3.32d) and MR angiography (MRA) (Fig. 3.15) have better sensitivity for detecting RAS, reported in the range of 93–94% and 77–93% respectively [256, 257]. Improved detection rates need to be balanced with the potential risks of ionizing radiation (for CTA) and sedation or anesthesia (for MRA). CTA image acquisition is much faster than MRA, and therefore conducive for non-sedated exams in the school-age child. Dose reduction, motion tolerant and image enhancement technology in newer MRI scanners will potentially offer no radiation exposure while maintaining image quality. However, currently MRA remains susceptible to even small amounts of motion that can occur in both sedated and non-sedated patients.

Catheter directed angiography remains the gold standard for the diagnosis of renovascular hypertension when there is a suggestive history and absence of non-invasive imaging findings [257]. In patients with appropriate histories as many as 40% of patients will have findings of clinically significant stenosis on angiography. Once identified, the lesion can be treated using angioplasty, stenting or ethanol ablation.

Angioplasty has, however, been the mainstay of endovascular therapy for renovascular hypertension and MAS. Lesions are confirmed with

aortography or selective renal angiography, crossed using supportive wires and treated with balloon angioplasty following heparinization (Fig. 3.33). Adjunctive devices such as drug-eluting and cutting balloons have reportedly improved outcomes in small series [258–260]. Branch segmental artery stenoses may be difficult to traverse and current limitations of angioplasty devices may preclude treatment. In these cases, directed ethanol ablation may offer a mechanism to reduce renin secretion from the affected area of kidney parenchyma [261].

Angioplasty using a combination of traditional, high pressure and cutting balloons has high rates of technical angiographic success, up to 94% in reported series [262]. Features associated with favorable technical and clinical outcomes include short segment stenosis (<10 mm), and residual stenosis of <10% following angioplasty [259, 262]. Clinical responses vary among children and are lower than comparable disease in adults. This is most pronounced in FMD, where angioplasty alone has been reported to be beneficial in 93–98% of adult patients. In contrast, pediatric cases series have reported benefit in 56–72.8% of cases and cure in 23–39% of cases. Variability in the response rates may be due to regional variation and heterogenous disease etiology [259, 262, 263].

Renal artery angioplasty is well tolerated with infrequent life-threatening complications, even in the pediatric population. Potential complications include contrast toxicity (contrast-induced nephropathy), thrombosis, stent migration, vascular spasm, dissection, perforation and resulting hemorrhage with imminent threat to the kidney. Contrast toxicity is best managed by setting weight-based contrast notifications throughout the procedure and maintaining patient hydration. Hemorrhagic complications are more frequent when using cutting angioplasty balloon but can be treated with angioplasty as either a temporizing or a definitive therapy. Covered stent deployment is reserved for cases refractory to angioplasty and when appropriate sized devices are available but is associated with higher rates of re-stenosis [263]. If hemorrhage continues after a trial of balloon occlusion, then urgent engagement of the surgical team may be the only remaining option. Surgical interventions have been reported for primary or salvage therapy in the event of failed angioplasty or restenosis [264].

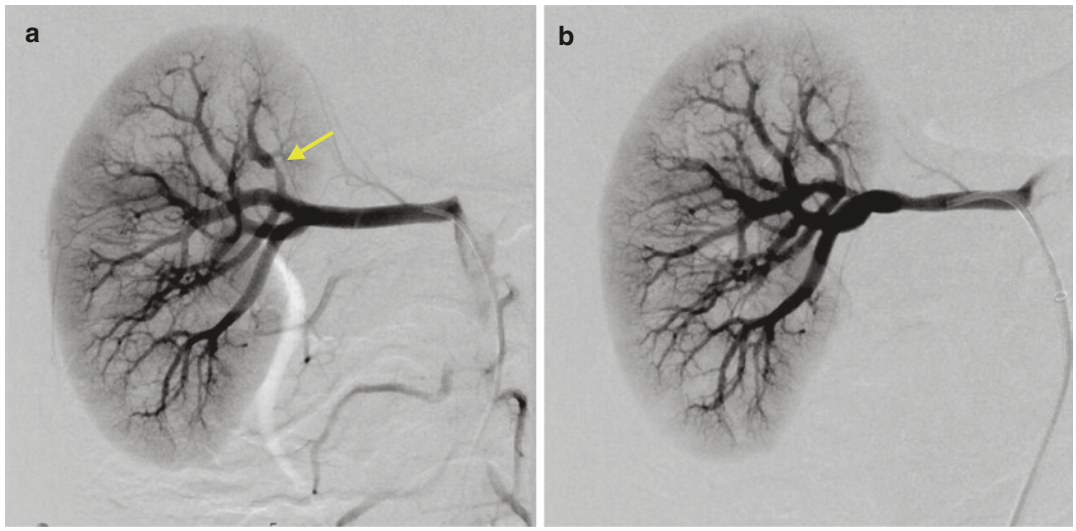

Fig. 3.33 Catheter directed angiography. Catheter directed angiography in a 9-year-old male with newly detected hypertension without hereditary risk factors. (**a**) Focal segmental branch stenosis in right upper pole with wedge-shaped parenchymal perfusion defect. (**b**) Repeat angiography following right upper pole segmental angioplasty showing improved lumen diameter and perfusion

Cystic Kidney Diseases

Kidney cysts can be seen at any age in the pediatric group. Cysts can be sporadic and unassociated with any systemic disease or may occur in a wide range of genetic and non-genetic diseases [265–267].

Kidney and bladder US is the primary imaging modality recommended for initial assessment of kidney cysts in children [267]. On US, a kidney macrocyst will appear anechoic with an imperceptible wall, whereas multiple microcysts may manifest as heterogeneous parenchymal echogenicity (e.g. "salt-and-pepper sign" in autosomal recessive polycystic kidney disease [ARPKD]) or just increased echogenicity without distinct cysts (Fig. 3.34) [267]. When distinct macrocysts are present, important characteristics to note include the number of cysts, location (e.g. cortical, medullary), sizes of larger cysts, and whether any complex features such as septations or debris are present [267]. The collecting system should be identified to ensure that a calyceal diverticulum or dilated calyces are not confused with cysts [267].

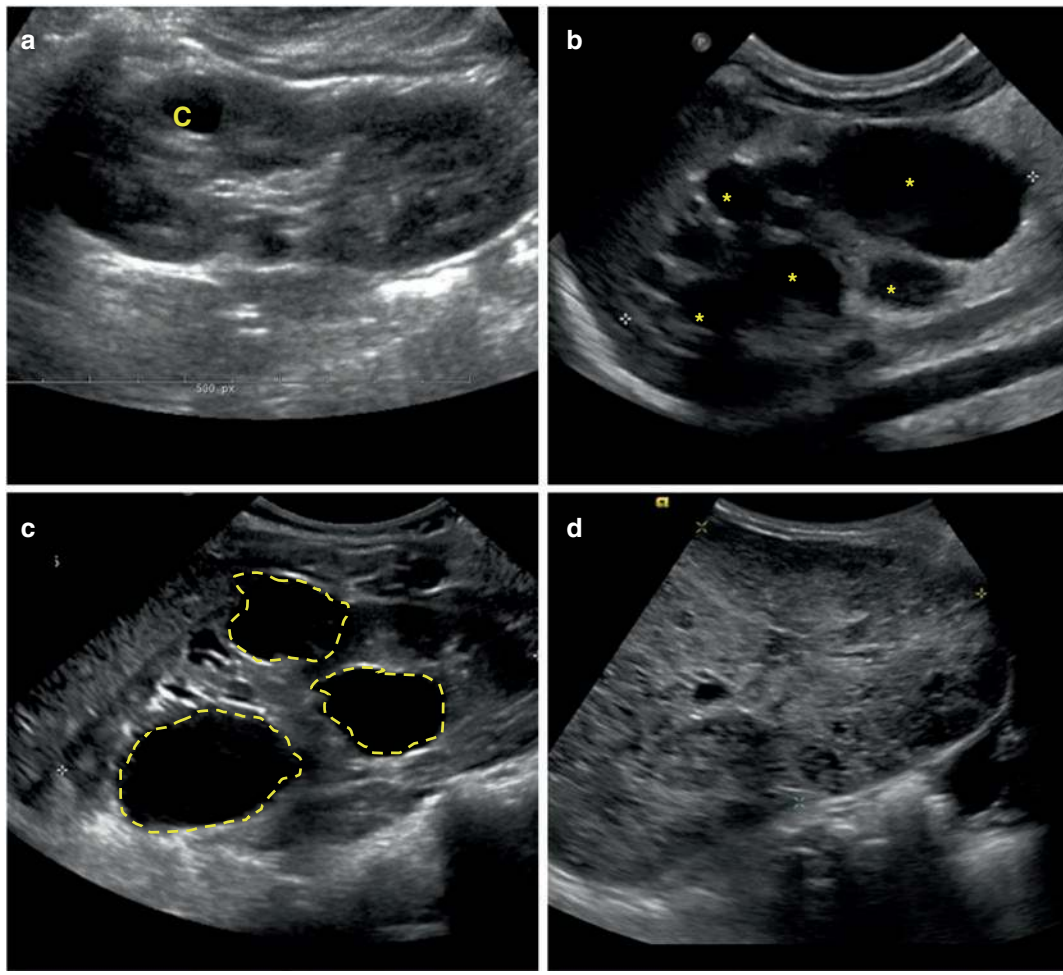

Fig. 3.34 US of cystic kidney diseases. US appearance of kidney cysts. (**a**) Simple cyst (c); (**b**) Multicystic dysplastic kidney, with numerous disorganized cysts (*) without intervening normal parenchyma; (**c**) ADPKD, with enlarged kidney with multiple macrocysts (dashed lines); (**d**) ARPKD, with numerous microcysts causing "salt-and-pepper" appearance of parenchyma and few resolvable cysts

Other imaging modalities such as CT or MRI are not routinely recommended for initial evaluation of kidney cysts in children due to cost and availability, as well as due to associated risks of ionization radiation (for CT) and need for sedation in younger children (for MRI). However, CT or MRI may be indicated in select circumstances, such as evaluation of complex cystic lesions with suspicion for malignancy or detection of angiomyolipomas in patients with tuberous sclerosis [267].

On CT, a cyst will have attenuation equal or near that of simple fluid and will have an imperceptible wall. There should be little to no change in the attenuation of the cyst after contrast administration. The Bosniak classification system was developed to assess the risk for malignancy based on cyst appearance on CT in adults, using factors such as cyst wall thickness, presence of septa, calcifications, or solid components, and degree of enhancement [268]. Studies in children suggest that modified versions of the Bosniak classification based on US appearance of cysts can adequately stratify malignancy risk [269–271]. CEUS also appears promising to classify kidney cystic lesions in native and transplant kidneys [57, 61, 62].

Non-genetic causes of kidney cysts in infants and children include cystic dysplasia, multicystic dysplastic kidney [272], medullary sponge kidney [273], cystic tumors or malformations, cystic nephroma, and solitary simple or acquired cysts [265, 267, 274]. Although simple cysts are common in adults, the reported incidence of simple cysts in children is as low as 0.2% [274]. Solitary cysts can also result from previous insult to the kidney, such as trauma or infection. In most cases of solitary cysts, the appearance of the remainder of the kidney is often normal.

Genetic causes of kidney cysts include autosomal dominant polycystic kidney disease (ADPKD), ARPKD, *HNF1B*-associated disease, nephronophthisis, and other genetic ciliopathies [267]. ADPKD is characterized by the development of multiple macrocysts throughout the kidney cortex and medulla (Fig. 3.34c), with progressive enlargement of the cysts and the kidneys throughout the lifetime [275, 276]. ARPKD is typically characterized by enlarged, echogenic kidneys, sometimes with visible macroscopic cysts or dilated tubules predominantly in the medulla. Echogenic foci and the "twinkling sign" suggestive of calcifications can also be seen (Fig. 3.34d) [267, 277]. Juvenile nephronophthisis is characterized by relatively normal-sized or small kidneys with increased echogenicity and often cysts at the corticomedullary junction [267]. Imaging is also useful to assess for other complications or extra-renal manifestations of genetic cystic kidney diseases, such as angiomyolipomas in children with tuberous sclerosis, liver fibrosis in children with ARPKD, or liver cysts in children with ADPKD [278].

Kidney Stones and Nephrocalcinosis

Kidney Stone Imaging

US is typically the first-line modality in the evaluation of pediatric stone disease. Kidney stones can range from a few millimeters in size in a renal calyx to several centimeters in size filling the renal pelvis (staghorn calculus) (Fig. 3.35). They are most reliably detected by US when present in the kidney or bladder but may also be seen in the ureter when not obscured by overlying bowel gas. Kidney stones appear on greyscale US as echogenic foci, which may produce acoustic shadowing deep to the location of the stone. Small stones may be difficult to differentiate from vessels or portions of renal sinus fat, particularly if distal shadowing is not present. The twinkle artifact, or presence of alternating colors, is an imaging feature of kidney stones on color flow Doppler US (Fig. 3.35). Proper optimization of US settings is necessary to detect smaller stones, particularly in the selection of the focal zone and pulse repetition frequency. In addition to detecting stones, US may also assess for UTD suggestive of urinary obstruction due to stones. The bladder can also be evaluated for ureteral jets, the absence of which is suggestive of obstruction.

CT may also be indicated in the evaluation of stone disease if the US is equivocal or non-diagnostic. CT has a higher sensitivity than US

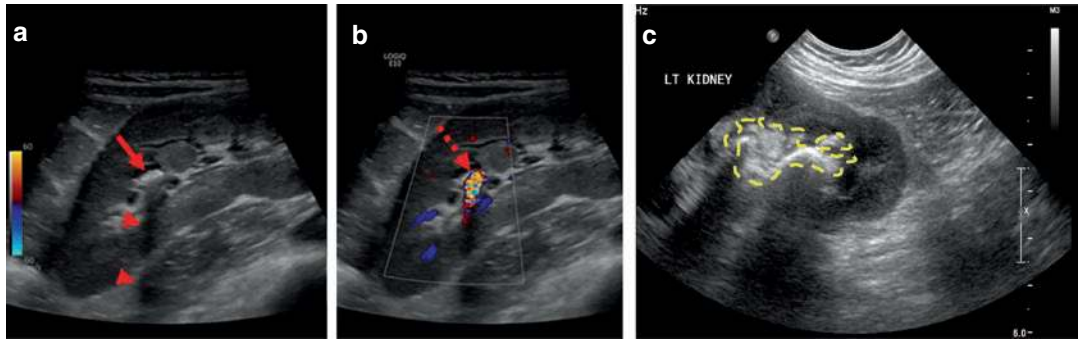

Fig. 3.35 US of kidney stones. (**a**) Greyscale longitudinal US of a non-obstructing stone, appearing as an echogenic focus (arrow) in the upper pole of the kidney with posterior acoustic shadowing (arrowheads). (**b**) Color Doppler longitudinal US demonstrates twinkle artifact (dashed arrow) associated with the echogenic focus, compatible with a kidney stone. There is no urinary tract dilation to suggest obstruction. (**c**) Obstructing staghorn calculus demonstrated by a large echogenic focus (dashed outline) occupying the collecting system of the left kidney

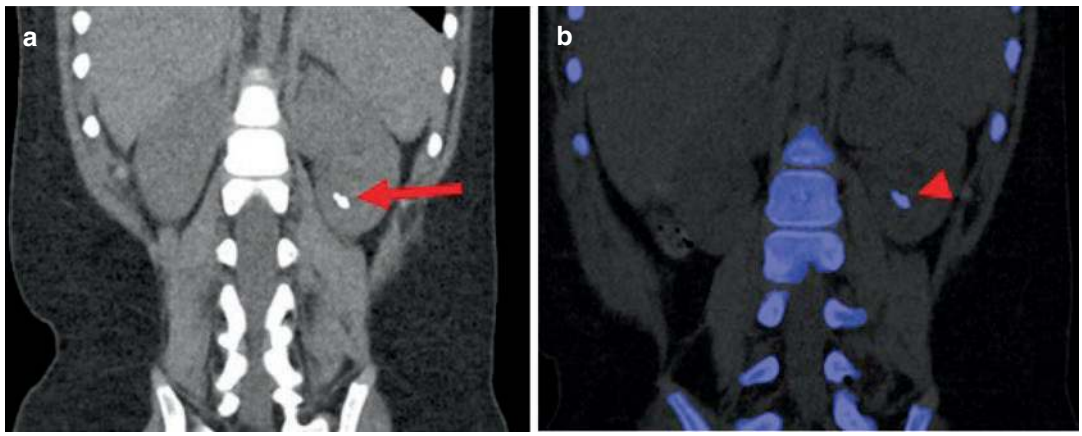

Fig. 3.36 Dual-energy CT (DECT) for detecting kidney stone composition. Non-obstructing kidney stone on DECT in an 8-year-old female. (**a**) Coronal DECT images demonstrate a dense focus in the left kidney lower pole (arrow), compatible with stone. (**b**) Color coding demonstrates that the stone is blue, correlating with non-uric acid/urate-composition, which is compatible with a cystine or calcium-based stone

for the detection of stones smaller than 3 mm [279], and can detect nearly all stones, except for matrix stones or medication stones, such as indinavir stones [280]. CT may also be necessary to evaluate for ureteral stones that may not be visualized on US, as well as complications of stone disease, including abscess and pyonephrosis. Stone evaluation is further enhanced by DECT, which can be used to determine stone composition based on the attenuation ratios (Fig. 3.36). Determining stone composition can help guide management, as certain compositions are less amenable to extracorporeal shock wave lithotripsy, and knowledge of the stone composition can help guide dietary recommendations and medical management [281]. In children, kidney stone size and Hounsfield attenuation values on CT have been shown to predict successful shock wave lithotripsy [282].

Nephrocalcinosis

US is often the modality of choice to assess nephrocalcinosis. While cortical nephrocalcinosis can be seen, it is a rare finding in children [283]. Medullary nephrocalcinosis is well described in children and can be accurately diagnosed and fol-

lowed using US. Typically, medullary nephrocalcinosis appears as echogenic pyramids, often outlining the rim of the pyramid, with a normal appearing cortex (Fig. 3.12c). A pattern of peripheral increased echogenicity followed by progression toward the center of the pyramid has been described as the Anderson-Carr progression of nephrocalcinosis [172].

Conclusion

The role of radiologic imaging and interventions in the diagnosis and treatment of diseases of the kidneys and urinary tract continues to evolve. US, CT, MRI, and nuclear medicine remain the most common modalities for assessing the urinary tract, while new technologies such as CEUS, ceVUS, and MRU are starting to change practice patterns. Interventional radiology is playing an increasing role in the management of urinary tract and renovascular disease. Close collaboration between nephrologists, urologists, and radiologists will continue to allow optimal care for children with kidney and urinary tract abnormalities.

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4 Molecular Diagnosis of Genetic Diseases of the Kidney: Primer for Pediatric Nephrologists

Aoife Waters and Mathieu Lemaire

Introduction

Over the past decade, remarkable advances have been made in our understanding of the human genome through the development of genotyping arrays and next-generation sequencing techniques. These technological advances have allowed us to examine the consequences of various types of genomic variations on the phenotype of patients in an unparalleled manner. Identification of mutations in novel genes associated with rare renal diseases has forced dramatic pathophysiological revisions owing to the discovery of links to unexpected biochemical pathways or structural scaffolds. These provide not only a firm molecular diagnosis for hitherto "idiopathic" conditions, but also offer hope for new therapeutic strategies for diseases that would otherwise have an unfavourable prognosis. The last few years have witnessed great improvements in our understanding of the role of common genetic variation in the pathogenesis of complex renal diseases, although the promises of clinical utility are far from reality. In this chapter, we will outline developments made in various fields related to genomic medicine, with a particular emphasis on how these will translate into the daily clinical practice of pediatric nephrologists.

General Genetic/Genomic Concepts

Deoxyribonucleic Acid (DNA)

In 1962, the Nobel Prize in Medicine was awarded to Watson and Crick together with Maurice Wilkins for their discovery of the structure of deoxyribonucleic acid (DNA). DNA is a critical participant in the execution and regulation of biological processes that are critical for living organisms to develop and survive. Constituting the double helix structure of DNA are two twisting, paired strands that are packed full of information via the systematic arrangement of four nucleotide bases—adenine (A), thymine (T), guanine (G), and cytosine (C)—the "genetic alphabet" [2]. Opposite strands anneal together specifically, via the pairing of bases: A binds to T, and C to G. If one strand's nucleotide sequence reads ATTCGG, the other strand will read TAAGCC; we refer to these as the positive and negative strands, respectively.

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Gene

A gene refers to segments of DNA that carry the sequencing code for all building blocks necessary for cells to thrive. Two sequential processes are key: transcription and translation. Transcription is the process by which a ribonucleic acid (RNA) strand is made to match exactly the nucleotides that make up a particular gene. It is critical to preserve both nucleotide identity and order because it is a key determinant of the function of RNAs. The most well-known RNA molecule made this way is the messenger RNA (mRNA): it is the key player in the translation (or encoding) of proteins. During this process, which occurs in ribosomes, the sequence of mRNA molecules is read in triplets of nucleotides, known as codons, and this specifies which amino acid we add next during protein synthesis (Fig. 4.1). The recent discovery of a dizzying array of noncoding RNA molecules has added an unexpected level of complexity: the RNA world is much more than mRNAs [3]. We are only starting to understand how these noncoding RNAs carry cellular functions by themselves, without requiring translation into proteins [4].

Genome

A genome represents an organism's complete DNA sequence. The Human Genome Project, an international effort to sequence the entire human genome (6 billion nucleotides), was completed in 2001 after more than 10 years of work and billions in research funding [5]. DNA from 13 anonymous individuals of European descent was used to build what is now referred to as the human ref-

1st base	2nd base								3rd base
	T		C		A		G		
T	TTT	(Phe/F) Phenylalanine	TCT	(Ser/S) Serine	TAT	(Tyr/Y) Tyrosine	TGT	(Cys/C) Cysteine	T
	TTC		TCC		TAC		TGC		C
	TTA	(Leu/L) Leucine	TCA		TAA	(X) STOP	TGA	(X) STOP	A
	TTG		TCG		TAG		TGG	(Trp/W) Tryptophan	G
C	CTT		CCT	(Pro/P) Proline	CAT	(His/H) Histidine	CGT	(Arg/R) Arginine	T
	CTC		CCC		CAC		CGC		C
	CTA		CCA		CAA	(Gln/Q) Glutamine	CGA		A
	CTG		CCG		CAG		CGG		G
A	ATT	(Ile/I) Isoleucine	ACT	(Thr/T) Threonine	AAT	(Asn/N) Aspargine	AGT	(Ser/S) Serine	T
	ATC		ACC		AAC		AGC		C
	ATA		ACA		AAA	(Lys/K) Lysine	AGA	(Arg/R) Arginine	A
	ATG	(Met/M) Methionine	ACG		AAG		AGG		G
G	GTT	(Val/V) Valine	GCT	(Ala/A) Alanine	GAT	(Asp/D) Aspartic Acid	GGT	(Gly/G) Glycine	T
	GTC		GCC		GAC		GGC		C
	GTA		GCA		GAA	(Glu/E) Glutamic Acid	GGA		A
	GTG		GCG		GAG		GGG		G

Amino acid properties: Nonpolar | Polar | Basic | Acidic | Nonsense

Fig. 4.1 DNA codons and associated amino acids. The three nucleotides (codons) that make up the 20 different amino acids (and stop signal) are presented, along with the single-letter database codes. To obtain the various codons, one simply needs to integrate the value of the first (left), second (top) and third (right) bases. To obtain the RNA codons, Key properties of amino acids are indicated in the color legend at the bottom. (Figure credits: Modified from Wikipedia, https://en.wikipedia.org/wiki/DNA_and_RNA_codon_tables#Translation_table_1)

erence genome. In principle, the human reference genome should be a repository containing a representative whole-genome sequence for a prototypical human. After the publication of the draft sequence in 2001 [6], a high-quality reference sequence followed in 2004 [7]; the Genome Reference Consortium continues to improve the quality and coverage of low-complexity, repetitive, and hard to resolve regions [8].

While the exact number of genes remains unknown, most experts estimate that there are ~20,000 protein-coding genes that occupy about 1% of the human genome. Other constituents of the genome include RNA genes, regulatory sequences, and repetitive DNA sequences. Ongoing research by the Encyclopedia of DNA Elements (ENCODE) project suggests that ~80% of the human genome is functionally active: non-protein-coding DNA must be implicated in a significant number of regulatory processes including gene-gene regulation, gene-protein interactions and the transcription of nontranslated RNA [9].

Genetic vs. Genomic

It is now common, but incorrect, to use the terms "Genetic" and "Genomic" as synonyms. The former refers to investigations restricted to a small number of genes at once, while the latter applies to tests that involve genome-wide interrogation.

Other Omics

Many other "-omics" sciences are actively developed in parallel to genomics, such as proteomics [10], transcriptomics [11], epigenomics [12], and metabolomics [13]. New forms of -omics are still emerging: for example, epitranscriptomics, which is the study of mechanisms that regulate RNA functions [14], is relevant to novel forms of nephrotic syndrome [15, 16]. The new frontier is to study one or more omics patterns at single-cell resolution [17]: it is widely anticipated that it is a key development to better understand the pathophysiology of many kidney diseases [18]. Eventually, these methods will be combined to provide holistic insights into organ or cell functions [19]. It is, however, beyond the scope of this chapter to cover these methods in any detail.

Online Resources

Sources	URLs
MIT Pedigree	https://peds-renomics.clinic/MIT-pedigree
HapMap	http://hapmap.ncbi.nlm.nih.gov
Broad Institute's Primer on medical and population genetics	https://peds-renomics.clinic/BI-Primer-Genetics

Variations in the Genome

Several different classes of DNA sequence variations are observed when comparing the genomes of different individuals. A variation may also be classified as a mutation, which is defined as a change of the nucleotide sequence when compared to the human reference genome; a mutation is not necessarily pathogenic. These alterations may be caused by a number of mechanisms, including unrepaired DNA damage (caused by mutagens such as radiation or chemicals), DNA polymerase errors during replication, or insertion or deletion of DNA segments by mobile genetic elements. Below, we will describe the different kinds of mutations after a short discussion on their key characteristics. Implicit to these discussions is that these mutations are all germline, which means that all cells of an individual carry the mutation, and this mutation is passed on to subsequent generations according to Mendel's law of allele segregation. This contrasts with somatic mutations that are only present in a restricted subset of cells. The concept of somatic mosaicism will not be covered further here: it has relevance well beyond that of cancer genetics [20], most notably in nephrology, for autosomal dominant polycystic kidney disease [21].

How to Assess Genomic Variations

One of the key features that will allow genomic medicine to flourish in the clinic is the ability to reliably assess whether a given mutation is likely to affect protein function or not. When appraising a particular mutation, there are a number of important characteristics to consider systematically before deciding on its pathogenic potential [22]. A recent review reveals that the nephrology literature is replete with spurious gene-disease associations, or "diagnoses of uncertain significance" [23]. Below, we will briefly describe four such characteristics, including frequency, location, mutation type, and conservation. This section introduces many key concepts that will be used frequently throughout this chapter. Please refer to Fig. 4.2 for an illustration of the various concepts.

Is the Allele Common or Rare?

The first consideration is to assess how frequently the mutation is observed in the general population. The minor allele frequency (MAF) is the most useful piece of information for this. It is calculated by dividing the number of mutant alleles in a sample by the number of wild-type alleles (each individual has two alleles since humans are diploid organisms). MAF data for common variants is easily retrieved from online databases, such as dbSNPs. The MAF for rare and common variants should be less than 1% and between 1% and 49%, respectively; the major allele frequency is usually more than 50%. This concept is important because common mutations are expected to have a low probability to cause rare conditions. The frequency of deleterious alleles is expected to remain low in the general population because of reduced reproductive fitness and natural selection.

Coding vs. Non-coding Variants

The second distinction relates to the location of the mutation in the genome: is it in a region that is involved in protein coding or not? Because we have rich knowledge about protein-coding DNA (exons) and their functions, most studies and clinical tests tend to focus on these regions of the genome, collectively known as the exome. "Non-coding" DNA, which is composed of intronic and intergenic segments, does not contain only "junk" DNA as this is where one finds a host of regula-

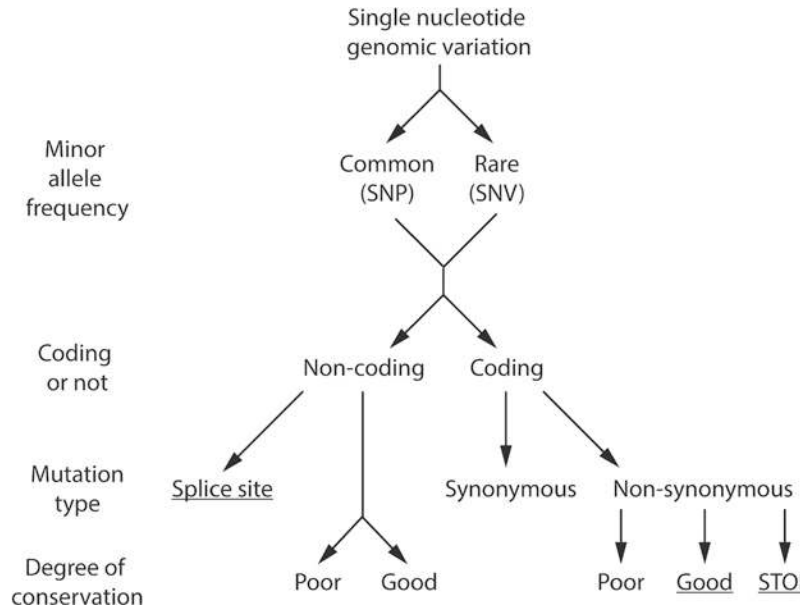

Fig. 4.2 An approach to assess a mutation. We present a simple workflow to assess mutations that include four steps. These are based on allele frequency, mutation location and type and degree of conservation. Underlined terminal nodes indicate the mutations most likely to cause human disease

tory elements such as promoters, enhancers, silencers and insulators, as well as microRNAs. There is mounting evidence that there is in fact no such thing as junk DNA as 80% of the entire genome plays an important role one way or another [24].

There is one type of intronic mutation that has a special status in clinical genetics: splice site mutations that change key nucleotides in one of the specific sites that are critical for splicing introns during the processing of precursor mRNA into mature mRNA. Abolishment of a splicing site results in the retention of introns in mature mRNA molecules and leads to the production of aberrant proteins (for an example relevant to nephrology, see Mele et al. [25]).

Mutation Types

Third, it is important to determine if an exonic mutation will result in the insertion of different amino acids in the encoded protein, in which case it is referred to as a nonsynonymous substitution (see Fig. 4.3). These changes are important in clinical genetics because they are most likely to alter the function of the encoded protein. To determine if a nonsynonymous mutation may be pathologic, one needs to assess if the physicochemical properties of the original amino acid are preserved (size, charge, and polarity) and the degree of amino acid conservation (see next section).

There are three instances where a substitution will be expected to be deleterious without requiring these analyses. First, changing any amino acid to a nonsense (or stop) codon will result in the production of a truncated protein. Second, substituting a start codon (which is always a methionine) for any other amino acid will cause problems because protein translation cannot be initiated properly: no protein is produced. Third, any mutations at a stop codon will also cause problems because the protein made will be longer than the wild type, and as a result, it will include extra amino acids that may interfere with the proper function of the protein.

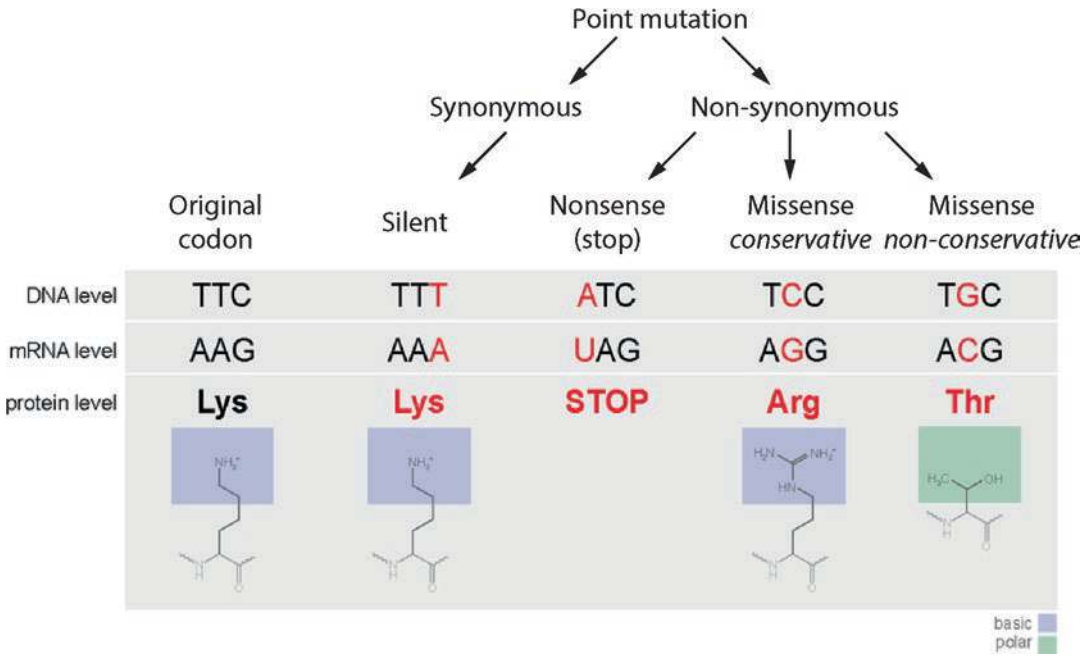

Fig. 4.3 Different types of point mutations. This figure illustrates how a single point mutation in a given codon can change the encoded amino acid in many ways. A conservative missense mutation is one that keeps some or all of the original amino acid properties intact. (Figure credits: Modified from Wikipedia, https://en.wikipedia.org/wiki/Synonymous_substitution)

Mutations that do not result in an amino acid change are referred to as synonymous variants (this is possible because many amino acids are generated from different codons; see Fig. 4.1). They are also known as silent mutations since in most instances (but not always [26]), they have little to no effect on the function of the encoded proteins.

Degree of Conservation

Finally, one needs to determine if the mutation occurs at a genomic position that is highly conserved when compared to the genomes of other species. It is possible to make this comparison rapidly since we have the entire genomic sequences of many different species (e.g., from humans to mice, frogs, and fruit flies). This analysis is most meaningful for coding segments because one can directly compare the amino acid sequences of the same protein in different organisms (orthologs) [27].

Such comparisons support the notion that the degree of interspecies amino acid variation is indirectly proportional to the functional importance of the amino acid. For example, key residues within the catalytic domain of protein kinases are conserved as a functional unit among all kinases of any species [28]. Kinases are enzymes that transfer a phosphate group from ATP to tyrosine, serine, or threonine residues on target proteins. Other approaches are needed to assess mutations found in noncoding regions because nearly all positions are poorly conserved across species [29].

Single Nucleotide Polymorphisms

Strictly speaking, an SNP is a single base substitution at a specific genomic locus which makes this position different from that of the Human Reference Genome [30]. However, common usage has SNPs also defined as a "common variant" on the basis of a population minor allele frequency of >1%. Single nucleotide mutations may occur anywhere in the genome. That the bulk of SNPs are located in noncoding segments is hardly surprising since they constitute ~99% of the genome. As discussed above, the vast majority of SNPs have long been thought to be benign because they are so common. However, the lack of clear clinical significance does not mean that SNPs are useless far from it.

Over the years, geneticists have long used a variety of genetic markers to study human genetic variation with greater depth and accuracy. SNPs emerged as the genetic marker of choice around 2000. It was preceded by three other "generations" of markers: restriction length fragment polymorphisms (~1980), variable number of tandem repeats (~1985), and short tandem repeat polymorphism (~1989) [31]. The rise of SNPs as markers is in large part due to the momentum created by the HapMap project [32], which aimed to catalogue millions of SNPs in hundreds of subjects from many ethnicities [33]. This resulted in marked improvements in the reliability, efficiency, and cost of high-throughput SNP genotyping (see the section below).

SNPs proved to be invaluable to study haplotype patterns in humans. Haplotypes are genomic segments on a chromosome that are defined by a combination of alleles that are consistently inherited together. This phenomenon is termed linkage disequilibrium (LD) [34] since these alleles are linked to each other more often than not because recombination events occur outside of the LD block. Because of the low diversity of genetic variation within LD blocks, genotyping a subset of SNPs provides a fingerprint of the underlying haplotype without having to genotype all alleles: these are known as "tag SNPs"[35]. The concepts described here will be useful for the sections on linkage analysis, homozygosity mapping, and genome-wide associations.

Single Nucleotide Variants

Single nucleotide variants (SNV) are often defined as alleles with a minor allele frequency of <1% in the general population. SNVs are found throughout the genome, both in noncoding (introns, intergenic segments) and coding (exons)

regions. As described earlier, coding SNVs exist in two "flavours": synonymous and nonsynonymous variants. Since there is almost no selective pressure on synonymous variants, they are expected to be scattered anywhere in the genome. In contrast, nonsynonymous variants are often enriched in well-conserved amino acid positions owing to the strong selective pressure they sustain. As a result, it is not surprising that highly penetrant disease-causing variants in monogenic conditions are overwhelmingly nonsynonymous SNVs. It is commonplace to refer to disease-causing SNVs as pathogenic mutations [36]: this topic will be covered in the section on Mendelian conditions.

When dealing with a rare condition, one needs to determine whether the mutation is truly rare (or novel) by interrogating a variety of publicly available variant databases such as dbSNPs [37]. Most of these resources have been generated using samples from subjects of European descent. As a result, interpretation of variants from patients that are not "European" is problematic because usually, one does not have access to the optimal control group. This phenomenon, called population stratification, makes the search for pathogenic mutations more challenging because variants that are common in one population may be rare (or even absent) in another [38]. Hence, the validity of the interpretation of pathogenicity for a given variant is deeply impacted by the patient's ethnic background. The magnitude of this problem will be lessened as more non-European subjects are sequenced through various efforts worldwide [39].

An additional layer of complexity comes from the fact that reported ethnicity is notoriously unreliable [40]. This is particularly true in localities where multiethnic families are common: the genetic makeup of a patient may be much more complex than predicted based on recent family history [41]. For example, some self-identified "Europeans" have substantial genomic portions that are derived from African ancestors, whereas 99% of the genome of some self-identified "African Americans" is of European origin [42]. To circumvent this problem, researchers use unbiased measures of ethnicity that rely on the SNPs genotyping data (such as principal component analysis) [43]. This approach is, however, not routinely used by clinical diagnostic laboratories to draw conclusions about the presence or absence of pathogenic mutations.

Structural Variations

We define structural variation as any genomic change that implicates more than a single base substitution at once. There are typically 5000–10,000 structural differences when comparing the genomes of two unrelated, apparently healthy individuals [44]. dbVar is a database equivalent to dbSNPs, but for variants >50 bp in length [45]. Below, we will briefly describe the most common types of structural variations while also providing, whenever possible, examples from the renal literature. We are only starting to grasp the magnitude, complexity, and ramifications of structural variations in human biology and diseases [46]. We still believe it is worthwhile for pediatric nephrologists to understand these topics since they will undoubtedly become "household" terminology in the clinic soon.

Insertions and Deletions (Indels)

There are two major types of structural variations that involve short genomic segments that are less than 1000 base pairs. These include situations where nucleotides are added or removed, which are respectively, known as insertions and deletions. Structural variants that affect more than 1000 base pairs are known as copy number variations (discussed in the next section).

Some events involve very short segments that are located within an exon. Whether such an alteration is deleterious for proteins depends largely on the number of nucleotides involved and the position of the alteration relative to the coding sequence. Any change that disrupts the normal sequence of bases is likely deleterious (it is key to remember that codons always include three bases) [47]. For example, any insertion that is not a multiple of three in terms of base number (for example, insertion of a single A) will dramatically change the interpretation of the mRNA

sequence: its reading frame will be shifted, and the protein produced, truncated (Fig. 4.4a). In contrast, after insertion of three bases (for example, insertion of AGGACG), it simply adds a few extra amino acids to the protein (in this case, arginine "AGG" and threonine "ACG"). This type of alteration is usually benign because if it does not alter the way the mRNA sequence is "read" after the insertion (i.e., the amino acid sequence of the rest of the protein is unchanged).

Frameshift mutations are commonly found in genes known to cause Mendelian conditions affecting the kidney. Insertion of a single cystine residue in the gene mucin1 (*MUC1*) was recently found in multiple unrelated families with medullary cystic kidney disease type 1 using a unique combination of sequencing and bioinformatic techniques [48]. What is fascinating about this story is that while multiple linkage studies pointed to a small genomic segment on chromosome 1 (where *MUC1* is located) [50], the disease-causing mutations remained elusive even to next-generation sequencing approaches. Indeed, for all families, the C insertion lies in an exon that is enriched in C residues, thereby making it very difficult to distinguish *bone fide* mutations from sequencing errors [48] (see Fig. 4.4b).

For structural events involving slightly larger segments (for example, deletion of two exons), the functional consequences are related to either the absence of an important domain (e.g., these exons contain a kinase domain) or an aberrant

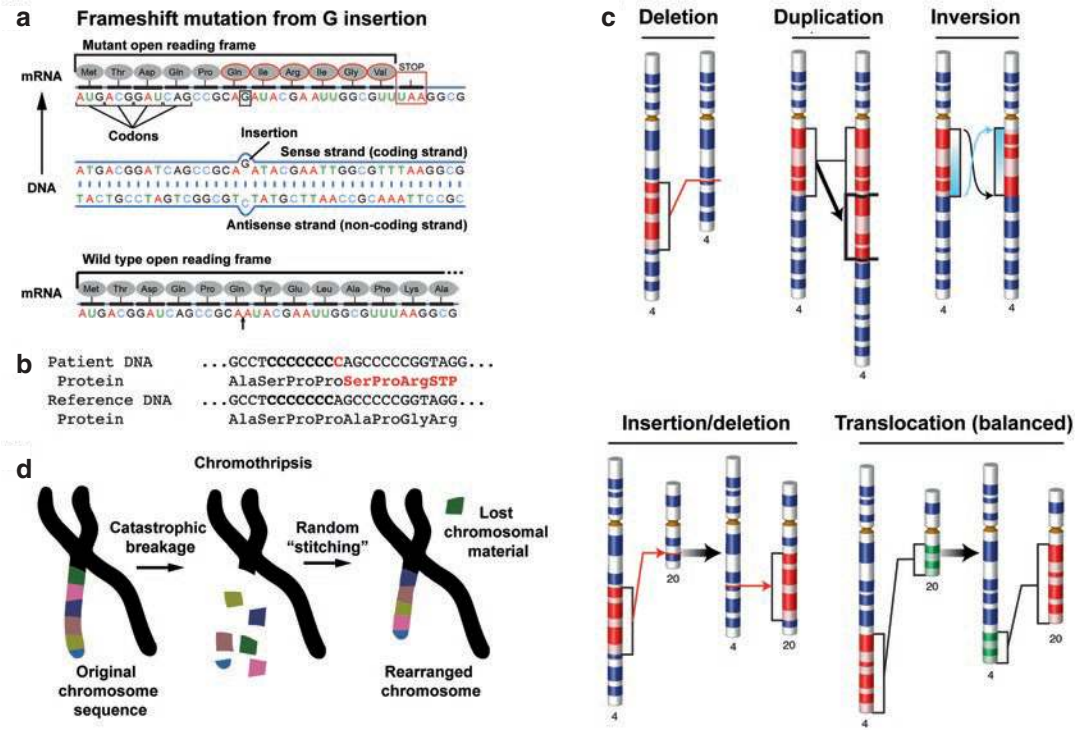

Fig. 4.4 Structural variations. Illustration of various types of structural variations. (**a**) Illustration of the impact of insertion of a single base in a coding exon. The shift in the reading frame causes a major change in the amino acid sequence. The encoded protein is truncated because a new stop codon is created by the frameshift. (**b**) Example of a C insertion (red) in a genomic segment that is highly enriched in cystine (bold). The impact on the amino acid sequence is presented below the DNA sequences. (**c**) Examples of large-scale structural changes. (**d**) This schematic presents the model by which the process of chromothripsis is explained. (Figure credits: Modified from (**a**, **c**) NHGRI digital image database. (**b**) Modified from Kirby et al. [48]. (**d**) Inspired from Fig. 1 in Tubio et al., 2011 [49])

tertiary protein structure (e.g., direct linking of the flanking exons results in a misfolded protein). These types of events are relevant to many conditions managed by nephrologists. For example, small deletions in *COL4A5* are described in patients with Alport syndrome [51], and a large duplication of the complement factor H-related protein 5 gene (*CFHR5*) gene is described in many patients with *C3* glomerulopathy [52].

Copy-Number Variations

Large-scale insertions or deletions are collectively referred to as copy-number variants (CNV; see Fig. 4.4c). They are detected using the same microarray-based technologies used for GWAS studies [53]. Most CNVs are predicted to be benign since the majority are common in the general population. Large hemizygous or homozygous deletions, especially when rare and leading to abrogation of a single gene, are the simplest CNVs to interpret phenotypically. Indeed, this copy number loss is equivalent to a gene knockout. Studies on patients with a large deletion of the X chromosome led to the discovery of *CLCN5* as the first molecular mechanism causing Dent's disease in an affected female with nonrandom X-inactivation [54]. Similar events were critical to the identification of *COL4A4* and *NPHP1* as the disease-causing genes for Alport syndrome [55] and nephronophthisis [56], respectively. Some syndromes associated with large chromosomal defects, such as Turner syndrome (loss of the X chromosome [57]) or Down syndrome (gain of chromosome 21 [58]), can also be associated with renal defects.

It is not straightforward to predict the functional impact of other types of CNVs, even those strongly associated with human diseases. In most instances, gene dosage is thought to be reflected proportionally in the levels of gene expression [59]. For example, mRNA and protein expression is higher when there is a copy number gain; important, the amino acid sequence of this protein is unchanged. The most prominent example of this in the renal literature comes from studies on the genetic basis of lupus nephritis: SLE patients with low copy numbers (0 or 1 per chromosome) of the gene encoding Fc receptor for IgG (*FCGR3B*) were more likely to develop kidney disease than those who had more than 1 [60]. It was recently shown that patients with congenital kidney malformations are much more likely to harbour large and rare heterozygous CNVs in any region of the genome when compared to unaffected controls [61]. Tumour-specific copy number gain and loss have been observed in clear cell renal cell carcinoma, particularly when there is no germline mutation in the gene VHL [62].

Copy-Neutral Variations

Copy-neutral variation, defined as genomic alterations that do not affect the overall number of copies of genes, includes inversion and translocation (see Fig. 4.4c). These may cause the disease in several ways. The function of genes may be abnormal if the inversion/translocation is accompanied by random loss of parts of the rearranged genomic segment that play an important role (i.e., it is "unbalanced"). Balanced copy-neutral variations can also be pathogenic. Indeed, if the boundaries of the structural change occur in the middle of a gene, it may disrupt the normal transcription of that gene (since contiguous exons are now far apart). Even if gene integrity is preserved, aberrant gene expression may be observed because of interference with the function of regulatory elements such as promoters, enhancers, silencers, insulators [63].

There are only a few examples of patients with renal conditions caused by balanced copy-neutral variations [64–66]. A unique type of disease-causing copy-neutral variation was also found by exome sequencing in a rare form of pheochromocytoma: the affected patient had homozygous *MAX* mutations because of uniparental disomy (a process by which two copies of a chromosome come from a single parent) [67]. One explanation for the apparent rarity of copy-neutral variants as the cause of disease is that commonly used genomic techniques are unable to detect these types of rearrangements. Thus, clinicians who have patients with a clear phenotype but without mutations in the known gene(s) should consider asking for copy-neutral variation testing.

Chromothripsis

Recent advances in DNA sequencing and bioinformatics now allow interrogation of genomes at an unprecedented resolution: it was instrumental in the discovery of yet another type of structural variation, namely, chromothripsis (which means chromosomes shattered to pieces). Chromothripsis is suspected when a chromosome is found to harbour two or more complex structural rearrangements (see Fig. 4.4d). This phenomenon was first described as a type of somatic structural variation present only in cancer cells [68]. Many similar reports followed shortly thereafter, suggesting that this mechanism is common in tumours [69]. The current working model states that chromothripsis arises from a single catastrophic event causing shattering of one or more chromosomes followed by the formation of a chromosome-like structure via random stitching of the fragments [70]. Chromothripsis was recently described in renal cell carcinomas [71].

Of utmost interest for pediatricians is the fact that a chromothripsis-like phenomenon has been reported as *a de novo* germline variation in patients with congenital diseases [72, 73]. Recent evidence suggests that the underlying mechanism is analogous to that described for cancer cells; the main difference being that the shattering process is much more circumscribed in so-called "constitutional chromothripsis"[74]. Chromothripsis was recently described as potentially causal in a child with multiple congenital anomalies, including vesicoureteral reflux [75]. However, there is still plenty to learn about the biological relevance of this process. Indeed, genetic investigations of a child with congenital anomalies ultimately uncovered parentally transmitted germline chromothripsis that was unlikely to be causal: besides his mother, 9 healthy carriers were found in this three-generation kindred [76].

Haploinsufficiency

Haploinsufficiency refers to a situation where adequate gene expression to preserve normal cell function requires the presence of two wild-type alleles being actively transcribed (diploid organisms have two copies of all autosomal genes) [77]. Disease occurs when one of the two alleles is absent or dysfunctional owing to a deletion event or a deleterious mutation, respectively [78]. Such conditions may arise *de novo* or via inheritance from an affected parent. Given their extreme dependence on gene dosage, it remains unclear why negative selection has not resulted in the disappearance of haploinsufficient genes [79].

There are several conditions that affect the kidneys that are driven by abnormalities in haploinsufficient genes. The most common is RCAD syndrome (renal cysts and diabetes) syndrome, a condition caused by either a heterozygous mutation in HNF1B or by the 17q12 microdeletion syndrome (this segment includes HNF1B) [80]. RCAD syndrome is characterized by its striking phenotypic variability among affected individuals (even between first-degree relatives with the same genotype) [81]. Recent data suggest that this phenomenon may be due to epigenetic modifications in many genes that occur in response to the haploinsufficiency state [82]. Other examples of haploinsufficient conditions that pediatric nephrologists should be aware of include SON deficiency syndrome [83] and various forms of CAKUT [84–86].

Online Resources

Sources	URLs
dbSNPs	http://www.ncbi.nlm.nih.gov/SNPs/
HapMap	http://hapmap.ncbi.nlm.nih.gov
dbVar	http://www.ncbi.nlm.nih.gov/dbvar/

Modes of Inheritance

An allele is one of a number of copies of the same gene or locus. Every person has two copies of every gene on autosomal chromosomes, one inherited from the mother and one inherited from the father. The occurrence of two copies of the same allele results in a homozygous genotype for that allele, whereas the presence of two different alleles results in a heterozygous genotype for each allele. How recessive or dominant genotype

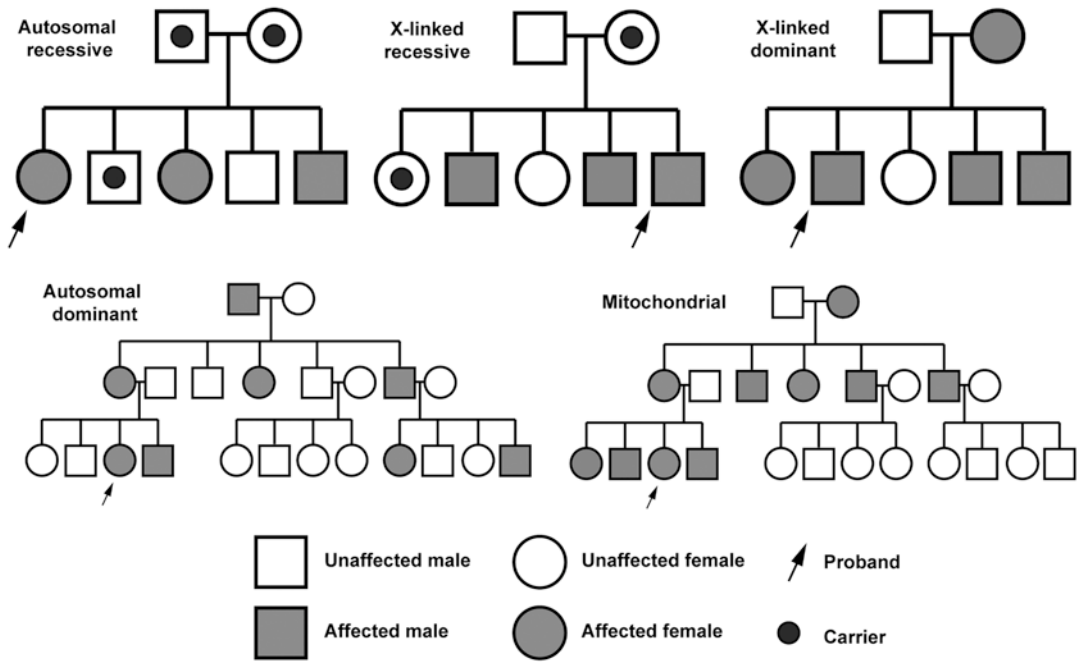

Fig. 4.5 Examples of pedigrees for most common types of inheritance. Examples of classic pedigrees for kindreds with patterns of inheritance consistent with autosomal recessive, autosomal dominant, X-linked recessive, X-linked dominant and mitochondrial

interactions can influence the expression of the characteristic traits of the underlying genetic variation are described in the following sections. Please see Fig. 4.5 for pedigrees displaying the typical inheritance patterns, and the sections below for clinical examples.

Dominant Genotypes

Dominant genotypes are seen when the heterozygous genotype is associated with phenotypic expression. Dominant genotypes arise when one allele dominates by masking the phenotypic expression of the other allele at the same genetic locus. For example, where a gene exists in two allelic forms (designated A and B), a combination of three different genotypes is possible: AA, AB, and BB. If AA and BB individuals (homozygotes) show different forms of some trait (phenotypes), and AB individuals (heterozygotes) show the same phenotype as AA individuals, then the allele A is said to dominate or be dominant to or show dominance to allele B, and B is said to be recessive to A. If instead, AB has the same phenotype as BB, B is said to be dominant to A. There are many autosomal dominant conditions that are relevant to pediatric nephrologists, such as autosomal dominant polycystic kidney disease (ADPKD) [87], tuberous sclerosis complex [88], or renal cyst and diabetes (RCAD) syndrome [80].

Recessive Genotypes

Recessive genotypes are seen when the homozygous genotype and not the heterozygous genotype are associated with phenotypic expression. Thus, both parents have to be carriers of a recessive trait in order for a child to express that trait. If both parents are carriers, there is a 25% chance for each child to show the recessive trait in the phenotype. Thus, if the parents are closely related (consanguineous), the probability of both having inherited the same allele is increased and as a result, the probability of children showing the

recessive trait is increased as well. Most pediatric nephrologists will regularly manage patients with autosomal recessive conditions that affect the kidneys, including cystinosis [89], autosomal recessive polycystic kidney disease [90], Bartter syndrome [91], or pseudohypoaldosteronism type 1 [92].

X-Linked Inheritance

X-linked inheritance means that the gene causing the trait, or the disorder is located on the X chromosome. Females have two X chromosomes, while males have one X and one Y chromosome. X-linked recessive (XLR) inheritance is a mode of inheritance in which a mutation in a gene on the X chromosome causes the phenotype to be expressed (1) in males (who are necessarily hemizygous for the gene mutation because they have only one X chromosome) and (2) in females who are homozygous for the gene mutation (i.e., they have a copy of the gene mutation on each of their two X chromosomes). Carrier females who have only one copy of the mutation do not usually express the phenotype, although differences in X chromosome inactivation can lead to varying degrees of clinical expression in carrier females since some cells will express one X allele and some will express the other.

In contrast, X-linked dominant (XLD) conditions occur when a single mutated X-linked allele is responsible for the manifestations of the disorder: as such, both males and females are usually affected. The exact pattern of inheritance varies, depending on whether the father or the mother has the trait of interest. All daughters, but no son of an affected father, will have the same condition. In addition, the mother of an affected son would be expected to be affected. Examples of X-linked dominant conditions that primarily affect the kidneys include nephrogenic diabetes insipidus [93], and Dent Disease [94]. X-linked Alport syndrome can be either dominant or recessive [95]. X-linked hypophosphatemia is a rare X-linked recessive condition caused by mutations in *PHEX* [96].

Mitochondrial

Inheritance from a single parent can give rise to disease (uniparental isodisomy). Some renal phenotypes arise solely by maternal inheritance and are characterized by mitochondrial dysfunction. Mitochondrial DNA (mtDNA) is derived from the mother because it exists in much higher concentrations in ova compared to sperm. Furthermore, sperm mtDNA tends to get degraded in fertilized ova and sperm mtDNA fails to enter the ovum in several organisms. Most patients with mitochondrial cytopathies will present with evidence of proximal tubular dysfunction; it can also be associated with a distal tubulopathy, or even a glomerulopathy [97].

Genetic/Genomic Methods

SNPs Genotyping

Because of the unique properties of SNPs, there has been considerable interest in developing high-throughput technologies that would allow simultaneous testing of a large number of SNPs cheaply and reliably. The starting point is always a solution containing the patient's fragmented DNA. The two most successful approaches apply this solution to a microarray seeded with specific oligonucleotide probes directed against the sequence surrounding the target common variants (with minor allele frequencies of at least 1%).

The first method relies on non-enzymatic hybridization of single-stranded DNA with probes: a perfect match generates a light signal, but a fragment harbouring a variant does not. The second uses DNA polymerase to add one of four fluorescent-labelled nucleotides (A, C, T, or G), thus extending the probe by one base specifically at the SNPs locus. SNPs genotyping was rapidly adopted by investigators as the method of choice to investigate the links between common genetic variants and human disease [98].

Current estimates show that a typical human genome (3 billion base pairs in total) harbours 5

million SNPs: this corresponds to roughly 1 SNP every ~1000 bases. While early versions of these arrays contained <100,000 SNPs scattered throughout the genome, current platforms routinely interrogate 1–2 million SNPs at once (1 SNP every ~2000 bases). This means that we currently have unprecedented precision in our assessment of common genetic variation. As described below, SNPs genotyping has proved useful for many types of investigations, such as assessment of linkage, genome-wide association, ancestral heritage, and copy-number variations. The most recent application of this technology is the generation of polygenic risk scores to estimate the likelihood that a given individual will experience a specific clinical outcome based on genome-wide SNPs data [99]. The polygenic risk profiling tools developed to predict renal outcomes may soon be used in clinical practice [100].

Linkage Analysis

For decades, genetic linkage analysis has proved to be a powerful method to uncover short genomic segments that contain disease-causing genes for Mendelian conditions. The concepts of co-segregation, phase, linkage disequilibrium, and haplotype, which were described above, are central to this analysis (see Fig. 4.6). The goal is to identify nonrandom segregation of disease phenotypes with discrete chromosomal segments [101]. When performed with the current technology, the first step is to obtain dense SNPs geno-

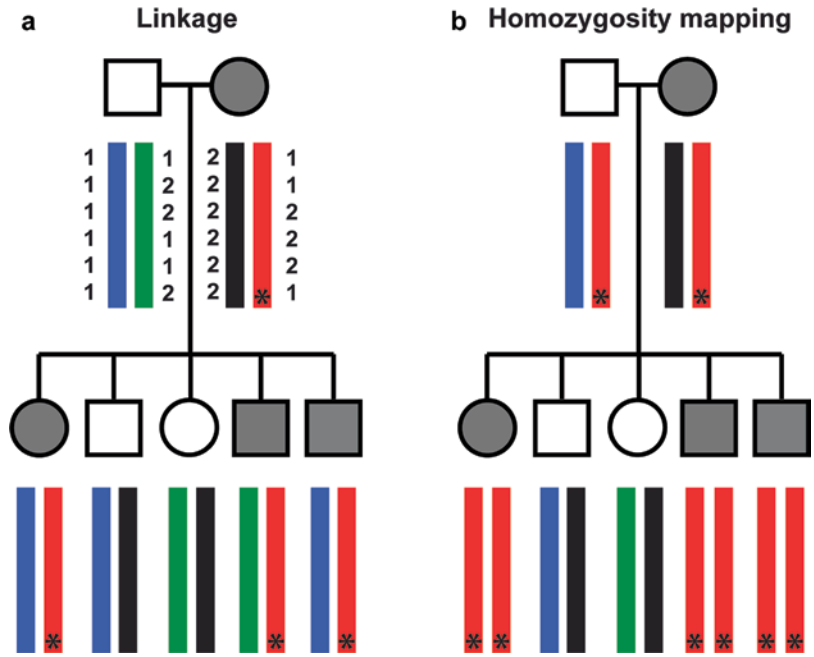

Fig. 4.6 Linkage analysis and homozygosity mapping. (**a**) Illustration of linkage analysis, with the disease haplotype (genomic segment harbouring the disease-causing mutation) in red. If an autosomal dominant pattern of inheritance with complete penetrance is suspected, all (and only) affected individuals should share the pathogenic heterozygous haplotype. Each coloured segment represents different haplotypes because they harbour different sequences of alleles at similar genomic positions (in this case, 6 loci are shown, each with 2 possible alleles, 1 or 2). (**b**) Homozygosity mapping seeks to identify homozygous segments that are present only in affected individuals (red). While a consanguineous union increases the probability of an autosomal recessive condition, outbred parents may share short genomic segments because of very distant common ancestors

typing data for all individuals in the family that provided a blood sample. Genotyping platforms typically record genotypes for millions of common variants (minor allele frequency >1% in the general population) that are scattered throughout the genome. On that basis, this approach is often referred to as a "genome-wide linkage scan."

Next is the identification of all series of contiguous SNPs that travel together (haplotypes) in all affected individuals, but not in healthy relatives (see Fig. 4.6). Using this approach, one can confidently exclude most of the genome and focus efforts on the incriminated genomic segments, which may contain any number of candidate genes (from zero to many). One key concept to understand is that the SNPs used for linkage analysis almost never turns out to be the disease-causing variant *per se* since the common variants on the SNPs genotyping platform cannot be the primary cause for a rare Mendelian condition. These SNPs are called tag SNPs because they effectively flag specific genomic segments (haplotypes) that contain the rare disease-causing variant.

The larger the number of samples from affected and unaffected individuals, the higher the precision of linkage analysis. This approach is thus most fruitful when dealing with a large kindred with multiple affected individuals, and it is not useful when dealing with families with a single affected individual. Obtaining a large number of blood samples from unaffected first-degree relatives is also key as one can exclude a larger number of "healthy" haplotypes. Combining linkage analysis performed on two or more unrelated kindreds with similar phenotypes allows for narrowing down the number and length of the target genomic segments since the disease-causing gene must lie within the segment that is shared between kindreds (if caused by mutations in the same gene).

Up until recently, most genes associated with Mendelian conditions were discovered using linkage analysis. This is true for renal conditions as well. Nowadays, gene discovery projects are done with whole-genome and whole-exome sequencing using analytical procedures that often include linkage analysis (see the section below) [102]. Instead of using common variants as genomic markers for the disease locus, one can directly find the disease-causing mutation (which is expected to be rare or novel since the disease under scrutiny is rare).

Homozygosity Mapping

Genetic analysis of consanguineous families with multiple individuals exhibiting a similar disease phenotype is in theory simpler than for outbred kindreds because of a much higher prior probability that the disease follows an autosomal recessive pattern of inheritance (see Fig. 4.6) [103]. Homozygosity mapping [104], which is the method of choice in this context, is a version of linkage analysis that is streamlined by restricting the playing field to genomic segments that are homozygous only in affected individuals [105]. The entire set of homozygous segments has been termed the "autozygome" because autozygosity refers to homozygosity in the context of consanguinity [106]. This method relies on identity-by-descent because one would expect to find a single founder mutation that originated from a common ancestor. As in traditional linkage analyses, testing of unaffected parents and siblings is critical to make sure that only affected individuals carry recessive genotypes (monogenic disorders typically have complete penetrance).

Ideally, the homozygosity mapping points to a single homozygous genomic segment. Interestingly, homozygosity mapping is at times problematic when investigating highly inbred families because there may be many shared homozygous segments [107]. Alternatively, isolated communities can accumulate distinct pathogenic mutations in the same gene, resulting in the unexpected identification of affected individuals with compound heterozygous mutations despite being the product of a highly consanguineous union. The main example for this exceptional scenario is relevant to pediatric nephrologists since it stems from research on congenital nephrotic syndrome [108, 109]. There is evidence from the renal literature that homozygosity mapping may also be useful to investigate a sub-

ject from an outbred family for whom a recessive pattern of inheritance is suspected [110].

Since most of these conditions are first diagnosed clinically during childhood, pediatricians should know about this approach. The history of kidney disease genetics is replete with examples of successful applications of identity-by-descent methodology to identify novel disease-causing genes. While homozygosity mapping is better known as a research method for gene discovery, it is emerging as a critical tool for clinical genetics as well. It is particularly useful when dealing with patients from consanguineous unions that have a condition with many possible genetic causes [111]. For example, this approach reduced diagnostic costs and streamlined patient care when applied to a large number of patients with a clinical diagnosis of Bardet-Biedl syndrome [112].

Genome-Wide Association Studies

In many ways, a genome-wide association study (GWAS) is very similar to a linkage study. Indeed, both test to see if there is a statistical association between any of 1–2 million SNPs and a particular trait that may be recorded as a variable that is either continuous (systolic blood pressure, in mmHg) or dichotomous (hypertension: yes or no) [113]. There are two major differences between these study designs [114]. First, while the focus of linkage studies is on families with affected and unaffected individuals, GWAS methodology actively prohibits the inclusion of close relatives in the same study to avoid bias. Second, linkage and GWAS studies use the same set of tag SNPs to flag haplotypes harbouring rare disease-causing mutations or common risk alleles, respectively.

Anyone reading an article reporting on the results of a GWAS for the first time is usually struck by the extremely low p-values required to identify valid associations: the typical genome-wide significance threshold for GWAS studies is 5×10^{-8}. Such low p-values are necessary to minimize the chances of reporting false-positive associations, which become increasingly common as the number of tests performed increases [115]. While on a different scale, this concept should be familiar to physicians ordering lab tests. The new p-value threshold is simple to calculate: 0.05 divided by the number of tests performed (in this case 1×10^{-6} SNPs). When reading an article, it is good practice for physicians to systematically calculate the number of tests performed and independently calculate the corrected p-value threshold, if applicable.

Flag SNPs with p values below the set threshold identify target haplotypes, each of which usually contains many genes. It is important to realize that a GWAS does not make it possible to pinpoint which of the genes contained in the haplotype is the culprit. This is critical because it influences the way GWAS results are reported in the literature. Early on, the tradition was to name a significant haplotype after one of the genes it contained, often based on the authors' best guess of pathogenic potential. This approach has backfired in the past because significant resources were allocated to the incorrect gene (see Box 4.1 for an exemplar taken from annals of Nephrology research history). Because of this problem, the authors now make it clear that the genes associated with haplotypes are merely signposts [116].

Box 4.1: The MYH9 vs. APOL1 Story

One of the best examples of an educated guess that misfired is from the nephrology literature. Simultaneous reports from two independent teams found a very strong genome-wide association between the haplotype containing the gene encoding myosin heavy chain 9 (*MYH9*) and glomerular disease in African-American adult patients [117, 118]. This was a reasonable guess given that many associated SNPs were clustered near or within *MYH9*, and given that autosomal dominant mutations in *MYH9* cause diseases with a complex, multisystemic phenotype that include an Alport-like glomerulonephritis that often leads to end-stage renal disease [119]. However, sequencing of all *MYH9* exons did not reveal the underlying risk allele(s).

Taking a deeper look at similar cohorts, a third team finally solved the riddle: the associated risk alleles were in a distinct gene named *APOL1*, located on the same haplotype [120]. These renal risk alleles are common in African Americans because they also confer a positive advantage via more efficient *APOL1*-dependent killing of trypanosome parasites. A prospective study later confirmed that African-American individuals harbouring the *APOL1* alleles do indeed have a higher risk of progression to ESKD or CKD over time [121]. Despite tremendous efforts to better understand the relationship between *APOL1* variants and kidney disease, the pathophysiology remains elusive [122, 123].

Polymerase Chain Reaction

Polymerase Chain Reaction (PCR) is the method of choice to amplify specific DNA segments. Most current DNA sequencing technologies depend on PCR amplification to generate a reliable signal that is translated into the DNA sequence itself through various ingenious means (see Fig. 4.7). PCR takes advantage of a DNA polymerase enzyme that is highly resistant to heat such that it is still functional following multiple rounds of heating/cooling. Heat is necessary to break the double-stranded DNA into single strands that can then be copied by the DNA polymerase using the supplied deoxynucleotide (dNTPs). The reaction also requires primer pairs that are synthesized in the laboratory: these are made out of a series of ~20 nucleotides that

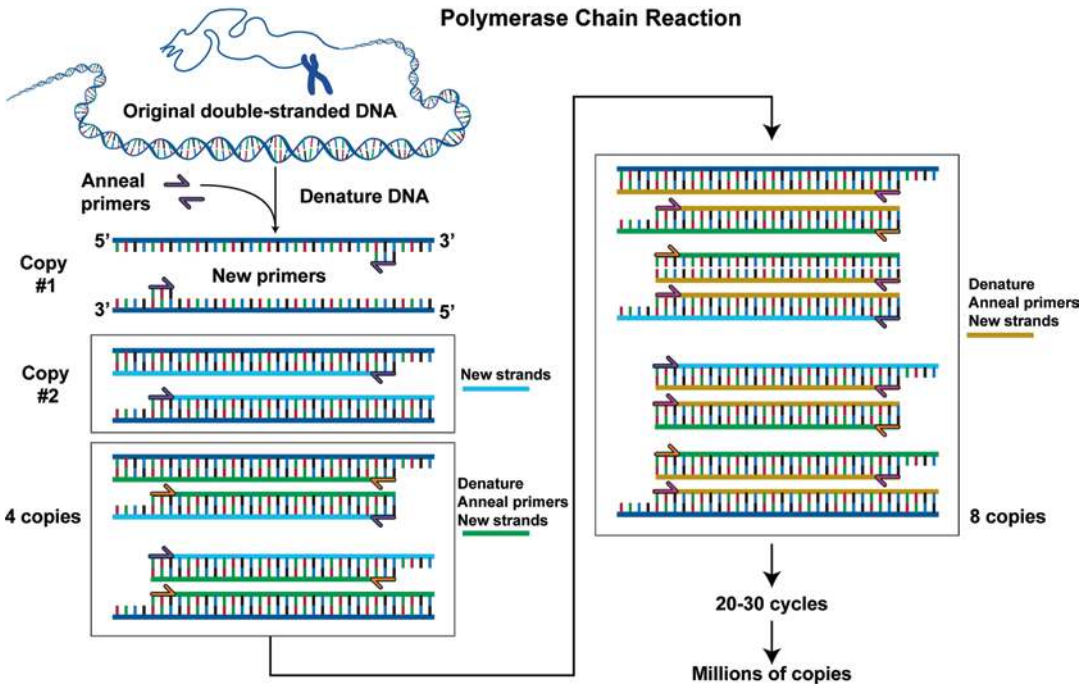

Fig. 4.7 Polymerase chain reaction. Illustration of the amplification process that occurs when DNA is subjected to PCR. Specific oligonucleotide primers, heat-resistant DNA polymerase and unlabelled nucleotides are added to the solution containing the starting DNA. The mix is subjected to heat to denature DNA. Once the temperature is reduced, the primers anneal to single-stranded DNA segments and new strands are synthesized by DNA polymerase enzyme. The first cycle generates two new strands, the second cycle four new strands. Ultimately, millions of copies of this segment are generated after 20–30 cycles. (Figure credits: Modified from NHGRI Image Gallery, https://www.genome.gov/genetics-glossary/Polymerase-Chain-Reaction)

match a very specific region of the genome. Now that the human reference genome is well established, it is simple to design primer pairs located around the target genomic segment of interest that is no more than 1000 base pairs in length.

The main drawback of PCR-based methods is that the DNA polymerase sometimes inserts the wrong nucleotide at a given position during the copying process. When one such amplified segment is subjected to DNA sequencing, one could be mistaken to think that it is a mutation when in fact there is none in the original DNA. It is thus important to validate all mutations deemed "pathologic" via PCR reamplification of a fresh sample of DNA followed by Sanger sequencing (method discussed below). The reason this step is useful is that most of these errors occur at random, which means that the same mistake is very unlikely to be observed at the same locus in a separate experiment.

DNA Sequencing Technologies

DNA sequencing determines the precise order of the four bases adenine, guanine, cytosine, and thymidine in a single strand of DNA. Sequences of individual genes or clusters of genes, full chromosomes, or the whole genome have greatly facilitated our understanding of the basic biological mechanisms of human disease. DNA sequencing was first developed in the 1970s [124] and a rapid sequencing method using the 'chain termination Method' was developed by Sanger in 1977 [125].

Sanger Method

DNA replication requires the presence of a single strand of DNA template, dNTPs, DNA polymerase, and DNA primers. Under normal conditions, the 3′-OH terminus of the dNTPs facilitates the formation of a phosphodiesterase bond between two nucleotides catalysed by the enzyme, DNA polymerase. The 'chain termination method' relies on the incorporation of dideoxy NTPs (ddNTPs) lacking a 3′-OH terminus such that the extension of DNA thereby ceases during the replication process (Fig. 4.8). Fluorescent-labelled ddNTPs are employed in automated sequencing methods which rely on wavelength determination to identify the different ddNTPs in a given sequence [126].

Next-Generation Sequencing Methods

Sanger sequencing can be laborious and expensive. A number of new sequencing technologies called next-generation sequencing (NGS) technologies have been developed that have significantly reduced the cost and time required for sequencing [127]. Unlike the Sanger method where a single predefined target is required for each sequencing reaction, NGS platforms allow for the sequencing of many millions of target molecules in parallel (Fig. 4.9). DNA molecules are immobilized on a solid surface and are sequenced in situ by stepwise incorporation of fluorescent-labelled nucleotides or oligonucleotides. "Clusters" of identical DNA are generated by the clonal amplification of template DNA, hence the term 'massive parallel deep sequencing' used to describe NGS. Platforms vary, with some covering fewer genomic regions than others, some are able to detect a greater total number of variants with additional sequencing, while others capture untranslated regions, which are not targeted by other platforms [128].

Massive parallel sequencing has greatly facilitated investigations of variations within the human genome. In January 2008, the 1000-Genome Project was launched with the objective of establishing a detailed catalogue of human genetic variation [129]. Utilizing the genomic sequences of 2500 anonymous participants from a number of different ethnic groups worldwide and using a combination of methods including low-coverage genome sequencing and targeted resequencing of coding regions, the primary goals of this project were to discover SNPs at frequencies of 1% or higher in diverse populations; to uncover rare SNPs with frequencies of 0.1–0.5% in functional gene regions; and to reveal structural variants, such as copy number variants, insertions, and

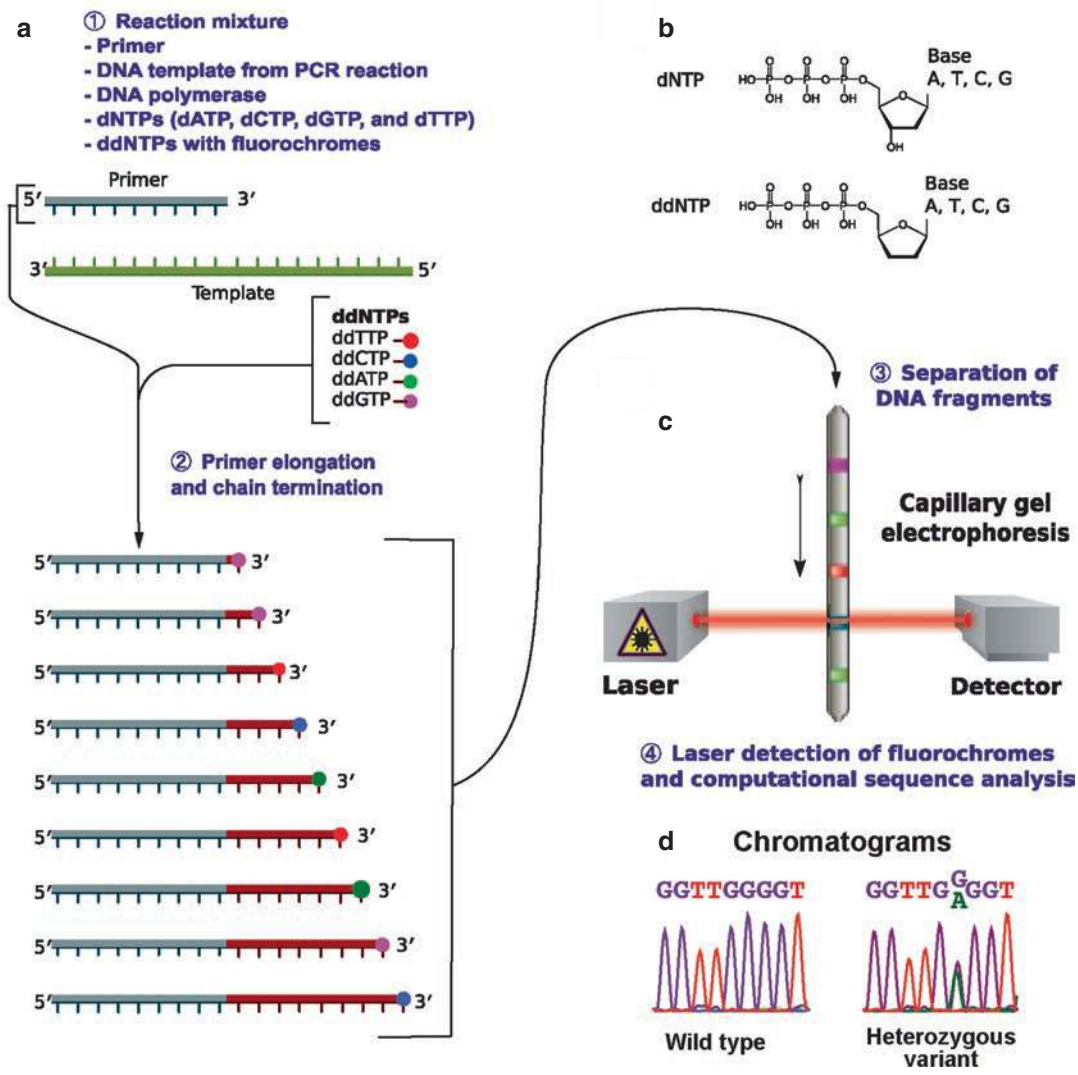

Fig. 4.8 Sanger sequencing with fluorescent-labelled ddNTPs. (**a**) Reagents necessary for Sanger sequencing. (**b**) Illustration of the structural difference between dNTP and ddNTP. (**c**) Capillary gel electrophoresis of elongated fragments and detection of the added fluorochrome-labelled base with laser detection. (**d**) Example of the output in the form of a chromatogram; the same sequence is presented from a subject that is wild-type and another that harbours a heterozygous G to A substitution. (Figure credits: Modified from a file that is licensed under the Creative Commons Attribution Share Alike 3.0 Unported license. The author of the original figure is Wikipedia user "Estevezj". It is available: https://en.wikipedia.org/wiki/Sanger_sequencing)

deletions. The pilot project involving more than 1000 genomes was completed in May 2011 [130]. This resource is publicly available and can be used by researchers to identify variants in regions that are suspected of being associated with the disease. By identifying and cataloguing most of the common genetic variants in the populations studied, this project has generated data that will serve as an invaluable reference for clinical interpretation of genomic variation.

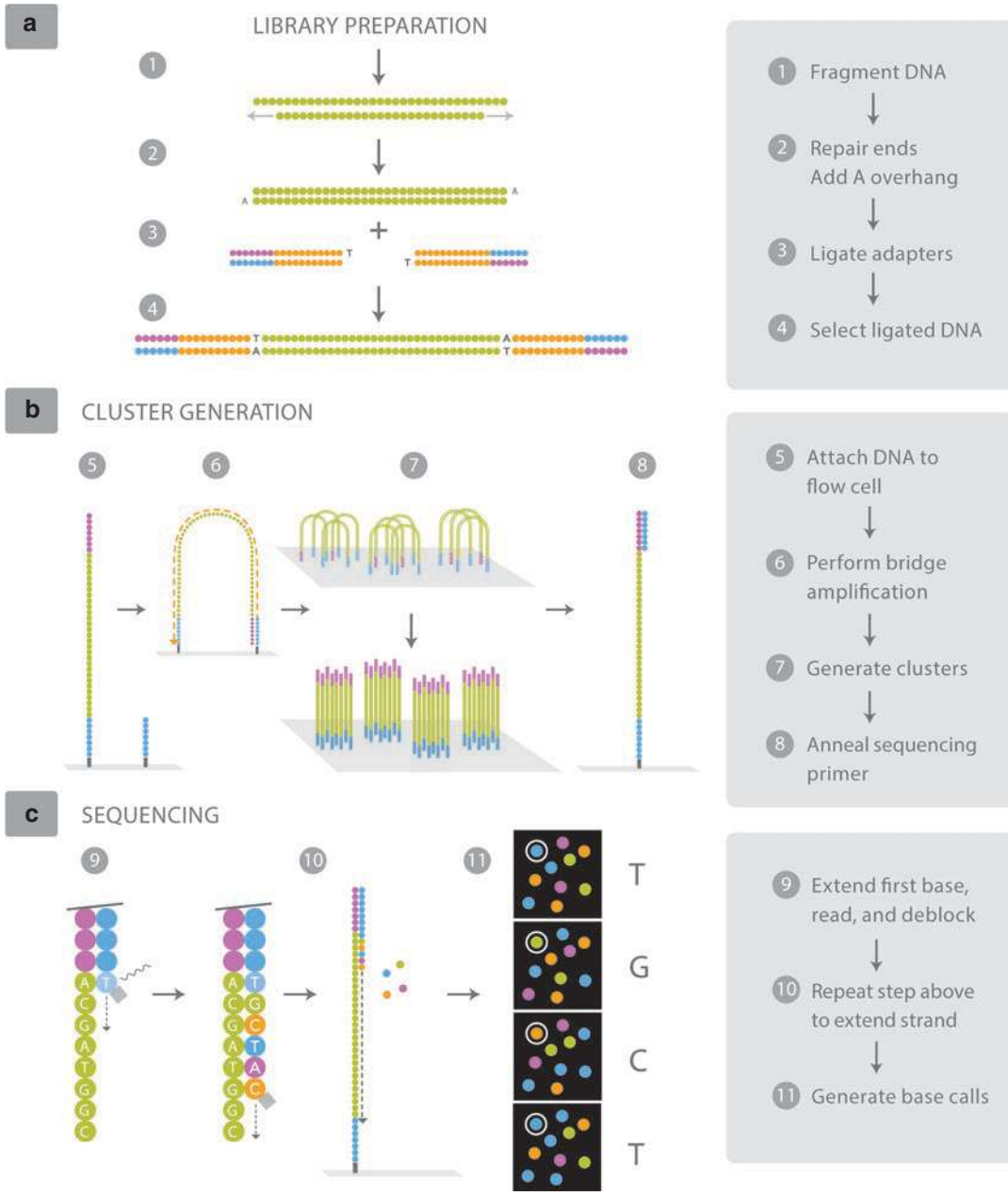

Fig. 4.9 Illumina® sequencing technology overview. This figure illustrates the steps necessary to sequence genomic DNA fragments using the Illumina® sequencing technology. These include (**a**) library preparation, (**b**) cluster generation and (**c**) sequencing. Additional details are provided in the gray boxes. (Figure credits: Image courtesy of Illumina, Inc. All rights reserved)

Third Generation Sequencing Methods

Newer or 'third generation' approaches have emerged that aim to sequence a single DNA molecule in real-time without prior amplification. The potential benefits of using single-molecule sequencing are minimal input DNA requirements, elimination of amplification bias, faster turnaround times, and longer read lengths that allow for some haplotyping of sequence information.

Whole-Exome Sequencing

Owing to the fact that about 85% of all disease-causing mutations in Mendelian disorders are within coding exons, the recent application of massive parallel deep sequencing with exon capture has shown the efficacy of this technique for the rapid identification of mutations in single-gene disorders [131, 132]. Almost 15 years have now passed since the targeted enrichment of an exome by hybridization of shotgun libraries was first described [133]. Two years later, the targeted capture and massively parallel sequencing of the exomes of 12 humans was published [134]. The following year, the first reports emerged on the use of whole-exome sequencing in gene identification [135]. Since then, investigators have discovered the genetic etiology of hundreds of Mendelian diseases.

Exome sequencing involves the targeted resequencing of all protein-coding sequences, which requires 5% as much sequencing as the whole human genome (Fig. 4.10) [134]. As the majority of Mendelian disorders are due to mutations that disrupt protein-coding sequences, the use of exome capture to identify allelic variants in rare monogenic disorders is well justified. Furthermore, highly functional variation can also be accounted for by changes in splice acceptor and donor sites, the sequences of which will also be targeted by exome capture.

The major advantage of whole-exome sequencing is that virtually all variants within

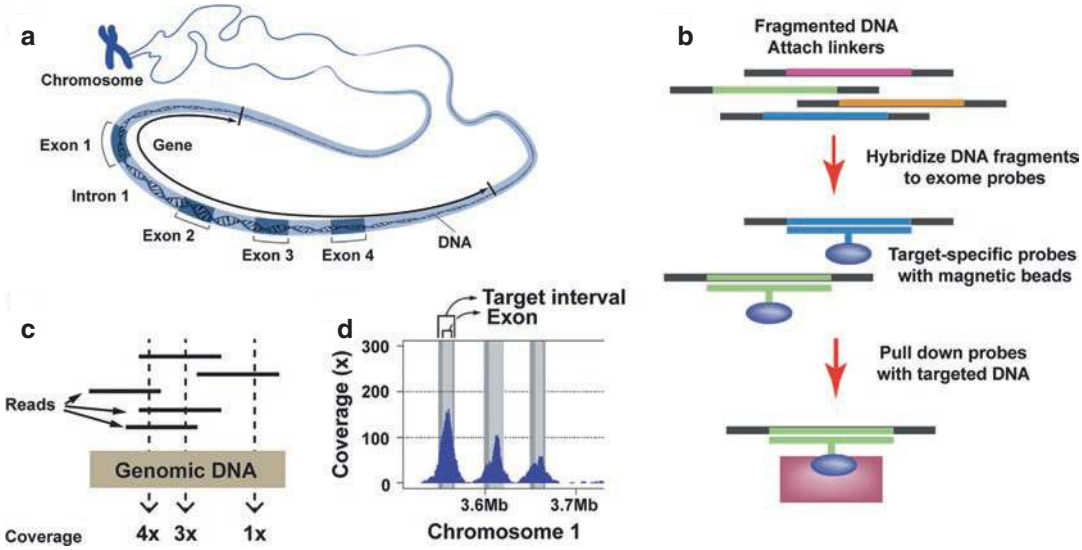

Fig. 4.10 Whole-exome capture and sequencing. (**a**) Representation of a gene and its exons and introns. (**b**) Illustration of the exome capture process. In this case, the oligonucleotide probes designed to specifically anneal to all exons in the genomes are in solution. They are easily pulled out from the solution, the probes are linked to magnetic beads. Another exome capture system has probes attached to a microarray. (**c**) Illustration of the concept of reads and coverage for a small genomic segment. These are key concepts in genomics. (**d**) Illustration of the skewed distribution of read coverage with exome capture to the exonic segments. (Figure credits: Modified from (**a**) NHGRI Image Gallery, https://peds-renomics.clinic/NHGRI-images. (**b**) Wikipedia, author SarahKusala, https://peds-renomics.clinic/Capture. (**d**) Dr. Murim Choi, Seoul National University)

an individual's genome are uncovered simultaneously. This allows for direct examination of the list of variants and candidate gene selection in the presence or absence of mapping studies. Variant listing depends on several factors that depend on the technology used. For example, the type of capture kit, the sequencing platform, and sequencing depth can influence the variant listing. Additionally, the lists produced will depend on the alignment algorithms and the stringency settings of the bioinformatics tools employed for identifying variants. Capture kits are continuously improving, initially covering 27 Mb and 180,000 coding exons to now up to 62 Mb of the human genome and over 201,121 coding exons. Each platform uses biotinylated oligonucleotide baits complementary to the exome targets to hybridize sequencing libraries prepared from fragmented genomic DNA. These bound libraries are enriched for targeted regions by pull-down with magnetic streptavidin beads and then sequenced.

Whole-Genome Sequencing

Whole-genome sequencing (WGS) involves sequencing the complete DNA sequence encompassing all 6 billion nucleotide bases in 23 chromosome pairs of a diploid human genome [136]. The main difference between WGS and WES is that WGS covers the entire genome, including all exons [137]. As a result, it includes the exome and allows the detection of mutations in noncoding DNA elements that are missed by WES. While we cannot efficiently analyse such mutations right now, it is fair to assume that we will be in a good position to do so in a few years. Once the tools exist to assess noncoding variants, re-analysis of "negative" WGS datasets would be very simple. On the other hand, patients who underwent WES studies that proved uninformative (i.e., no causative coding variant was identified) would have to be studied again with WGS.

Yet another significant advantage over WES is the much improved ability to uncover various types of CNVs, such as insertions, duplications, and deletions [138]. This is possible because of the introduction of paired-end mapping of sequence reads: a CNV is flagged when the 75 bp reads generated from both ends of genomic fragments of known length (~300 bp) are aligned farther (or closer) than anticipated (Fig. 4.11) [139]. The earliest example of genome-wide CNV interrogation using WGS data revealed 1000 large structural variations per genome, which is much more than anticipated [140]. However, the gold standard for a thorough investigation of structural variations remains comparative genomic hybridization (CGH) microarray techniques.

We will use one of the first published WGS studies (based on James Watson's DNA) to illustrate how comprehensive is the dataset created [141]. More than 100 million high-quality short-read sequences were produced, containing a total of 24.5 billion DNA bases. This allowed the investigators to "read" every base of Watson's genome on average seven-times—this is referred to as the "coverage", and the higher it is, the more confident you are that the mutations identified are true. After processing the data with various bioinformatics tools, a total of 3 million high-quality variants were ultimately identified, of which ~10,000 were nonsynonymous mutations (0.3%). More than 65,000 insertions and twice as many deletions were also found in these data.

When compared to WES, one of the biggest challenges of WGS is the substantially larger volume of data generated (estimated to be over 1 TB per genome). This has significant implications because the raw aligned data will typically be stored long-term. The issue is that sequencing costs have decreased much more rapidly than the costs associated with the infrastructure necessary to store and process these data [142]. For example, even when Watson's team was done processing his data back in 2008 [141], they would have to keep the data indefinitely in case reanalysis was deemed necessary. Consequently, teams interested in implementing WGS as opposed to WES have to be ready to face significant challenges with regards to storing data.

Another big challenge is the much higher number of variants identified in a WGS dataset. Indeed, it is not easy to pinpoint the disease-causing variant from the many hundreds of variants that remain after various bioinformatics

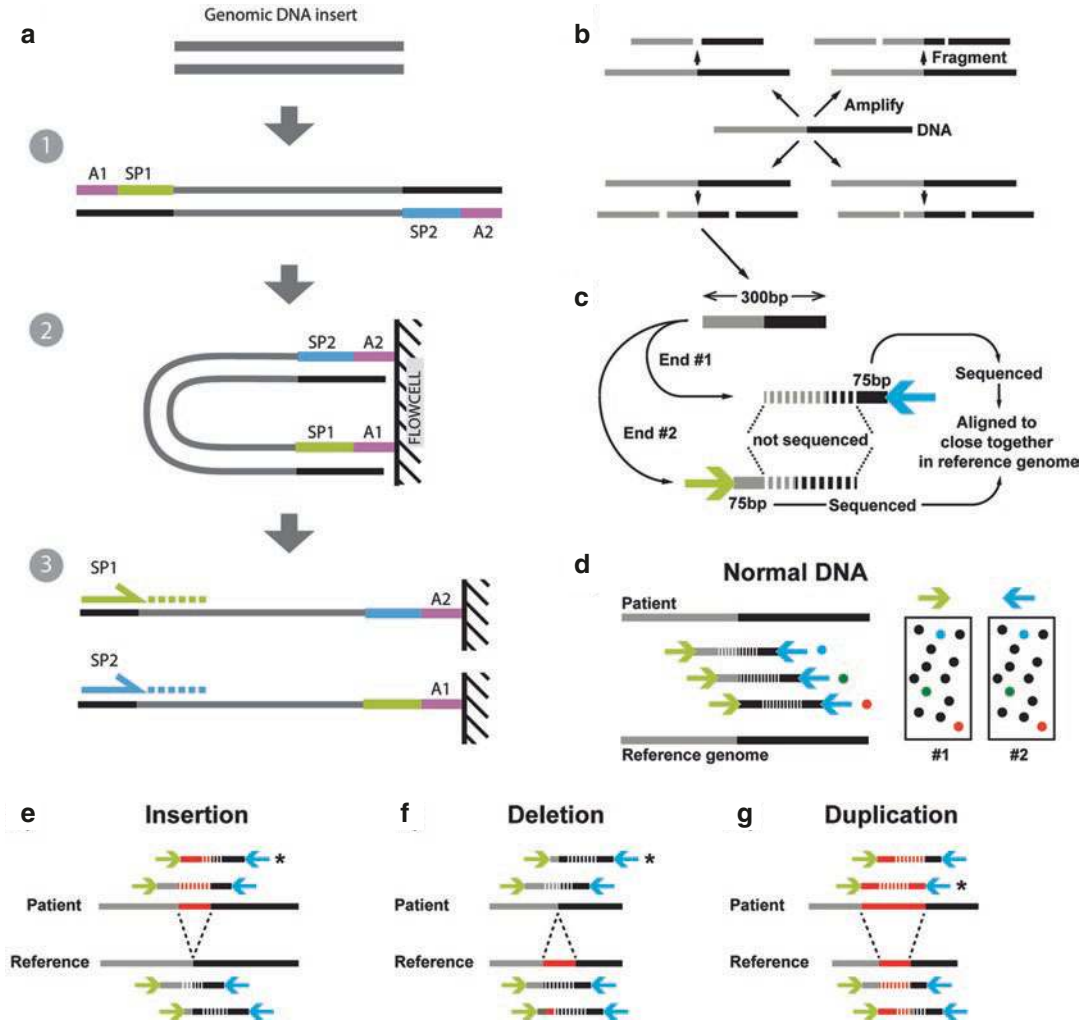

Fig. 4.11 Paired-end sequencing to detect structural variations. (**a**) Preparation DNA sample for standard next-generation sequencing using paired-end. Genomic DNA from the patient were amplified and then fragmented into small pieces. Only fragments with lengths ~200–250 base pairs are selected for sequencing (genomic DNA inserts). The steps described are as follows: (1) Adapter (A1 and A2) with sequencing primer sites (SP1 and SP2) are ligated onto DNA fragments; (2) Template clusters are formed on the flow cell by bridge amplification; (3) Clusters are then sequenced by synthesis from the paired primers sequentially. (**b**) Illustration of amplification of DNA, random fragmentation of amplified segments; in this case, fragments of similar sizes are illustrated. (**c**) Illustration of the two-step process leading to paired-end sequencing. The fragment size selected for is 300 base pairs, and 75 base pairs are sequenced at each end. The dotted lines represent segments that are not directly sequenced in that fragment. (**d**) Schematic showing how paired-end reads from a small genomic region from a patient with normal DNA align to the reference genome. The right-hand side shows the position of the clusters from which the data for each read come from. Each DNA fragment is linked to a specific cluster on the flow cell, thus linking the first and second sequencing data. Also shown are examples of how paired-end sequencing is useful to identify for insertions (**e**), deletion (**f**) or duplications (**g**). (Figure credits: Panel **a**: Figure modified from the original Illumina® paired-end sequencing workflow. All rights reserved)

filters. In comparison, one has to investigate ~50–100 coding variants after filtering WES data.

Furthermore, it will be essential for accurate and comprehensive characterization of disease phenotypes to greatly assist the analysis of an individual's phenotype. It has been shown that the presence of bi-allelic variants can greatly influence a disease phenotype and the data generated by WGS will likely increase our understanding of the molecular intersection of biologically relevant pathways.

As whole-genome is still expensive, currently over £2000 per genome, whole-exome sequencing remains the preferred strategy for molecular genetic diagnosis as this remains a more cost-effective strategy than Sanger sequencing in genetically heterogeneous conditions. However, limitations exist with both technologies and the major challenges posed by both strategies will involve the logistics of delivering genome sequence information to clinicians and how we as clinicians use the data, and how patients and their families deal with the incidental findings.

Online Resources

Topics	URLs
PCR	https://peds-renomics.clinic/PCR
Single base primer extension genotyping	https://peds-renomics.clinic/Genotyping
Linkage	https://peds-renomics.clinic/Linkage
Sanger sequencing	https://peds-renomics.clinic/Sanger-seq
Exome sequencing	https://peds-renomics.clinic/Exome
Illumina on YouTube	https://peds-renomics.clinic/YouTube-Illumina
NHGRI on YouTube	https://peds-renomics.clinic/YouTube-NHGRI

Clinical Implementation in Nephrology

Finding Pathogenic Mutations for Mendelian Disease

Nowadays, most pediatric nephrologists will send blood samples for genetic testing a few times a year for patients with a wide range of conditions. This may be done to establish a firm molecular diagnosis for a patient, to verify if the relatives are carriers. Non-invasive techniques are also emerging to obtain an antenatal molecular diagnosis from maternal blood [143]. The interested reader is directed to a recent review on the topic of genetic testing applied to nephrology [144].

The first set of investigations is usually to sequence genes known to cause a particular condition to uncover possible pathogenic mutations. Until recently, this was done using PCR amplification and Sanger sequencing of all exons of these genes. However, when the number of genes under investigation is less than 50, the same diagnostic procedure is usually now done using targeted exome sequencing because it is fast, cheap, customisable, widely available, and reliable [145]. The difference between the targeted exome and the whole exome lies in the number of genes investigated at once: all known genes known to cause disease X vs. all genes of the genome. This approach has been applied to various groups of kidney diseases with great success [146]. A gene panel was designed to allow for the rapid discovery of pathogenic mutations in ~4500 genes relevant to genetic conditions with neonatal or infantile onset: "RapSeq" includes many genes that cause renal diseases [147].

Whole-exome sequencing is usually reserved for conditions caused by >50 genes, cases that remain undiagnosed using targeted sequencing panels, or for patients with atypical phenotypes. Recent data suggest, however, that the added benefits of whole-genome sequencing may result in this approach soon becoming the standard test [148]. A clear advantage and yet another challenge of this approach is the ability to interrogate all genes and all noncoding regions at once, and the improved yields for structural variations.

The simplest scenario is when a mutation identified in your patient is described in other patients with the same phenotype. Unfortunately, the alternative scenario is much more common: the report states that there is a variant of "uncertain significance" in one of the genes tested. This means that the genetic testing company, using a systematic approach to evaluate novel variants, cannot determine with high certainty that your

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patient's mutation is causal [149]. With a little extra work, it is however possible to provide families with more information about the pathogenic potential of the variant. We provide a case that illustrates what a pediatric nephrologist can do to evaluate such a report (see clinical vignette). It emphasizes the usefulness of simple concepts that were discussed earlier in this chapter, such as co-segregation, allele frequency, and amino acid conservation.

Physicians need to be prepared to revisit the diagnosis since databases are evolving and should make this clear to the patient and his/her parents. Current protocols used by leaders in the field stipulate systematic rechecks of all datasets every 6 months, with automatic reporting to the ordering physician if new findings emerge [150, 151]. This is critical because as public databases are populated with an increasing number of pathogenic variants over time, the output of bioinformatic analyses performed changes. Table 4.1 shows the significant increase in the number of pathogenic genotypes in HGMD between 2012 and 2020 for congenital nephrotic syndrome caused by *NPHS1* mutations. Thus, an initial negative report from next-generation sequencing testing may yield a firm molecular diagnosis later on. A recent study on millions of genetic tests done for hereditary forms of cancer found that ~25% of variants of uncertain significance were later confidently labelled as pathogenic or benign [152].

Since the criteria for a mutation to be deemed pathogenic are very stringent, one may assume that the interpretation of such a mutation is unlikely to be overturned. However, the curation of mutation databases is surprisingly unreliable: sequencing of ~438 target genes in 52 parent-child trios revealed that ~25% of mutations flagged as "likely pathogenic" (on the basis of being already described as such) were likely to be benign since they were found in healthy, asymptomatic parents [153]. To address this issue (and many others in the field), the NIH created the Clinical Genome Resource, or ClinGen [154]. This group devised a comprehensive and systematic approach to evaluate the robustness of all gene-disease and mutation-disease causal links [155]. The assessment of renal genetic conditions will soon improve as ClinGen recently established a Clinical Domain Working Group on tubulopathies, and another one focused on kidney cystic and ciliopathy disorders (https://peds--renomics.clinic/ClinGen-kidney).

Clinical Vignette

A young patient with a clinical diagnosis of congenital nephrotic syndrome spent many months as an inpatient. Since the parents are first cousins, an autosomal recessive form of CNS was expected; the patient has a 2-year-old female sibling that is apparently healthy (Fig. 4.12). There is no prior family history of CNS. A few months back, your colleagues made sure to send blood samples from all first-degree relatives for genetic testing of the usual CNS gene panel, which includes Sanger sequencing of the genes *NPHS1* and *NPHS2*. You are seeing the patient and his parents today in the clinic to discuss the report issued by the diagnostic laboratory.

Table 4.1 Change in the number of pathogenic *NPHS1* mutations in HGMD between 2012 and 2020

	Yearly data for *NPHS1* in HGMD database								
Mutation types	2012	2013	2014	2015	2016	2017	2018	2019	2020
Missense/nonsense	87	97	113	125	145	152	152	163	180
Splicing	14	16	20	22	25	28	28	33	39
Regulatory	0	0	0	0	0	0	0	1	1
Small deletions	20	24	25	28	33	35	36	38	40
Small insertions	10	10	10	10	13	14	14	14	16
Small indels	2	2	2	2	3	3	3	3	4
Gross deletions	0	0	1	1	3	3	3	3	3
All mutations	133	149	171	188	222	235	236	255	283
% Freely available	62	66	72	67	79	76	75	78	79

Note: These data were compiled yearly by the author (ML) directly from the HGMD website

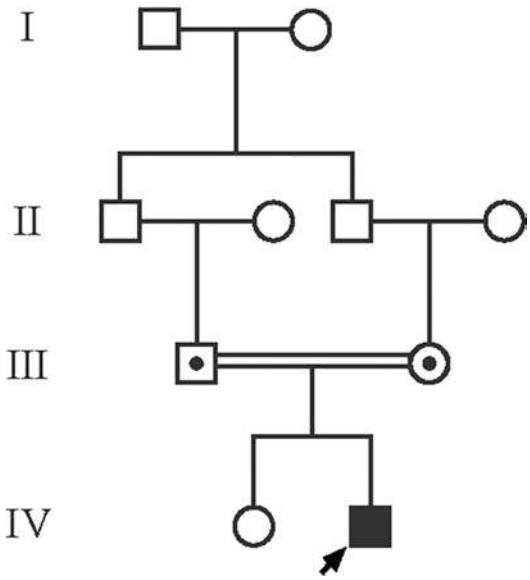

Fig. 4.12 Pedigree for a patient with Congenital Nephrotic Syndrome. Black and white symbols represent affected and unaffected subjects, respectively. Black dots and double lines denote heterozygous carriers and consanguineous unions, respectively. Black arrowhead identifies the proband

The report highlights potentially relevant alterations. The homozygous mutation in *NPHS1* (c.2276 T>C; p.I759T) is considered a "variant of uncertain significance" (VUS) because it has not been described before in other patients with CNS. It is important to note that mutations should always be described with "c." and "p.", which are the base change relative to the cDNA (mRNA) and the resulting amino acid change in the encoded protein, respectively. Not surprisingly, for a novel variant, there are no *in vitro* studies that have tested its functional impact experimentally. We confirmed that the two healthy parents and the unaffected sibling were heterozygous carriers: thus, the pattern of cosegregation is consistent with this mutation being potentially pathogenic. In preparation for our meeting with the family, we decided to investigate further to see if this mutation could be pathogenic. Indeed, finding a convincing mutation in a gene known to be associated with CNS would help our team provide a more accurate prognosis while also providing some closure for the family [156].

First, we visited the public version of the Human Gene Mutation Database (HGMD [157]) to make sure that this mutation remained novel (free registration required for access; Fig. 4.13a). We then confirmed that conclusion using ClinVar (Fig. 4.13b) [158]. These two resources present comprehensive lists of mutations causing Mendelian conditions curated by experts. A major advantage of ClinVar is that it also provides information on the variant of interest if it was reported as VUS by any genetic testing facility or if there are any data in control databases, such as the gnomAD browser (see the bottom of Fig. 4.13b) [159] or dbSNPs [37]. As shown in Fig. 4.13b (bottom panel), ClinVar also reported that the Broad Institute Rare Disease Group reported an unrelated case of congenital nephrotic syndrome with *NPHS1* p.I759T in compound heterozygosity.

To get a better understanding of the pathogenic potential of this variant, we also consulted gnomAD [159]: it was observed in the heterozygous state in 4 out of >125,000 individuals that were all of South Asian descent (like our patient; Fig. 4.13c). This database includes whole-exome and whole-genome data from adults that are unlikely to have a severe pediatric syndrome [159]. Another port of entry to access all this information is dbSNPs [37], particularly if the report includes the rs# associated with the variant (in our case, rs775313529; Fig. 4.13d).

The gnomAD page for the variant includes a wealth of information [159], including the likelihood of it being pathogenic based on two of the most widely used prediction tools, Polyphen [160] and SIFT [161]. There are now dozens of such bioinformatic tools, all claiming to be better than the others. All evaluate the pathogenic potential of missense mutations by using information about the degree of amino acid conservation between various species, the predicted impact of the specific amino acid change (based on physicochemical properties of amino acids, such as size, charge, and polarity) compounded by some advanced computing methodologies (machine learning). An alternative approach is to use software like Condel [162] because it inte-

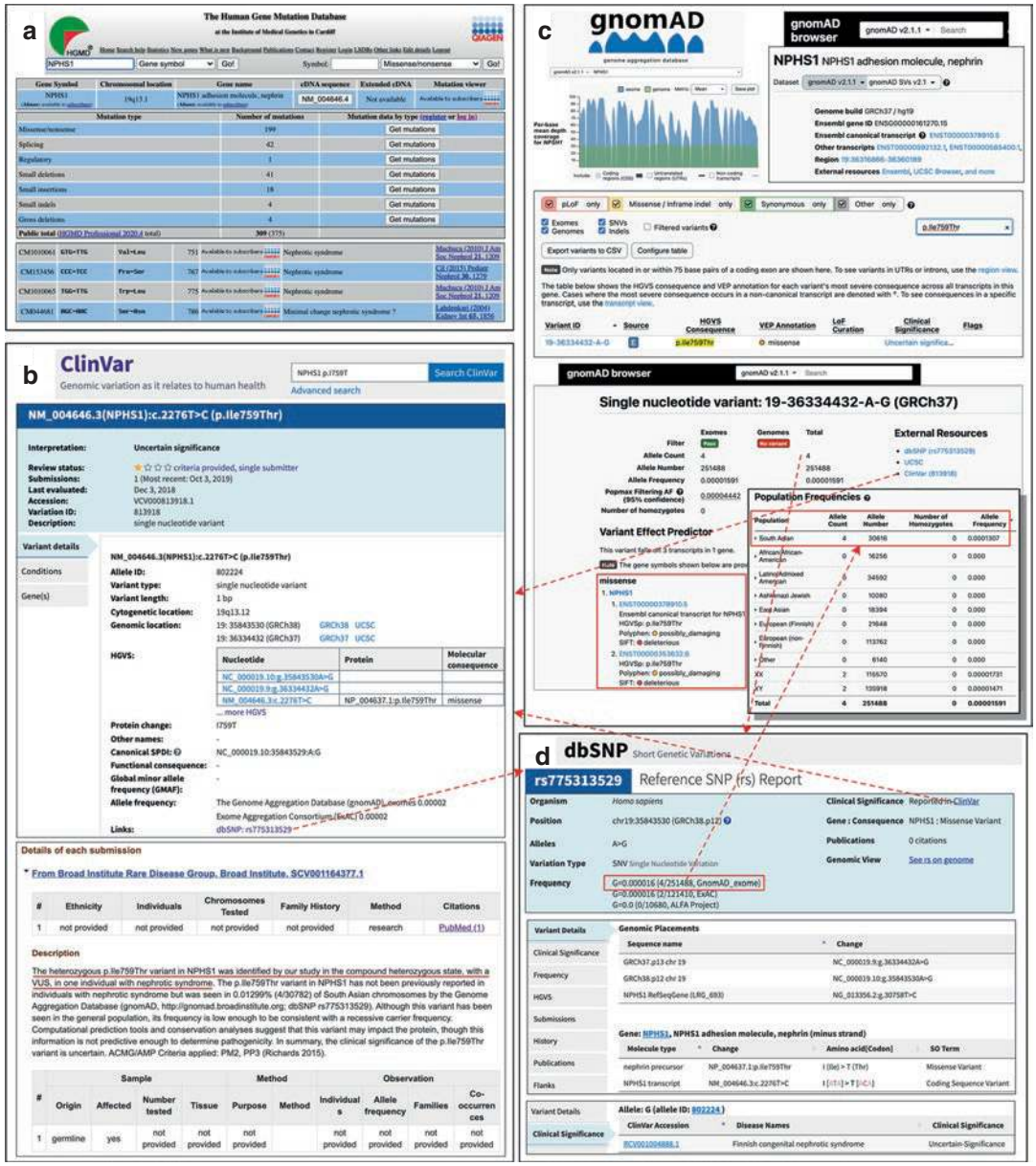

Fig. 4.13 Assessing a variant of uncertain significance in *NPHS1*. (**a**) A visit to the HGMD website suggest that the variant *NPHS1* p.I759T has yet to be reported in a patient. (**b**) While the ClinVar website corroborates this finding, it also suggest that there is one report of a patient with the same variant in the context of compound heterozygosity. (**c**) Data from gnomAD suggest that variant is rare and is only described in South Asian individuals. (**d**) dbSNP is the simplest point of entry to all these data if the genetic test report include the rs#. Note that most of these resources are densely linked to each other (red arrows)

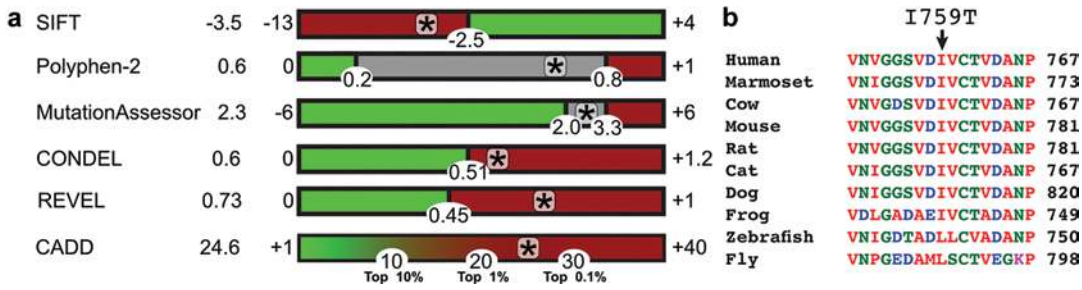

Fig. 4.14 Assessing mutations for pathogenicity with various prediction softwares. (**a**) The ranges of scores for SIFT, Polyphen2, MutationAssessor, Condel, REVEL and CADD. For a mutation to be considered as pathogenic, its score should lie in the red zone of the score gradients. Because all softwares use slightly different inputs and analytical methods, it is not unusual to generate contradictory predictions for the same mutation. (**b**) Multiple species protein sequence alignment was done using BLAST shows that position I759 is well conserved down to the fruit fly (note: leucine and isoleucine are interchangeable). Each letter represents an amino acid; the number on the right-hand side indicates the position of the last amino acid of the series presented in a given ortholog

grates the outputs of Polyphen [160], SIFT [161] and MutationAssessor [163] (Fig. 4.14a). Newer tools that attempt to further refine the output include CADD [164] and REVEL [165] (Fig. 4.14a). Interestingly, the isoleucine at position 759 is well conserved across orthologs, down to the fruit fly (Fig. 4.14b).

Overall, it is likely that this VUS will soon be upgraded to "pathogenic" on the basis that it was found in two unrelated patients with congenital nephrotic syndrome, familial cosegregation analysis was consistent with a recessive mode of inheritance (as expected), the mutated amino acid is well conserved, the mutation is only observed in the heterozygous states with very low MAF in South Asians (0.01%), and most prediction softwares predict that p.I759T should be deleterious.

Genetic Heterogeneity

Genetic (or locus) heterogeneity is recognized when a particular phenotype may be caused by mutations in different genes. Nephronophthisis is one of the best examples of locus heterogeneity in the renal literature, with disease-causing mutations reported in ~30 genes (Table 4.2). Functional studies have shown that the majority of the proteins encoded by these genes are localized and play important roles at the centrosome, basal body, and cilia. This led to the proposition that these structures are central in the pathogenesis of nephronophthisis [166]. It is important to note that locus heterogeneity is common among many monogenic renal conditions, such as atypical hemolytic-uremic syndrome [167], nephrotic syndrome [168], distal renal tubular acidosis [169], Dent's disease [170], or primary hyperoxaluria [171].

Pleiotropy is the mirror image of genetic heterogeneity: mutations in the same gene cause different phenotypes. Incidentally, nephronophthisis also provides many well-documented cases of pleiotropy (Table 4.1). For example, the study of patients with unique phenotypes within the nephronophthisis spectrum using whole-exome sequencing led to the identification of novel candidate genes that were previously associated with completely distinct disorders, such as *SLC4A1* or *AGXT* [172]. The first gene, which encodes the anion exchanger 1 protein, was previously associated with hereditary spherocytosis [173] or distal tubular renal acidosis [169], while the second one is a well-established cause for primary hyperoxaluria (it encodes an enzyme involved in glyoxylate metabolism) [171].

Interestingly, a substantial number of patients with nephronophthisis (~40%) remain genetically undefined. The same is true for many other renal conditions. This will prove to be a fertile

Table 4.2 Genetic heterogeneity among nephronophthisis-associated ciliopathies

Genes	Nephronophthisis syndromes											OMIM #
	BBS	CED	COACH	JATD	JBTS	LCA	MKS	MSS	MORM	NPHP	SLS	
AGXT										■		604285
AH1					■							608894
ANKS6										■		615370
ARL13B					■							608922
CC2D2A			■		■		■					612013
CEP164						■				■		614848
CEP290	■				■	■	■			■	■	610142
CEP41					■							610523
GLIS2										■		608539
IFT122		■										606045
IFT140				■				■				614620
IFT43		■										614068
INPP5E					■				■			613037
INVS										■	■	243305
IQCB1										■	■	609237
MRE11										■		600814
NEK8										■		609799
NPHP1					■					■	■	607100
NPHP3							■			■	■	608002
NPHP4										■	■	607215
RPGRIPL1			■		■		■					610937
SDCCAG8	■										■	613524
SLC4A1										■		109270
SLC41A1										■		610801
TMEM138					■							614459
TMEM216					■		■					613277
TMEM67	■		■		■		■					609884
TTC21B				■						■		612014
WDR19			■	■						■		608151
XPNPEP3										■		613553
ZNF423										■		604557

BBS Bardet-Biedl Syndrome, CED Cranioectodermal dysplasia, COACH syndrome characterized by cerebellar vermis hypo/aplasia, oligophrenia, congenital ataxia, ocular coloboma, and hepatic fibrosis, JATD Jeune asphyxiating thoracic dystrophy, JBTS Joubert Syndrome, LCA Leber's Congenital Amaurosis, MKS Meckel-Gruber Syndrome, MMS Mainzer-Saldino syndrome, MORM syndrome characterized by mental retardation, truncal obesity, retinal dystrophy, and micropenis, NPHP nephronopthisis, SLS Senior Loken Syndrome

ground to display the utility of whole-exome and/or whole-genome sequencing and will undoubtedly lead to the identification of many other unexpected candidate genes [174, 175].

Finding Risk Alleles for Complex Diseases

One of the promises of genomic medicine is for knowledge about a patient's genome to play a central role in the management of complex medical conditions. Classic examples of complex diseases include essential hypertension, diabetes mellitus type 2, steroid-sensitive nephrotic syndrome, and congenital malformations of the kidney and/or urinary tract. For example, pediatric nephrologists could optimize the prevention and/or treatment of steroid-sensitive nephrotic syndrome, thus realizing the guiding principles of personalized medicine. While this will undoubtedly change the face of clinical medicine, as described below, we are unfortunately years away from enacting this scenario.

In contrast to Mendelian conditions that are caused by highly penetrant mutations in a single gene, complex diseases are not rare and are caused by multiple factors. Detailed epidemiological

studies have shown that for many of these conditions, environmental and genetic factors are the major determinants of trait expression. Current models implicate the interaction of multiple low penetrance variants in many genes. These are based on studies of heritability, defined as the fraction of phenotypic variation that is likely explained by genetic variation [176]. Heritability is estimated from the phenotypic correlations among related individuals, using families with multiple affected individuals as well as twin studies. A prime example of a complex trait with high heritability is adult essential hypertension [177].

Finding the genetic basis for complex traits could have significant public health implications. First, it may help identify at-risk individuals early on, thereby perhaps allowing for the prevention of long-term, largely preventable health consequences. Second, it may be an important tool to decide the type and dose of medication that is best suited for a given patient, which would be a major advance towards the realization of personalized medicine. Below, we will discuss the two competing theories of complex trait genetics and the methodologies employed to find associations, while providing examples from the renal literature.

Complex Diseases: Associated with Common and/or Rare Variants?

There are two predominant explanatory models of complex disease genetic causation: the complex disease, common variants (CDCV) and the complex disease, rare variants (CDRV) hypotheses (see Fig. 4.15) [178]. Both assume that multiple genes are implicated in the pathophysiology of common traits. The first hypothesis states that the genetic landscape of common traits mostly comprises common variants (minor allele frequency >1%), each with a very small contribution to the overall phenotype. The alternative hypothesis suggests that rare variants (minor allele frequency <1%) with larger effects are largely responsible. Up until recently, the technology to perform the comprehensive genomic analyses required to unravel the genetic architecture of complex diseases did not exist: the predominant working model was CDRV.

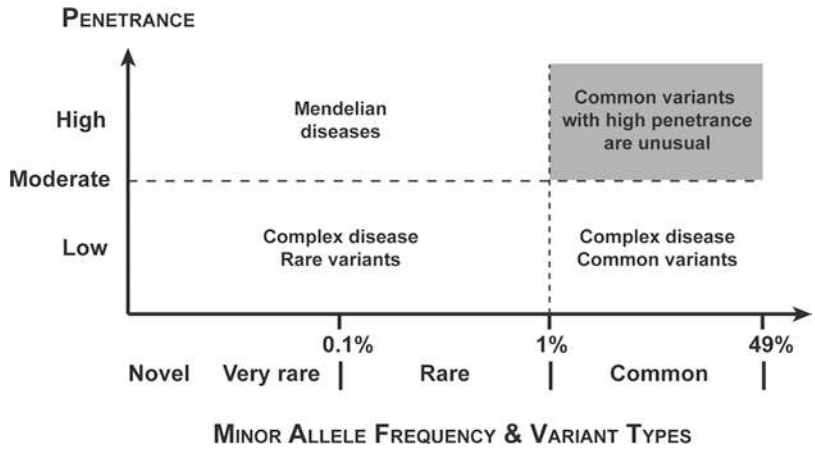

Fig. 4.15 The relationship between minor allele frequency and penetrance to explain the genetic basis of complex diseases. This graph illustrates the relationship between minor allele frequency and penetrance of phenotype. Typically, common variants that have a phenotypic effect have low penetrance. On the other hand, rare variants are expected to result in a much higher penetrance. This is due to the fact that penetrance is one of the main factors driving the selective pressure for/against a particular phenotype. Most variants identified thus far for Mendelian conditions are in the left upper quadrant, while those associated with complex diseases fit in the right lower quadrant

The CDCV hypothesis was the first to be tested rigorously owing to technological and methodological developments related to SNPs genotyping. This opened the gates for a flurry of genome-wide association scans (GWAS) performed on common SNPs. Reliable investigations of the CDRV hypothesis were beyond reach until the emergence of cheap high-throughput sequencing and the development of exome capture.

Clinical Implications of Genome-Wide Association Studies

Most of the GWAS studies focused on renal conditions include only adult subjects [113]. Some studies were focused on dichotomous disease outcomes, such as hypertension, diabetic nephropathy, IgA nephropathy, CKD, ESKD/FSGS and kidney stones [113]. Others tested the association with relevant continuous variables like blood pressure, eGFR, serum creatinine, creatinine clearance, albumin-to-creatinine ratio, or serum cystatin C [113]. Unfortunately, there are few variants showing robust association with a given trait that is replicated across distinct studies. These SNPs typically explain very little of the phenotypic variability observed in patients: this phenomenon, which is not unique to nephrology studies, has been termed "missing heritability" [179]. Many of these studies have, however, provided unique insights into the biological pathways that cause disease that were hitherto immune to detection [180].

There are now a few published GWAS that enrolled children with kidney disease [181]. As a result of this research lag, children may not benefit from the realization of genomic medicine as rapidly as adult patients [182]. Indeed, it is likely that advances percolating from adult studies will not translate into concrete measures for children for two main reasons. First, the important pediatric public health problems that could be addressed "genomically" are not on the adult medicine "radar" (e.g., nephrotic syndrome). Second, even when diagnoses are congruent (e.g., hypertension), it is unclear how useful results from large adult studies will apply to pediatric care since the underlying pathophysiology is often distinct (due to growth and development) [183, 184].

Thankfully, this state of affair is changing. The first GWAS, which included more than one thousand children with CKD, was inconclusive because of low power [185]. A number of small GWAS (sample sizes <500) focused on nephrotic syndrome yielded statistically significant SNPs, particularly in the HLA genes [186–189]. The largest GWAS published in 2020, which included many of the subjects from earlier cohorts, confirmed this association and also uncovered new loci of interest in the regions of *NEPHS1-KIRREL2* and *TNFS15* [190].

Source of Missing Heritability: Rare Variants?

A substantial proportion of the genetic contribution to complex traits thus remains unexplained. The next logical step is to determine the impact of rare variants on such phenotypes using whole-genome or -exome sequencing [191]. Unfortunately, because the variants are rare, the sample sizes required to draw conclusions are prohibitively large (10,000–100,000 subjects at a minimum) [192, 193]. As a result, few studies testing the CDRV hypothesis have been published [194]. Other aspects that could also play a role in the pathophysiology of complex traits include epigenetic factors [195], gene-gene interactions (epistasis) [196], and/or gene-environment interactions [197]. An alternative explanation recently put forward is that heritability may have been overestimated all along [198]. Finally, the complexity of the underlying genetic architecture of these diseases may be such that it is not possible to unravel with our current experimental tools [199].

Pharmacogenetics/Pharmacogenomics

Most current studies attempt to link genetic polymorphisms in a small number of genes with

known functions in drug metabolism. As such, they establish the pharmacogenetic profiles for patients. Very few studies, particularly when enrolling pediatric subjects, perform pharmacogenomic profiling, which involves genome-wide interrogation. One of the major promises of personalized medicine is that drug prescriptions will be tailored based on a patient's pharmacogenetic profile. This promise is within reach because of the extensive knowledge accumulated about two of the key determinants of drugs' pharmacokinetic and pharmacodynamics properties.

First, a group of enzymes add specific chemical groups to the parent compound: this way, drugs are metabolized to their active form, or they are modified to enhance excretion. Cytochrome P-450 oxidases (CYP) and uridine diphosphate-glucuronosyltransferase (UGT) are two of the most prominent families of enzymes of this class. The second class are transporters that mediate the efflux of drugs outside of cells, thereby limiting their therapeutic benefits. Many great examples are provided by transporter proteins that are part of the ATP-binding Cassette (ABC) superfamily. The other key development is the increasing knowledge about the physiological impact of SNPs in genes encoding proteins that play important roles in both of these processes. Below, we will describe issues that relate specifically to the realization of pharmacogenetics for pediatric nephrology patients, while also providing concrete examples from the literature.

Four main issues plague the field of pediatric pharmacogenetics in general. First, the vast majority of pharmacogenetics studies are done exclusively on adult subjects. Unfortunately, these findings cannot be directly extrapolated to children because of the impact that growth and development have on pharmacologic parameters. Second, there are currently very few studies reporting specifically on the association of genetic polymorphisms with pharmacologic parameters in pediatric subjects. Third, the predictive power of most pediatric studies is effectively limited by small sample sizes (typically less than 100). Finally, all studies conducted thus far are retrospective and have not been able to test if the inclusion of pharmacogenetic data influences outcomes. It is therefore not surprising that there are currently very few tests that are ready for prime time for pediatric patients.

In both adult [200] and pediatric nephrology [201], the most active research in pharmacogenetics relates to key agents used in current renal transplantation cocktails. Calcineurin inhibitors were logical candidates for such studies since they have proved to be valuable as steroid-sparing agents but are known to cause significant level-dependent nephrotoxicity. A single study with 104 pediatric kidney transplant recipients treated with cyclosporin showed no evidence that genotyping for polymorphisms in genes from the CYP and ABC families helped optimize patient care [202]; these results largely echo the consensus opinion derived from similar adult studies [200]. In contrast, the implementation of pharmacogenetic profiling is probably closer to reality for tacrolimus: testing for polymorphisms in *CYP3A5* identified in adult studies [200] were found to be predictive when tested in 30 teenage kidney transplant recipients [203]. There are also data from two small studies on pediatric kidney transplant recipients treated with MMF that report promising associations with UGT polymorphisms that require corroboration [204, 205].

Two additional hurdles will complicate the implementation of pharmacogenetics specifically in renal transplant protocols. First, the benefits of genotyping above and beyond the current gold standard will have to be demonstrated. This may prove a difficult task since therapeutic drug monitoring (TDM) of various immunosuppressive medications allows personalization of doses within days to weeks [200]. There is evidence that at least in adult patients, pharmacogenetics leads to more rapid optimization of drug dosage, but it does not appear to translate into better outcomes [206]. Second, the ability of any predictive indices, including pharmacogenetics, to impact clinical outcomes will always be hampered by the polypharmacy that is inherent to most renal transplant drug cocktails because of complex drug-drug interactions [200].

Warfarin is yet another medication relevant to pediatric nephrologists that has been extensively studied for pharmacogenetic applications.

Studies in adult subjects have shown that polymorphisms in the genes encoding the target of warfarin, *VKORC1* (vitamin K epoxide reductase complex subunit 1) or its main metabolizing CYP (*CYP2C9*) are helpful to predict warfarin disposition [207]. Data emerging from pediatric studies testing the same polymorphism are unfortunately not as clear [208].

While pharmacogenetics offers the potential of individualized treatment strategies, it is critical to obtain solid evidence of clinical utility before widespread clinical implementation. A great source of information for interested physicians is the Clinical Pharmacogenetics Implementation Consortium (CPIC), a project aimed at addressing "some of the barriers to the implementation of pharmacogenetic tests into clinical practice".

Online Resources

Sources	URLs
Clinical Pharmacogenetics Implementation Consortium	https://cpicpgx.org/
Renal genes	http://www.renalgenes.org/
HGMD	http://www.hgmd.cf.ac.uk/
ClinVar	http://www.ncbi.nlm.nih.gov/clinvar/
The gnomAD browser	https://gnomad.broadinstitute.org/
SIFT	http://provean.jcvi.org/index.php
Polyphen2	http://genetics.bwh.harvard.edu/pph2/
MutationAssessor	http://mutationassessor.org/
BLAST	http://blast.ncbi.nlm.nih.gov/

Barriers to Implementation in the Clinic

There is a lot of hype and high expectations that remain largely unrealistic. Apart from the diagnosis of rare diseases and some rare applications in pharmacogenomics, the real-world impact of genomics has yet to be shown in the clinic. Notwithstanding the knowledge limitations that are inherent in the implementation delays, there are a number of other issues that will hamper this process, some of which are discussed below.

Patients' Health Literacy and Numeracy

Adequate health literacy skills are necessary for patients to understand a discussion about genomic issues and to appropriately consent for testing. Health literacy is defined as "the degree to which individuals can obtain, process, and understand the basic health information and services needed to make appropriate health decisions" [209]. A survey of general US adults demonstrated poor health literacy in ~30–40% [210]. Not surprisingly, the situation is even worse when these skills are assessed in the context of genomics issues [211]. Apparent familiarity with genetic concepts is common in adults, but physicians should be alert to the fact that understanding of basic genetic concepts is often limited [212].

Health numeracy skills are also critical for patients to grasp the predictive nature of most discussions that relate to genomic health decisions. Indeed, statistics and probabilities are an integral part of these discussions. Health numeracy is defined as "the degree to which individuals have the capacity to access, process, interpret, communicate, and act on numerical, quantitative, graphical, biostatistical, probabilistic health information needed to make effective health decisions" [213]. A recent survey revealed that ~50% of adult subjects have basic or minimal numeracy skills [210]. In contrast to health literacy, this subject has been little studied [211].

While these issues cannot be ignored, they should not jeopardize patient care. Given that time is limited in the clinic, it may not be possible for the treating physician to spend time explaining basic concepts. The critical point is to rapidly assess if the patient (or their caretaker) has at least a basic understanding of the issues at stake and take measures to try to address perceived inadequacies. If the family has access to the Internet and is motivated to learn independently, the physician may provide the address of reputa-

ble websites focused on teaching basic genetic concepts to a general audience (see the list below).

Online Resources for Patients

Sources	URLs
Genetic Science Learning Centre	http://learn.genetics.utah.edu/
NHGRI educational material	https://www.genome.gov/about-genomics
MedLine Plus—Genetics	https://medlineplus.gov/genetics/
All about genetics (Kids Health)	http://bit.ly/all-about-genetics
CDC Family health history	http://bit.ly/CDC-family-health-history

Physicians' Health Literacy and Numeracy

Another important issue that is emerging is the gap between what physicians will need to know to implement genomic science in clinical practice, and what they actually know [1, 214]. Given the scarcity of healthcare professionals with expertise in genetics and genomics [215], which is unlikely to change in the next few years, it is possible that these inadequacies may hamper the deployment of genomic medicine when it is ready for widespread implementation. There are many resources in the literature [1], in print and online (see the list below) that may help bridge that gap for practicing physicians.

While most physicians agree that a good understanding of basic statistical concepts is necessary in contemporary medicine, very few feel confident of their own skills [216]. This is a long-standing problem that has been repeatedly documented in Europe and North America [217–219]. Up until recently, these skills were deemed important by physicians espousing the principles of evidence-based medicine. The advent of genomic medicine will hopefully trigger a renewed interest for physicians to acquire basic quantitative skills. Medical schools have adapted their curricula to reflect these changes such that current physicians-in-training will be better prepared [220–222].

Online Resources for Physicians

Book from AAP: Robert A Saul, Medical Genetics in Pediatric Practice, 2013, 503 p., American Academy of Pediatrics. ISBN: 978-1-58110-496-7.

Mobile Apps:

Human Genome; iPhone
GeneticCode; iPhone
Gene Screen (CSHL); iPhone
Gene tutor; iPhone & Android
Genetics 4 Medics; iPhone ($5), Android (free)

Type	Sources	URLs
Genetic testing	Gene Reviews	http://Genetests.org
	NCI—Gene testing	https://peds-renomics.clinic/NCI-genetic-testing
	Lab tests Online	https://peds-renomics.clinic/lab-test-genetics
	NIH Genetic Testing Registry	http://www.ncbi.nlm.nih.gov/gtr/
Online tutorials	Open Helix	http://openhelix.eu/
	OMIM	https://www.omim.org/
	Medline Plus—Genetics	https://medlineplus.gov/genetics/
	Genetics in Primary Care (AAP)	https://peds-renomics.clinic/AAP-Genetics
Free resources	European Rare Disease	http://www.orpha.net/
	CDC—Public Health Genomics	http://www.cdc.gov/genomics/
	Human Gene Mutation Database	http://www.hgmd.cf.ac.uk/
	Gene Forum	http://www.geneforum.org/

Test Costs and Gene Patents

Both governmental programs and insurance companies will usually agree to defray the costs of tests that are ordered by physicians, are relevant to the patient's condition, and may lead to a con-

crete change in the plan of care. Ideally, the cost of tests would not play a major role in this decision, but up until recently, it did for a few genetic tests that were prohibitively expensive (thousands of dollars). These high prices were driven in large part by the strict enforcement of gene patents held by a few companies. For example, the price of BRCA1 and 2 tripled once the company Myriad Genetics decided to exercise a strict monopoly on its gene patents [223].

Since ~40% of the human genes have been patented [224], this has the potential to be a major hurdle in the development of personalized medicine based on genomic testing [225]. This situation changed dramatically following the US Supreme Court judgment that invalidated the gene patents held by Myriad Genetics [226].

Demonstration of Efficacy and Cost-Effectiveness

With the gene patent barriers now down and cheap sequencing being available, the next big obstacle to the promised widespread clinical implementation of genomic tests [227] will be the demonstration of efficacy and cost-effectiveness for common conditions [228]. At the request of the CDC, a committee of experts proposed to use a specific set of criteria to assess whether a particular genetic test ought to be implemented in the clinic. It is referred to as ACCE, an acronym that reflects the four criteria that need to be fulfilled: Analytical validity, Clinical validity, Clinical utility, and associated ethical, legal, and social implications.

As seen in Fig. 4.16, this is an ambitious task that requires the integration of data from many different spheres of expertise. Genomic tests will also need to be analysed using the ACCE multifaceted approach: this will likely prove to be a lengthier process since, in theory, each gene-disease combination will undergo a similar in-depth examination. The CDC also formed another committee, named Evaluation of Genomic Applications in Practice and Prevention (EGAPP), that is aimed at the prospective integration of published data within the ACCE framework [229].

It is sobering to review the most up-to-date EGAPP recommendations, which are focused only on common diseases with a significant public health burden: there is “insufficient evidence to recommend for or against the use” of well-studied genetic tests for breast cancer, cardiovascular disease, depression, diabetes, and prostate cancer [230]. In fact, only genetic testing for KRAS mutations in colorectal cancer fulfils all requirements. Children are typically not mentioned in articles on this topic; if they are mentioned, it is as part of the subjects that are excluded [231].

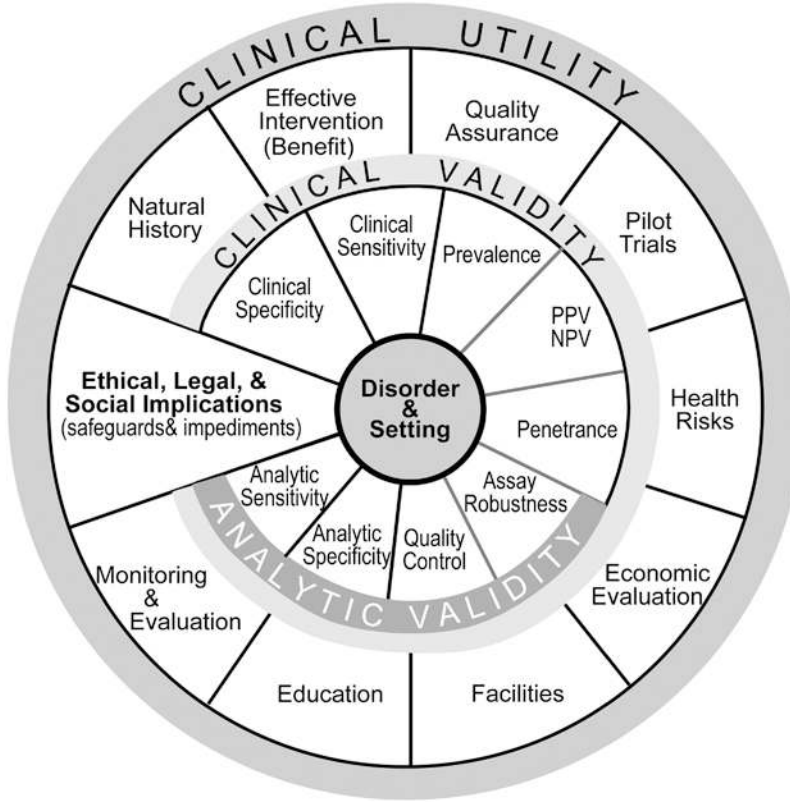

Fig. 4.16 Illustration of the ACCE framework used to evaluate genetic tests. Evaluation of genetic tests with ACCE is based on four criteria: analytic validity, clinical validity, clinical utility and associated ethical, legal and social implications (ELSI). It is meant to be an up-to-date source of information for policymakers to allow informed decision-making. This figure illustrates the various components that are studied for each category. Analytical validity is defined as how accurately and reliably the test measures the genotype of interest. Clinical validity is defined as how consistently and accurately the test detects or predicts the intermediate or final outcomes of interest. Clinical utility is defined as how likely the test is to significantly improve patient outcomes. ELSI is defined as the ethical, legal, and social implications that may arise in the context of using the test. (Figure credits: CDC, https://www.cdc.gov/genomics/gtesting/acce/index.htm)

Ethical and Legal Issues

As the clinical use of sequencing technologies becomes increasingly widespread, practicing nephrologists should expect to have to deal with a new set of issues when discussing results with their patients. With the price of whole-exome and/or exome sequencing decreasing over time, these technologies have now supplanted current approaches that are more targeted, such as Sanger sequencing. This is a dramatic change in practice since clinicians will now have to deal with genome-wide data that are not restricted to genes known to be associated with a given condition. With these changes comes a complex set of issues that every physician is likely to encounter in the near future. These include dealing with mis-attributed paternity and handling incidental genetic findings. Physicians should also be able to discuss how genomic medicine may affect patient privacy and how these results may lead to genetic discrimination. Below, we provide a brief introduction to these concepts that will provide interested physicians with a good starting point or springboard to learn more about these topics. The interested readers are directed to more in-depth reviews on these topics [232, 233].

Mis-attributed Paternity

One important consideration when performing genetic testing is to be cognizant of the fact that misattributed paternity, also referred to as non-paternity, is observed in ~10% of tested individuals (range 1–30%) [234]. In the clinical arena, this problem will be encountered in two main scenarios. First, in the context of HLA testing when assessing parents as potential organ donors [235]. The other situation is when diagnosing a genetic condition, particularly if it is a recessive disease and the father does not harbour the mutation [236].

The treating team should strongly consider consulting a medical geneticist or a genetic counsellor since they are trained to handle these situations. In addition, they can help to calculate and interpret the paternity index, the most useful measure of paternity testing [237]. This method relies on genotyping of 10–15 additional loci for each member of the trio, the assumption being that: each parent shares ~50% of the variants with their child. Rarely, a paternally inherited de novo mutation may explain these findings when paternity testing confirms that the father is indeed genetically related to the patient.

In most jurisdictions, there are no guidelines, rules, or laws that dictate what a clinical team should do in these circumstances. Proponents of nondisclosure emphasize the importance of non-maleficence, while those that advocate for disclosure invoke respect for patient autonomy and truth telling [238]. Treating physicians should be aware that nearly all genetic specialists in the US [239] or abroad [240] have consistently favoured disclosure, but only to the mother. This was also the recommendation from a report published by the Institute of Medicine in 1994 [241].

Medically Actionable Variants

Another dilemma that stems from the introduction of genome sequencing in the clinic is how incidental genomic findings, also known as "incidentalomas", should be handled. This dilemma is particularly acute when dealing with so-called "medically actionable pathologic mutations". These are defined as variants in genes known to be associated with severe Mendelian diseases for which there is good evidence of benefit from specific preventive measures or therapies. A debate on whether one has a duty to report such findings has been raging for a few years in the genetic research community: a recent consensus opinion states that such findings should be reported to subjects who have a priori consented to receive information about incidental findings [242]. The debate is now overflowing to the clinical world.

Recently published policy guidelines put forth by the American College of Medical Genetics recommend mandatory reporting of variants found via clinical sequencing and deemed to be likely pathogenic in 57 genes associated with 24 monogenic conditions [243]. In stark contrast to the research guidelines mentioned above, these incidental findings would have to be reported to patients even if they did not consent to receive this information. While providing valuable information to patients and their families, the enactment of these guidelines would add extra work that may not be accommodated easily by the current clinical workforce. Interrogation of whole-genome sequencing data from 500 Caucasians and 500 African-American "healthy" adults revealed that ~1–3% of subjects tested harbour at least 1 mutation in one of these genes [244]. Adjudication of which incidental findings are actionable is not straightforward [245].

Importantly for pediatricians, these recommendations make no exception regarding children, even for genes that cause adult-onset conditions [243]. This suggestion is based on the fact that it may be critical for the parents themselves to know about these incidental findings. It is predicted that more restricted reporting of such variants will be recommended for children once clinical sequencing becomes more widespread: indeed, this argument will be moot when the parents also have their own sequencing data [243]. Table 4.3 provides a list of genes that are relevant to the practice of pediatric nephrologists: all follow an autosomal dominant pattern of inheritance and are the primary cause for a variety of cancers [243]. A recent study on more than 2000 adults

Table 4.3 Conditions and/or syndromes with clinically actionable mutations that are relevant to pediatric nephrologists

Conditions	Gene	OMIM #	Patients affected	Mode of inheritance	Clinical impact
VHLS	VHL	193300	Child/adult	AD	Known and expected
MEN type 1	MEN1	131100	Child/adult	AD	Known and expected
MEN type 2	RET	171400, 162300	Child/adult	AD	Known
HPPS type 1	SDHD	168000	Child/adult	AD	Known and expected
HPPS type 2	SDHAF2	601650	Child/adult	AD	Known
HPPS type 3	SDHC	605373	Child/adult	AD	Known and expected
HPPS type 4	SDHB	115310	Child/adult	AD	Unknown
TSC type 1	TSC1	191100	Child	AD	Known and expected
TSC type 2	TSC2	613254	Child	AD	Known and expected
Wilms tumour	WT1	194070	Child	AD	Known and expected
NF type 2	NF2	101100	Child/adult	AD	Known and expected

HPPS hereditary paraganglioma-pheochromocytoma syndrome, *MEN* multiple endocrine neoplasia, *NF* neurofibromatosis, *TSC* tuberous sclerosis complex, *VHLS* von Hippel-Lindau syndrome

with CKD who underwent exome sequencing revealed that 1.6% had a medically actionable variant [144].

Privacy

All physicians are keenly aware that patient privacy is paramount. Most are also cognizant of data that could be used as unique identifiers: name, birth date, home address, social security number, etc. When stripped of these data, samples or datasets are deemed deidentified. Like most pediatric subspecialists, pediatric nephrologists routinely take care of patients with very rare conditions. For such patients, it is unclear how "deidentified" the information really is if the diagnosis is part of the data that may be shared. For example, the inclusion of this information in public databases could lead to the de facto identification of a specific patient, particularly if the condition is associated with a visible phenotype. For this reason, some jurisdictions have added rare (UK) or unique (USA) characteristics of patients to the list of unique identifiers [246].

One recent challenge to the privacy of patients stems from the emergence of genomic medicine because genomic data is not considered as a unique identifier per se. This is likely to become even more challenging as the pressures from the research community grow to have as many genomes available publicly as possible [247]. Given the fact that humans differ at ~0.1% of the 3.2 billion bases of the genome, and given a world population of 6 billion, current estimates show that genotyping data from 30 to 80 alleles would be sufficient to provide unique genomic fingerprinting for every individual [248]. This number is amazingly small when compared to the datasets from whole-exome and whole-genome sequencing, which provide thousands of such alleles. Thus, reidentification of a deidentified dataset is simple if one can genotype a sample obtained from an individual that may then be used to find a perfect match against all available genomic datasets (or near-perfect match for close relatives).

In a recent tour-de-force, it was shown that it is possible to trace the original subject linked to a particular genomic dataset by combining the analysis with data from publicly available genealogical databases [249]. The "investigator hackers" were able to do this with the following publicly available information in hand: whole-genome sequencing data, gender (males), age when the samples were provided, and state where the men lived at the time (Utah).

As front-line responders deal with keen parents who are very likely to use the internet to find health information [250], pediatricians should be prepared to answer questions about the impact of genomic medicine on the privacy of their patients. The current status is that there is still considerable uncertainty in the field, but the consensus

appears to be that complete deidentification of genomic datasets is far more complex than expected [251].

Genetic Discrimination

The rapid developments in the sequencing and analysis of genomic information have forced a debate about the potential real-life consequences for patients when it is used in the clinic [252]. The reporting of diagnostic and/or incidental findings opens the door to genetic discrimination, which is defined as an "adverse treatment that is based solely on the genotype of asymptomatic individuals" [253].

Knowledge about genetic or familial risks for a variety of diseases has been used to justify health insurers' refusal of at-risk patients [254] or employers' dismissal of potential or current employees [255]. Most European countries have enacted legislation against this type of discrimination since the 1990s [256]. The US congress followed suit in 2008 with the passage of the Genetic Information Nondiscrimination Act (GINA) [257]. Canada remains the only G8 country without such legislation, and Canadians are routinely refused life and/or disability insurance because of genetic risk factors [258]. Similar problems also occur in other developed nations with national health care systems, such as Japan [259] or Australia [260].

All pediatricians ordering genetic tests for their patients should seek information about the current legal framework in their country as these may have immediate implications for the family as a whole. These issues should ideally be discussed with the family before ordering the tests.

Direct-to-Consumer Testing of Presymptomatic Minors

The first direct-to-consumer (DTC) genetic testing companies started to operate more than 10 years ago. Up until recently, they existed in a legislative void: because the tests used were developed internally (known as "home brews"), they are exempt from the tight regulations that apply to most diagnostic tests [261]. As a result of this, they were able to offer tests of questionable value, without oversight and without interaction with a healthcare professional before or after testing [262]. Once it became clear that thousands of people were paying for these services, regulatory bodies in many countries started to pay closer attention to the products offered by these companies [263], but changes in regulation have been slow to come [264]. The interested reader is directed to recent exhaustive reviews for more details on this complex topic [265, 266]. In this discussion, we will focus on issues that relate to DTC testing when applied specifically to children.

Many direct-to-consumer (DTC) genetic testing companies, most notably 23andMe, agree to perform pre-symptomatic or predictive genetic testing on children [267]. This is in direct contradiction with the professional guidelines promulgated by most professional organizations, which state that such tests should only be performed once the child can provide informed consent for themselves [268]. Additional concerns raised by the behaviour of DTC companies are that there is no requirement for these findings to be medically actionable (i.e., at a minimum, a way to prevent or treat the condition must exist to offer such tests to minors) [267]. The FDA has been investigating to determine whether tighter regulations are necessary for these companies.

A pediatrician should expect to be asked for advice regarding performing DTC genetic testing on their patients, or they may be asked to help interpret the results of such tests [269]. It may also lead to new consultations for asymptomatic children because a number of renal conditions are included in mainstream DTC reports (for example, carrier status for ARPKD, primary hyperoxaluria type 2, and tyrosinemia type 1). Since DTC companies do not spend time explaining the ethical and legal issues that stem from such testing (discussed above), the onus will be on the treating physician to do so. Unless clinically indicated, pediatricians should strongly consider refraining from ordering additional diagnostic tests triggered solely from the results

of DTC genetic testing as the clinical validity of many of the findings has yet to be established [270]. In a significant turn of events, the US Federal Drug Administration (FDA) asked 23andme to stop marketing these tests to consumers starting in November 2013; the FDA will now require DTC genomic companies to undergo regulatory clearances that are typical for genetic tests used in the clinic.

Online Resources

Sources	URLs
FDA letter to 23andme	https://peds-renomics.clinic/FDA-vs-23andme
23andme website	https://www.23andme.com/health/

Glossary

Alleles Alternative forms of a gene at the same locus.

Alternative splicing Formation of diverse mRNAs through differential splicing of an mRNA precursor.

Autosome Any chromosome (1–22) other than the sex chromosomes X and Y.

cDNA, complementary DNA DNA sequence that contains only exonic sequences and was made from an mRNA molecule.

Centimorgan Length of DNA that on average has 1 crossover per 100 gametes.

Cis Location of two genes/changes on the same chromosome.

Codon Three consecutive bases/nucleotides in DNA/RNA that specifies an amino acid.

Compound heterozygote Individual with two different mutant alleles at a locus.

Consanguineous Mating between individuals who share at least one common ancestor.

Conservation Sequence similarity for genes present in two distinct organisms or for gene families; can be detected by measuring the sequence similarity at the nucleotide (DNA or RNA) or amino acid (protein) level.

Crossover Exchange of genetic material between homologous chromosomes during meiosis.

Digenic inheritance Two genes interacting to produce a disease phenotype.

Diploid Chromosome number of somatic cells.

Domain Segment of a protein associated with a specialized structure or function.

Dominant Trait expressed in the heterozygote.

Downstream Sequence that is distal or 3′ from the reference point.

Empiric risk Recurrence risk based on experience rather than calculation.

Epigenetics Term describing non-mutational phenomena (e.g., methylation and acetylation) that modify the expression of a gene.

Euchromatin Majority of nuclear DNA that remains relatively unfolded during most of the cell cycle and is therefore accessible to transcriptional machinery.

Exon Segment of a gene (usually protein-coding) that remains after splicing of the primary RNA transcript.

Expressivity Variation in the severity of a genetic trait.

Genotype Genetic constitution of the organism; usually refers to a particular pair of alleles the individual carries at a given locus of the genome.

Germline Cell lineage resulting in eggs or sperm.

Germline mutation Any detectable, heritable variation in the lineage of germ cells transmitted to offspring while those in somatic cells are not.

Gonadal (germline) mosaicism Occurrence of more than one genetic constitution in the precursor cells of eggs or sperm.

Haplotype Group of nearby, closely linked alleles inherited together as a unit.

Heterozygote Person with one normal and one mutant allele at a given locus on a pair of homologous chromosomes.

Homozygote Person with identical alleles at a given locus on a pair of homologous chromosomes.

Imprinting Parent-specific expression or repression of genes or chromosomes in offspring.

Intron Segment of a gene transcribed into the primary RNA transcript but excised during exon splicing, thus does not code for a protein.

Isodisomy, uniparental Inheritance of two copies of one homologue of a chromosome from

one parent, with loss of the corresponding homologue from the other parent.

Karyotype Classified chromosome complement of an individual or a cell.

Lyon hypothesis (X inactivation) Principle of inactivation of one of the two X chromosomes in normal female cells (first proposed by Dr. Mary Lyon).

Mendelian Following patterns of inheritance originally proposed by Gregor Mendel.

Monogenic disorder Caused by mutations in a single gene.

Mosaicism Occurrence of more than one genetic constitution arising in an individual after fertilization.

Multifactorial disorder Caused by the interaction of multiple genetic and environmental factors.

Mutation Change from the normal to an altered form of a particular gene that has harmful; pathogenic effects.

Oligogenic inheritance Character that is determined by a small number of genes acting together.

Penetrance Frequency with which a genotype manifests itself in a given phenotype.

Phenotype Visible expression of the action of a particular gene; the clinical picture resulting from a genetic disorder.

Pleiotropy Multiple effects of a single gene.

Polymerase chain reaction (PCR) Amplification of DNA using a specific technique that allows analysis of minute original amounts of DNA.

Polymorphism Usually used for any sequence variant present at a frequency greater than 1% in a population.

Recessive A trait expressed only when both alleles at a given genetic locus are altered.

Recombination Separation of alleles that are close together on the same chromosome by crossing over of homologous chromosomes at meiosis.

SNPs (single nucleotide polymorphism) Usually used for any sequence variant present at a frequency greater than 1% in a population.

Somatic Involving the body cells rather than the germline.

Syndrome, genetic Nonrandom combination of features.

Teratogen Any agent causing congenital malformations.

Trans Location of two genes/changes on opposite chromosomes of a pair.

Transcription Production of mRNA from the DNA template.

Translation The process by which a protein is synthesized from an mRNA sequence.

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Tools for Kidney Tissue Analysis

5

Anette Melk

Introduction

The gold standard for renal tissue analysis is the kidney biopsy. It is routinely performed to allow histological diagnoses of kidney diseases and determine the extent of damage in native and allograft kidneys. However, there has been controversy over the use and interpretation of kidney biopsies. Issues include sampling errors and reproducibility between different observers. More importantly, histopathological assessment has failed to predict progression or regression of kidney diseases reducing the value of kidney biopsies as a guide for clinical therapeutic approaches. Because of this, researchers have always tried to use new methods in order to add more validity and prognostication. Needle biopsies were performed already in the 1930s, but kidney biopsies as clinical diagnostic tool were introduced in the 1960s when Jones silver stain and the new techniques of electron microscopy (EM) and immunofluorescence (IF) became available. In the 1970s, immunohistological methods were applied to identify, localize and semi-quantify immune deposits, extracellular matrix proteins and cellular infiltrates. The developments in the late 1980s and 1990s have been focused on methods to measure RNA and DNA. With the ongoing advances in kidney imaging this non-invasive, indirect technique may become the ultimate way of analyzing kidney tissue in the future.

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Kidney Biopsy

Indication

There are multiple indications to perform a kidney biopsy. The most frequent indications for native kidney biopsies in the pediatric population are nephrotic syndrome, non-nephrotic range proteinuria, asymptomatic hematuria and acute kidney injury [1, 2]. Other indications are unexplained kidney failure, nephritic syndrome, and potential kidney involvement in case of systemic diseases. Kidney transplant biopsies are performed in case of allograft dysfunction, suspected rejection, virus-associated nephropathy, and suspected *de novo* or recurrent kidney diseases. Some transplant centers advocate for the use of protocol biopsies in pediatric kidney transplantation [3, 4].

Absolute contraindications to kidney biopsy include uncorrected severe coagulopathy, uncontrolled severe hypertension, active renal or perirenal infection, or a skin infection at biopsy site. The following conditions represent relative contraindications to kidney biopsy: complex anat-

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omy of the kidney that may increase the risk of the biopsy (e.g. multiple cysts, anatomical abnormalities as in horseshoe kidneys), small kidneys or the presence of a solitary native kidney.

Procedure

In children, kidney biopsies were done using open exposure of the kidney until 1962 when White in England and in 1970 Metcoff in the United States described a modified needle biopsy procedure for children including infants [5–7]. Today percutaneous kidney biopsies in children are done under ultrasound guidance and have become a routine procedure. A report on the 22-year experience on 9288 native kidneys biopsies (715 from children) from the Norwegian Kidney Biopsy Registry came to the conclusion that the percutaneous kidney biopsy is a low-risk procedure at all ages [8]. However, in case of contraindications or after failed attempts at percutaneous kidney biopsy, open (surgical), laparoscopic or transvascular biopsies may be performed.

Despite differences in details, the procedures for a kidney biopsy are relatively standardized. In preparation for the biopsy the patient and the parents have to be informed about the possible risks of the biopsy and the potential therapeutic consequences. For safety, laboratory values that should be obtained include hemoglobin, platelet count, prothrombin time, partial thromboplastin time and bleeding time if uremic. In addition, serum creatinine, electrolytes and a urine dip stick analysis are useful as baseline parameters in case of occurring complications. Prior to performing the biopsy the nephrologist should be aware of the patient's kidney anatomy. Ultrasound examination is used to exclude contraindications.

Small children will receive general anesthesia. In larger children the procedure can safely be performed with mild sedation allowing for cooperation of the patient, but many centers use general anesthesia even in larger children. Briefly, the patient is placed in prone position with a foam roll under the upper part of the abdomen. The kidneys are localized by ultrasound from the back. The kidney, of which the lower pole is easiest to reach, is chosen for biopsy. In most cases, this will be the right kidney. The exact position of the kidney during inspiration is determined and after marking the intended entry position of the needle on the skin, the skin is cleaned with an antiseptic solution. If the patient is only sedated, the skin, the subcutaneous tissue and the muscle are infiltrated with a local anesthetic. After a small incision, the needle mounted on the semi-automated spring loaded biopsy gun is carefully introduced under ultrasound guidance until the kidney is almost reached. Manually operated needles have been widely replaced by biopsy guns because of the easier use and lower complication rates. The patient is then advised to take a breath and hold the air. In case of general anesthesia, the anesthetist will hold the patient in deep inspiration. The needle is quickly advanced to the capsule of the kidney and the biopsy is taken (Fig. 5.1). Ideally, the whole procedure is followed on the ultrasound screen to visualize the path of the biopsy needle.

After the procedure, the patient usually stays in bed for 24 h with compression of the puncture site. However, during the past several years, kidney biopsies have been performed on an outpatient basis, where stable patients are discharged after about 8 h [9]. To assure brisk diuresis the patient receives either a glucose/sodium chloride solution intravenously and/or is asked to drink a lot. Urine is collected in single portions and is examined with urine dip sticks. Hemoglobin levels and ultrasound controls are performed in most centers after 4–6 h and after 24 h. Hourly controls of blood pressure and heart rate have to be done.

The primary major complication remains macroscopic hematuria that occurs in 0.8–12% of biopsies performed in pediatric patients. Other complications may include subcapsular or perirenal hematoma, possible need for transfusion, infection, and pain requiring medication. Arteriovenous fistulas are diagnosed more often in recent years because of the improvement in Doppler ultrasound technique. Table 5.1 provides on overview of the efficacy and most frequent complications with regard to different biopsy techniques over a period of three decades [10].

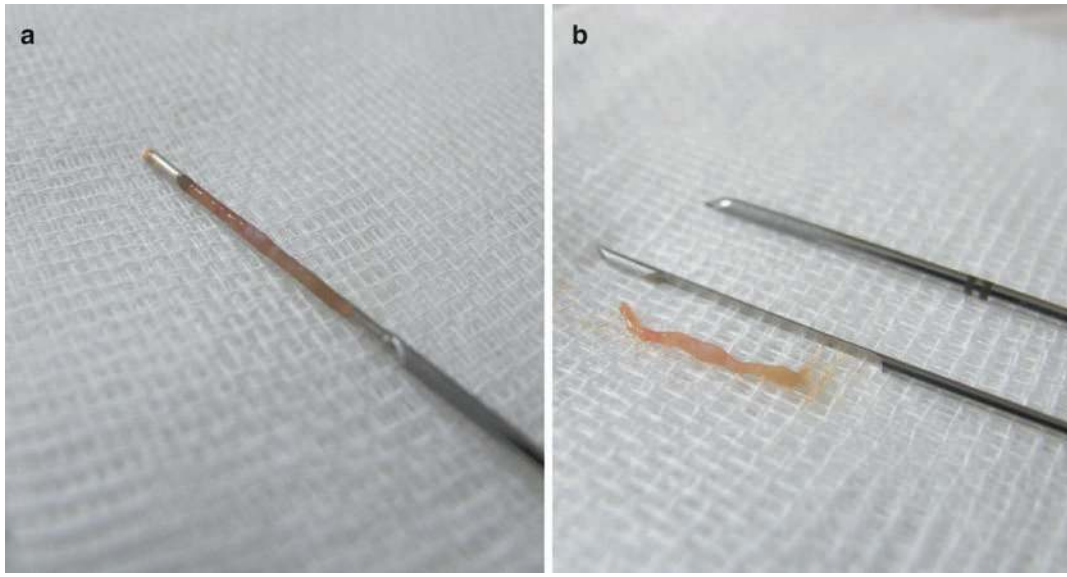

Fig. 5.1 Kidney biopsy needle and biopsy specimen. (**a**) Biopsy specimen still captured in the notch of the stylet. (**b**) Biopsy specimen sitting next to the inner needle and outer trocar

Table 5.1 Historical evolution of percutaneous biopsy technology in children (modified after [10]). While the efficacy significantly improved over time, the rate of complications did not change

Periods	1969–1974	1974–1985	1985–1990	1990–1992	1992–1996
Needle	Silverman	TrueCut	TrueCut	Biopsy	Biopsy
Localization of kidney	Radiocontrast imaging	Radiocontrast imaging	Pre-biopsy ultrasound	Pre-biopsy ultrasound	Ultrasound guidance
Efficacy					
No. of passes per session, %	3.04	2.98	2.86	2.60	2.45
Tissue-yielding punctures, %	78	87	90	87	94
No. of glomeruli per session	22.3	24.3	26.4	28.4	33.7
Complications					
Microhematuria, %	21.7	32.5	26.3	47.0	40.3
Macrohematuria, %	2.7	16.7	15.8	8.3	4.4
Perirenal hematoma, %			32.3	46.7	55.8

Processing of Biopsy Specimens

As important as accurate performance of the biopsy is adequate processing and interpretation of the specimen [11, 12]. The Renal Pathology Society has published practice guidelines that address specimen handling and processing [13].

Prior to fixation, each core should be examined for the presence and number of glomeruli by light microscopy with tenfold magnification. Based on sample size and location this examination should lead to a decision on whether more kidney tissue is needed. One should take into account the number of glomeruli as well as the suspected diseases process. It cannot be emphasized enough that adequacy of sample size is crucial for the validity of a biopsy specimen. In order to diagnose a focal disease process such as focal segmental glomerulosclerosis (FSGS) recognition of a single abnormal glomerulus is required.

Table 5.2 Minimum number of harvested glomeruli required to allow concluding a minimal fractional involvement (e.g. % sclerotic glomeruli in FSGS, % crescents in extracapillary GN) (from [100])

Number of harvested glomeruli	Minimal number of abnormal glomeruli (absolute and %) required to reliably estimate extent of involvement		
	≥80%	≥50%	≥20%
8	8 (100)	7 (88)	3 (38)
10	10 (100)	8 (80)	4 (40)
12	12 (100)	9 (75)	5 (42)
15	14 (93)	11 (73)	6 (40)
20	19 (95)	14 (70)	7 (35)
25	23 (92)	17 (68)	9 (36)
30	28 (93)	20 (66)	10 (33)
35	32 (91)	23 (66)	11 (31)
40	36 (90)	26 (65)	12 (30)

The probability to make this diagnosis depends on the fraction of affected glomeruli per kidney as well as on the glomeruli present in a given biopsy specimen. The same holds true for the assessment of the extent of a disease with variable pathologic involvement among glomeruli. Corwin and colleagues have published estimates on the minimum number of abnormal glomeruli that have to be present in a biopsy core that contains a certain number of glomeruli to infer with 95% confidence that a disease process involves 20%, 50% or 80% of the kidney (Table 5.2). Glomeruli within the juxtamedullary region are the ones to be involved first with FSGS, highlighting the importance that this area is represented in the sample. Overrepresentation of global sclerosis, however, may result from subcapsular cortical specimens and needs to be considered when dealing with wedge biopsy taken during an open biopsy of the native kidney or at implantation of a kidney transplant. It is also of note that the number of glomeruli that are retrieved per centimeter core length decreases with age (Fig. 5.2) [10].

Appropriate processing and potentially fixation of the biopsy should be done as soon as possible because small cores can dry out fast. The choice of fixatives should be discussed with the local nephropathologist. In pediatric kidney diseases, light microscopy (LM) alone can be insufficient to make a diagnosis. Therefore, specimens for IF and EM should be obtained. Because of the

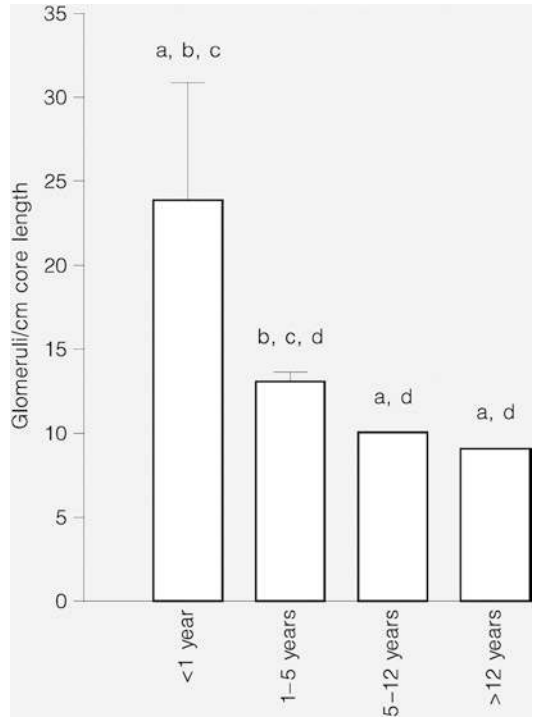

Fig. 5.2 Number of glomeruli per centimeter core length derived from native kidneys shown for different age groups. (From [10])

superior morphology, some nephropathologists prefer fixation with Bouin's alcohol picrate solution. This, however, may lead to problems if immunohistochemistry (IHC) staining needs to be performed. Fixation with paraformaldehyde or buffered formalin (4%, pH 7.2–7.4) followed by paraffin embedding is therefore common practice in most pathology departments. For IF, kidney tissue should be "snap-frozen" in liquid nitrogen or can be placed in tissue transport media or isotonic sodium chloride solution if transported to the laboratory immediately. Specimens for EM are usually fixed in a solution containing 0.1–3% glutaraldehyde.

LM specimens should be sectioned at a thickness of 2 μm or less by an experienced technician. Subtle pathologic changes are more easily detected in thinner sections and section thickness is an important issue for glomerular pathology, especially when assessing for cellularity.

A number of histochemical stains are used to evaluate kidney biopsies (Fig. 5.3). Typically,

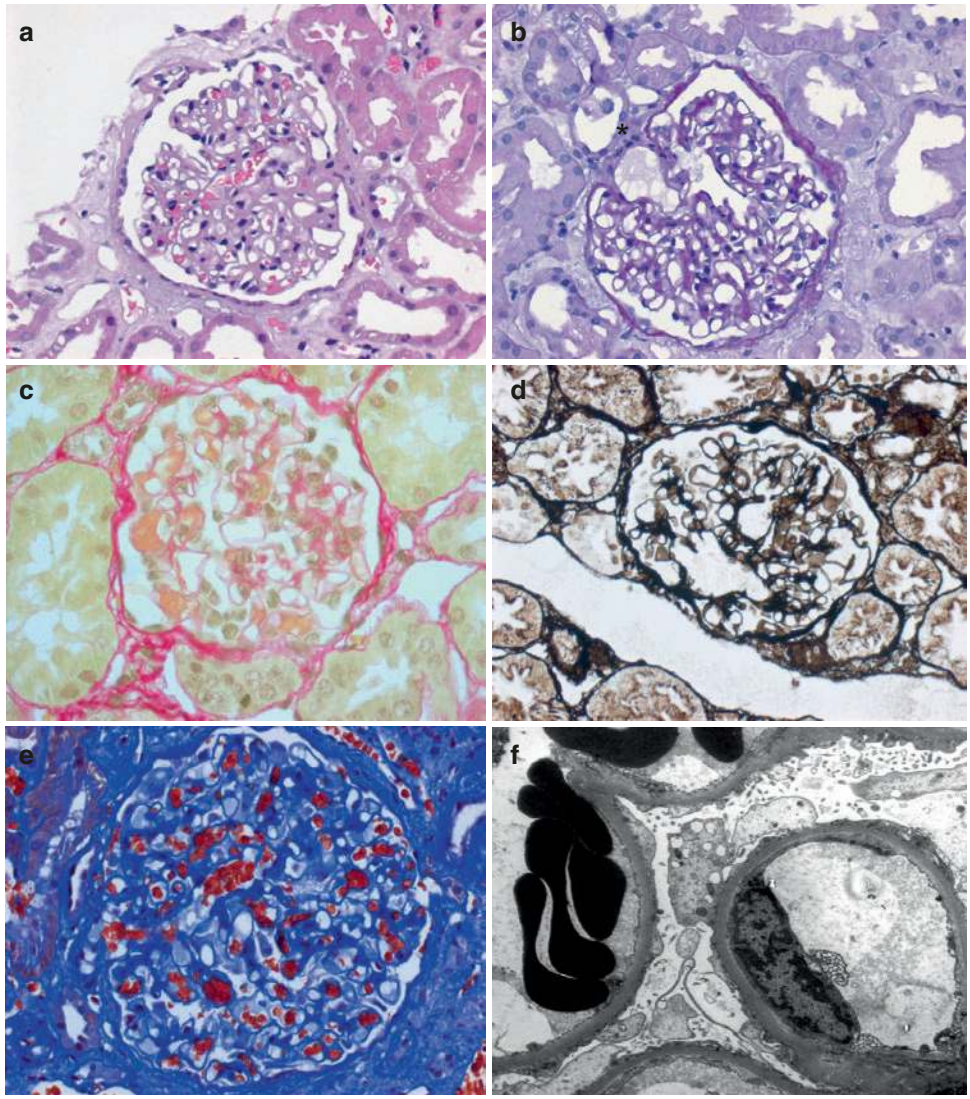

Fig. 5.3 Overview of the different stainings that are used to evaluate a kidney biopsy. Representative light (**a**–**e**) and electron microscopy (**f**) of minimal change glomerulopathy (MCGN) with mild hypercellularity. (**a**) Hematoxylin eosin stain (HE): the HE stain is the work horse of pathology; it is useful to get a first idea about glomerular changes such as proliferation and matrix deposition. In the case of MCGN the glomerulus shows a slightly increased number of mesangial cells, open capillaries, no evidence of intra- or extracapillary proliferation and normal thickness of glomerular basement membrane (GBM). (**b**) Periodic acid-Schiffs (PAS) stain: PAS staining is helpful to analyze changes in glomerular cell number and GBM in more detail. In the case of MCGN mild segmental hypercellularity (*) and normal thickness of GBM without any irregularities is seen. Some podocytes (arrows) are slightly enlarged and appear detached from the GBM. (**c**) Sirius red stain (fibrous tissue stain): this fibrous tissue stain is helpful to analyze the amount of fibrous tissue (fibrosis) of glomeruli and most importantly the interstitial tissue (interstitial fibrosis). In the case of MCGN a normal amount of fibrous tissue is found in Bowmans capsule and no increase in mesangial matrix is visible. (**d**) Silver stain: the silver stain is most useful to detect thickening and irregularities of the GBM. In the case of MCGN no thickening and spike formation is seen. (**e**) Acid fuchsin-Orange G stain (SFOG): this stain is helpful to detect protein deposition, i.e. immune complex formation, which appear in bright red within the mesangial matrix or the GBM. In the case of MCGN no immune deposits are present. (**f**) Electron microscopy of a capillary loop shows typical changes in MCGN, i.e. normal thickness of GBM with no evidence immune complex deposition, but with effacement of podocyte foot processes and loss of endothelial fenestration. (Pictures are courtesy of Dr. Amann, Erlangen)

biopsy specimens are stained with hematoxylin and eosin (H+E), periodic acid-Schiff (PAS) or periodic acid-methenamine silver (Jones silver) and Masson's trichrome. H+E is used for general morphology and reference, whereas the other three stains provide a clear distinction of extracellular matrix from cytoplasm. Depending on indication Congo red stain (amyloid), Kossa stain (calcifications), elastic tissue stain (loss of elasticity in arteries, arterial thickening) and other stains are used. IF uses fluorophore-labelled antibodies to detect and localize immunoglobulins (IgA, IgG, IgM) and their light chains (κ, λ), components of the classical or alternative complement pathways (C1q, C3c, C4), albumin and fibrinogen. In transplant biopsies, staining for C4d, a fragment within the complement pathway, has become a major diagnostic tool in antibody-mediated rejection [14]. The pattern of fluorescence positive stain should be noted, such as mesangial versus capillary staining and linear or granular staining. The granular pattern has an EM counterpart and corresponds to extracellular, electron dense masses.

EM studies do not need to be part of the routine work up of every kidney biopsy. However, EM plays an important diagnostic role in almost 50% of cases and is essential for a correct diagnosis in up to 21% [15, 16]. Even though it is sometimes possible to omit EM after evaluation of LM and IF, specimens for EM studies should always be procured. EM can localize deposits (mesangial, subendothelial, or subepithelial). EM is able to detect changes in cell structure (e.g. fusion of podocyte foot process, podocyte vacuolization) and alterations of the basement membrane (thickening, thinning, splicing, duplication and other irregularities). The definite diagnosis of e.g. minimal change nephropathy, Alport's disease or thin basement membrane disease requires EM [17, 18].

Histopathological Assessment

Abnormalities in kidney structures can occur in all four compartments of the kidney: glomeruli, tubules, interstitium and vasculature [19, 20]. Numerous consensus classifications have been developed for specific diseases [21–25] and those classifications, as well as the specific pathological changes seen with certain diseases, are discussed in the relevant chapters of this textbook. This chapter provides a general overview on the various histopathological features that can be encountered while reading a biopsy [12, 26, 27].

Glomerular changes are the primary pathological event in many kidney diseases. If glomeruli are affected it needs to be decided whether these changes are diffuse (defined as ≥50% of glomeruli involved) or whether the disease process is focal (defined as <50% of glomeruli involved). By assessing single glomeruli a decision has to be made whether the disease process involves only part of the glomerulus, i.e. is segmental, or the whole glomerulus, which is considered global. Glomerular sclerosis refers to an increase in extracellular material (e.g. hyaline) within the mesangium that leads to compression of the capillaries. The capillary basement membrane has a wrinkled appearance and adhesions to Bowman's capsule are found. Depending on the expansion of the sclerotic lesion, it can be either segmental or global. Hypercellularity is a descriptive term reflecting an increased number of cells (e.g. mesangial cells, endothelial cells, inflammatory cells) in the mesangial space, internal to the glomerular basement membrane or in the Bowman's space, which is called mesangial, endocapillary or extracapillary hypercellularity respectively. Glomerular diseases involving hypercellularity are often called "proliferative". Changes of the basement membrane that can be seen by light microscopy involve thickening of the basement membrane and basement membrane double layering. Whereas the first is caused by the accumulation of basement membrane material, the latter is meant to be caused by peripheral interposition of mesangial material. Severe glomerular diseases show the formation of crescents. Crescents are located in the urinary space of the glomerulus and consist of cells and extracellular material. The proportion of glomeruli affected by crescents is of enormous prognostic importance in acute glomerulonephritis/vasculitis. The most subtle glomerular damage is

effacement of foot process and refers to the loss of normal podocyte morphology with an undivided cytoplasmic mass covering the basement membrane. Effacement of foot processes cannot be seen by light microscopy, but is easily found by electron microscopy.

Tubular cells can show various signs of damage. This includes loss of brush border (in proximal tubules), flattening of the tubular epithelium, and detachment of tubular cells from the basement membrane, necrosis and apoptosis. Mitosis of tubular cells is often found after an episode of acute tubular necrosis as a sign of repair. Tubulitis, an important feature in acute allograft rejection, refers to the presence of inflammatory cells that have crossed the tubular basement membrane and infiltrate the tubular epithelium. Tubular changes when chronic occur as tubular atrophy. Atrophic tubules have a reduced diameter and a thickened basement membrane. Tubular atrophy is often accompanied by interstitial fibrosis. Another type of tubular pathology is the accumulation of droplets containing various substances. This for example can occur in patients with heavy proteinuria or with various storage diseases. A foamy appearance of the tubular epithelium is called vacuolization and can occur with several conditions. Intranuclear inclusions can indicate viral infection (see below), can occur non-specifically with different nephropathies, and can also reflect regeneration after acute tubular necrosis. The occurrence of coagulated proteins or formed elements in the tubular lumen is described as tubular casts (e.g. RBC casts).

Interstitial changes are edema, fibrosis and infiltration by inflammatory cells. Edema indicates acute diseases whereas fibrosis is the sequelae of chronic kidney damage. In both cases, tubules are no longer "sitting back to back" but are separated by interstitial material. The degree of interstitial fibrosis is of prognostic importance in chronic kidney diseases. In many instances, the degree of interstitial fibrosis found in a primary glomerular disease is a more powerful predictor of outcome than the glomerular changes itself. Infiltration of inflammatory cells can be the cause of kidney disease such as acute interstitial nephritis or acute transplant rejection, but an interstitial infiltrate can also be a mere accompanying phenomenon, e.g. in fibrosis.

The kidney vasculature can also show a range of pathological changes. Inflammation (vasculitis) may affect any vessel. If only the arterial subendothelial space is affected, this subtype is called endothelialitis, a finding peculiar to vascular rejection. Direct damage of endothelial cells, e.g. through *E. coli* toxins in hemolytic uremic syndrome (see Chap. 24), leads to thrombotic microangiopathy with endothelial cell swelling and intimal edema that is followed by platelet fibrin thrombi. Hypertension can lead to vascular changes affecting all parts of the arterial wall. This includes fibrous intimal thickening and medial hypertrophy. Overall, the vessel walls can be thickened and hyalinized, in extreme cases leading to complete obstruction of the vessel lumen. Very high blood pressure can result in fibrinoid necrosis, an endpoint also seen in other thrombotic microangiopathies.

Pathologists summarize the findings seen in the different kidney compartments based on their assessment using LM, IF, IHC and EM. Recently, a minimum reporting standard for nonneoplastic biopsies has been proposed to facilitate optimal communication between pathologists and nephrologists and eventually to optimize patient care [28].

Protein Analysis

Proteins in kidney tissue are classically analyzed by immunostaining, using either IF or IHC for visualization. Both techniques use antibodies or antisera directed against the protein of interest. They can be performed both in native and formalin-fixed tissues. As fixatives can mask protein epitopes, antigen retrieval steps become necessary, particularly for nuclear antigens. Detection and visualization of unlabeled primary antibody that specifically binds the target epitope is achieved by a secondary antibody, which for IF carries a fluorophore or for IHC either peroxidase or alkaline phosphatase. In order to further enhance the signal, especially if the target antigen is rarely expressed, amplification systems

such as the streptavidin-biotin-peroxidase system are used for IHC. Direct IF or IHC, for which the primary antibody is linked to a fluorophore or peroxidase, is also possible.

Both IF and IHC have advantages and disadvantages. IF is most often performed on frozen sections. It is a technically easy and fast procedure. Processing, sectioning and staining can be performed within 1–2 h. Even though the cost of the procedure itself is low, storage is difficult as the fluorophores fade over time. A broad range of suitable antibodies is available and background staining is usually not a problem. However, in case of frozen sections an additional sample for assessment of histopathological details may be required to provide good morphology. IHC can be done using the same tissue as for LM. It provides much better morphological details and causes permanent staining, in contrast to IF. IHC is highly sensitive because of the possibility to enhance the signal by certain amplifiers, but can be technically challenging (e.g. due to higher background staining) and expensive.

Some of the applications of IF and IHC in non-neoplastic kidney biopsies have already been described above as they are part of the standard work-up of biopsy specimens. Some pathology centers prefer to use IHC even for the standard workup because of the possibility to store and archive the slides for future comparisons. In addition, IHC is used to detect subtypes of type IV collagen (see Chap. 16) and viral antigens, especially in allograft biopsies, although PCR techniques are currently taking over because of their higher sensitivity. A practical example for virus detection with IHC is BK polyoma virus. Demonstration of typical smudgy tubular cells with enlarged nuclei gives direct evidence for viral tissue invasiveness [29]. A pleomorphic infiltrate with lymphocytes, plasma cells and PMNs is highly suspicious of BK virus nephropathy. Diagnostic confirmation can be achieved by IHC using a monoclonal antibody directed against simian virus 40 (SV-40) large T antigen, which is common to all known polyoma viruses [19]. In case expansile/dysplastic plasma cells are found in the interstitium of an allograft biopsy specimen, staining for Epstein-Barr virus (EBV) may be useful to make the diagnosis of post-transplant lymphoproliferative disorder (PTLD) as most but not all PTLDs are EBV positive [30].

While proteomic tools represent a major technological advancement in protein analysis, they have not been included in the routine work-up of kidney biopsies to date. Proteomics has been coined the “non-invasive kidney biopsy”, reflecting that most proteomics approaches nowadays are performed in urine. Proteomic analysis in kidney tissue has been used to identify biomarkers enabling diagnosis, disease monitoring, and treatment of kidney malignancies, especially renal cell carcinoma [31–33]. Proteomic tools may become increasingly interesting to study microdissected structures from biopsies [34, 35] as demonstrated for different glomerular diseases [34–37] and amyloidosis [38–41]. For example, mass spectrometry, by providing the molecular composition of micro-dissected deposits, is a sensitive and specific technique accurately diagnosing kidney amyloidosis [39, 40, 42]. The fact that formalin-fixed tissue even from archived tissue blocks can be used for such analyses will support future use of this approach [43, 44]. The Kidney Precision Medicine Project has recently proposed to combine traditional and digital pathology with transcriptomic, proteomic, and metabolomic analysis of the kidney tissue not only to create a reference kidney atlas, but also to characterize disease subgroups [37].

RNA Analysis

Transcriptomic analysis allows to gain insight in physiologic and pathologic processes within the kidney. RNA expression can be evaluated from as little as 10% of a biopsy core [45]. The use of RNase inhibitors stabilizes and protects the integrity of RNA in unfrozen tissue samples. Importantly, kidney tissue can be stored in these agents allowing for later micro-dissection and immunohistochemical analysis [45]. Hence, RNA expression can be evaluated from fixed and processed kidney biopsy samples, allowing for molecular analysis in combination with routine histology assessments. RNA can be isolated from

frozen sections, but also from formaldehyde-fixed, paraffin-embedded tissues [46]. This methodology enables RNA analysis even after years of storage, allowing for a correlation of expression profiles from archived materials with the subsequent clinical disease course.

Even with these sensitive methods of RNA detection, the different compartments of the kidney contribute or respond differently to diseases or injury and signals may be underestimated or even missed. Manual dissection or sieving allows to compare glomeruli with tubulointerstitium [47]. Laser-assisted microdissection allows to select a defined histological structure from a given biopsy slide, allowing direct correlation of information from histology and gene expression for the same nephron segments. While the technique has been used successfully in fixed kidney tissue [48, 49], the challenge is to retrieve sufficient high-quality material. Several technical reports have been published [46, 50–52]. Laser-assisted microdissection is often used in combination with RNA amplification protocols with 1000-fold linear amplification efficiency to generate gene profiles that are specific for a certain nephron segment. This approach has been used to generate expression profiles of single glomeruli derived from biopsies of lupus nephritis [53]. The study revealed considerable kidney-to-kidney heterogeneity, whereas glomerulus-to-glomerulus variation within a kidney was less marked.

High throughput gene expression technologies emerged in the mid 1990s and a number of different microarray platforms became available. A microarray chip assembles a number of gene-specific probes (clones or oligonucleotides) spotted on a small surface area with high density. The underlying principle of microarray is that after labeling transcripts from a specific sample and hybridizing them to an array, the amount of sample material bound to the specific complementary probe set is measured. As variation across the different platforms is an important issue, a number of studies have compared different platforms. Some claimed significant divergence across technologies, whereas others found an acceptable level of concordance [54, 55]. Nowadays chips are commercially available from different suppliers and the method is highly standardized, which allows for comparison and exchange of data between different laboratories as long as the same chips are utilized. In contrast to those high density arrays, low density arrays allow for the simultaneous evaluation of a few hundred genes and are based on a reverse transcriptase-polymerase chain reaction (RT-PCR) technique.

Gene expression profiling has been performed investigating large cohorts of patients with native kidney diseases or after transplantation [56, 57]. The major challenge of any of those expression analyses remains the extraction of biological insight from such information. Ideally, one would like to recognize specific patterns or pathways involved in certain diseases (Fig. 5.4). Approaches combining conventional histological assessment with molecular analysis for transplant biopsies, called the molecular microscope [58], have created a new understanding of transplant disease states and their outcomes [59–61]. Despite strong associations described for certain genes with specific diseases, none of those genes have made it into routine diagnostics to date.

The introduction of single cell RNA sequencing has revolutionized the field of tissue transcriptomics [62] (Fig. 5.5). While methods that measure RNA in small biopsy samples had previously been applied to analyze diseased native kidneys as well as allografts, single cell RNA sequencing allows to monitor gene expression in thousands of individual cells in a single experiment. Importantly, a combination with other Omics approaches in single-cell multi-omics analyses (mRNA plus genome, mRNA plus DNA methylation, mRNA plus chromatin accessibility, and mRNA plus protein) is often applied to gain a comprehensive understanding of cellular pathways [63–67]. The single-cell transcriptomic landscape of certain kidney structures (e.g. glomeruli [68]) or diseases [69, 70] have been described. A pre-requisite for an accurate assessment of gene expression on a single cell level is, however, the creation of a single cell suspension that preserves the viability of cells and mirrors the cellular composition in the kidney, which can be a challenge. Data from such experiments is generally made available to the public, e.g. as

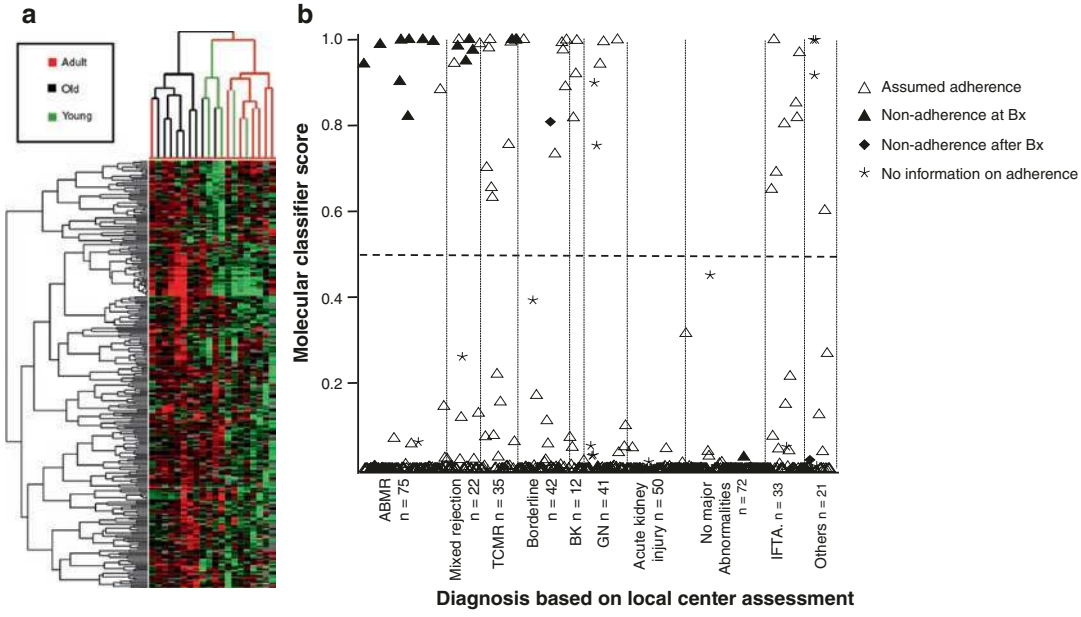

Fig. 5.4 (**a**) Dendrogram used in gene array analysis. Hierarchical cluster of genes that are differentially expressed based on kidney age (Y, young kidneys, A, adult kidneys, O, old kidneys). The color from green to red reflects increasing gene expression. Based on such gene array analysis in large cohorts, researchers have described molecular classifiers shown in (**b**) relationship between a molecular classifier score and diagnoses based on local center assessment. Molecular classifiers are able to distinguish antibody- and T-cell-mediated rejection. The order within each diagnostic category is random. Horizontal line represents arbitrary threshold of 0.5 for defining high versus low scores. Symbols indicate information about medication adherence. (Figure kindly provided by Dr. Einecke, Hannover)

single cell data sets or atlases, allowing exploration and comparison of own data against these existing data sets.

New technologies usually come along with some challenges. An important limitation of single cell RNA sequencing is the low coverage. Compared to traditional experiments using bulk RNA, the number of reads per sample is considerably lower for single cell experiments. This is especially important for genes with low expression levels, e.g. the senescence gene $p16^{INK4a}$, for which rarely any or only few corresponding reads are captured. Considerable variation in gene expression between two cells of the same type may occur as a result of the stochasticity in capturing and therefore require appropriate statistical modelling. Filtering of low-quality cells and especially of so-called doublets (libraries representing more than one cell captured in a single bead) are important quality control steps. Another important limitation is the loss of spatial information caused by the dissociation of the tissue. While techniques to overcome these limitations have been described [71–73], the need to work with fresh materials remains a major pre-analytical challenge and prevents large scale collection and analysis of human samples. Isolation protocols for archived tissues have been published; these also promise less bias between cell types [74]. Other approaches propose the use of nuclei, so-called single nucleus RNA-sequencing allowing the use of frozen tissue [75]. As further techniques are being developed, bioinformatic integration of multi-omics datasets from single-cell analyses represents a major challenge [76]. Innovative computational tools will be required that will accommodate the current limitations [77].

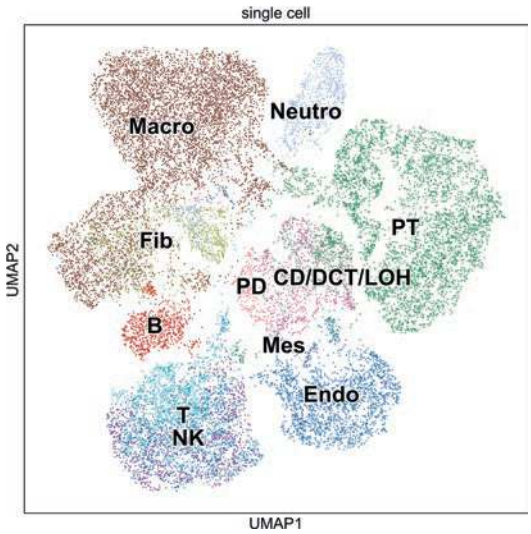

Fig. 5.5 Visualization of data from a single cell RNA-sequencing experiment using mouse kidney tissue. The high-dimensional data from such experiments is typically presented as so-called t-SNE (t-Distributed Stochastic Neighbor Embedding) plot. This technique assembles cell populations based on their expression profile. *B* B cells, *CD* collecting duct cells, *DCT* distal tubular cells, *Endo* endothelial cells, *Fib* fibroblasts, *LOH* loop of henle cells, *Macro* macrophages, *Mes* mesenchymal cells, *Neutro* neutrophils, *NK* natural killer cells, *PT* proximal tubular cells, *Podo* poocytes, *T* T cells. (Figure is retrieved from unpublished data, Drs. Melk and Schmitt)

DNA Analysis

Detection of viral DNA by PCR using sequence specific primers for a large panel of viruses such as BK virus, CMV, EBV, other herpes viruses, and hepatitis B virus is possible [78–81], but rarely used in clinical practice. Measurements of viral load to diagnose and monitor affected patients, especially after transplantation (see also Chap. 69), are preferentially performed in blood and urine [82–84]. Pathologists typically combine this information, the histopathological features and the available immunostaining methods to make the diagnosis of a virus-associated process in the kidney.

Molecular cytogenetic techniques such as chromosomal comparative genomic hybridization (CGH) are performed on tumor tissue. These techniques have improved the diagnosis of chromosomal aberrations e.g. in Wilms' tumor, but have only a limited resolution across the whole genome. The development of genomic arrays allows the assessment of the whole genome at a much higher resolution at a sub-microscopic or sub-band level [85, 86].

Indirect Measurements

Even though the complication rate with kidney biopsies is low, taking a biopsy is still an invasive procedure. Therefore, methodological progress should aim for indirect and non-invasive methods to assess the status of a kidney *in vivo* in order to minimize the need for biopsies. The kidney is well suited for such indirect measurements, as urine represents an easily accessible direct readout of the organ of interest. Indeed, proteinuria has been used for decades as a biomarker of disease activity, however it lacks disease specificity. Ideally, one would like to use urinary markers that are highly sensitive and specific for individual disease entities to screen and diagnose a kidney disease.

Urinary extracellular vesicles have become such attractive tools in biomarker development. These vesicles are secreted membrane-coated structures that allow conclusions on the cells of their origin through extraction of protein, mRNA, miRNA and lipid [87]. Urinary exosome signatures have been proposed to diagnose kidney transplant rejection [88], but also for autosomal dominant polycystic kidney disease [89]. In addition, some of the technologies discussed above have been applied to cells derived from urine. Recently single cell profiling was applied to human urine samples and shows that almost all cell types from the kidney and urinary tract can be detected and quantified in urine [90].

Transcriptome studies of urine cell extracts have identified a number of candidate biomarkers; e.g., CD3ε mRNA, IP-10 mRNA, and 18S rRNA levels in urinary cells have been postulated to detect or predict the outcome of acute rejection in kidney allograft recipients [91]. However, such measurements have not entered

routine diagnostic practice to date, highlighting the challenges in biomarker development. More advances have been made for the use of the urinary proteome [92].

Imaging methods as indirect tools to evaluate kidney tissue are also discussed elsewhere (see Chap. 3), Novel functional magnetic resonance imaging (MRI) techniques allow for detailed assessments of both kidney structure and function. While arterial spin labeling measures perfusion [93], diffusion-weighted MRI enables the assessment of fibrosis and microstructure and blood oxygen level dependent imaging detects hypoxia [94, 95]. A high cortical relaxation rate, which indicates lesser oxygenation, has been associated with faster eGFR decline [96, 97]. A longitudinal study on patients with CKD could not confirm an association between a low apparent diffusion coefficient (ADC), used as a an indicator of tissue fibrosis, and changes in eGFR over a period of 12 months after adjustment for albuminuria [98]. A much smaller study in transplant patients suggested that ADC may detect fibrotic changes and thereby disease progression earlier than through eGFR decline [99]. While it is not predictable when MRI biomarkers become available for clinical use, it is conceivable that with further technological progress applications assessing indicating oxygenation and fibrosis could replace the need for histopathological assessment in certain clinical settings.

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Part II

Disorders of Kidney Development

Functional Development of the Nephron

6

Aoife Waters

Abbreviations

ACE	Angiotensin converting enzyme
ACEI	Angiotensin converting enzyme inhibitors
ADH	Antidiuretic hormone
ANP	Atrial natriuretic peptide
AQ2	Aquaporin-2
AT1	Angiotensin type 1 receptors
BK	Bradykinin
CA	Carbonic anhydrase
cAMP	Cyclic adenosine monophosphate
CCD	Cortical collecting duct
CD	Collecting duct
cGMP	Cyclic guanosine monophosphate
COX-2	Cyclooxygenase type-2
ENaC	Epithelial sodium channel
ET	Endothelin
FGF-23	Fibroblast growth factor 23
GA	Gestational age
GFB	Glomerular filtration barrier
GFR	Glomerular filtration rate
GH	Growth hormone
IGF-1	Insulin growth factor-1
KK	Kallikrein
NaCl	Sodium chloride
NAG	*N*-acetyl-β-D-glucosaminidase
NCC	Sodium chloride co-transporter
NHE3	Sodium-hydrogen antiporter 3
$NKCC_2$	Sodium-potassium-chloride cotransporter
NO	Nitric oxide
PGs	Prostaglandins
PTH	Parathyroid hormone
RAS	Renin–angiotensin–aldosterone system
ROMK	Renal outer medullary potassium channel
RVR	Renal vascular resistance
TALH	Thick ascending limb of loop of Henle
TRPV5	Transient receptor potential cation channel subfamily V member 5
TTKG	Transtubular potassium gradient
VMNP	Vasomotor nephropathy

General Overview of Antenatal, Perinatal, and Postnatal Fluid and Electrolyte Homeostasis

Fluid and electrolyte homeostasis in the fetus is controlled by the placenta. As a result, the placenta receives a significant proportion of the fetal cardiac output (33%), whereas the fetal kidneys receive only 2.5% even in late gestation [1]. The low fetal renal blood flow (RBF) results in a low creatinine clearance which correlates well with gestational age (GA) (Fig. 6.1a, b). Urine pro-

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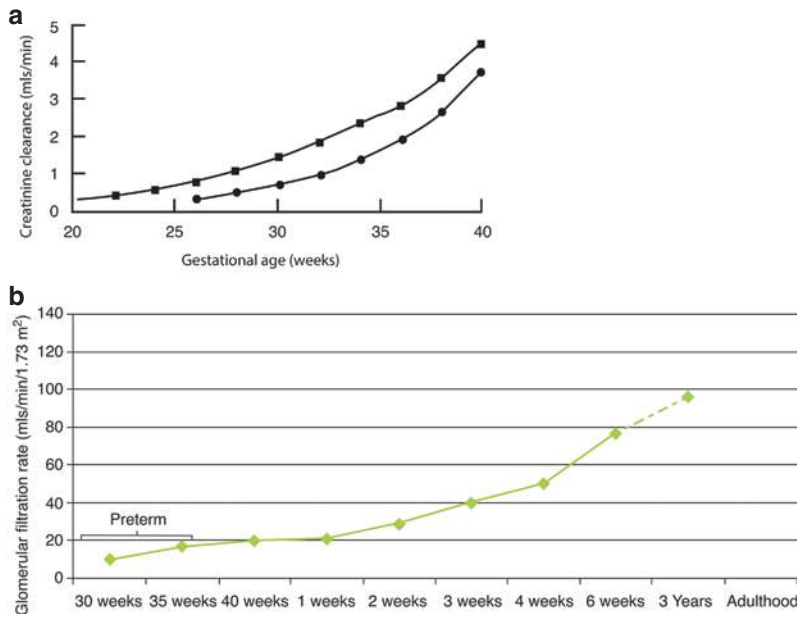

Fig. 6.1 (**a**) Creatinine clearance for human fetuses from 20 weeks. (■) and corresponding preterm neonates (●). Creatinine clearance increases with increasing gestational age (used with permission of John Wiley and Sons from Haycock [2]). (**b**) GFR doubles over the first 2 weeks of life in term infants and reaches almost 50 mL/min/1.73 m^2 between 2 and 4 weeks after birth and adult values by 2 years of age. (Used with permission of Vieux et al. [3])

duction begins at approximately 10 weeks of gestation in the human kidney. This coincides with the acquisition of the first capillary loops by the inner medullary metanephric nephrons. Subsequently, hourly fetal urine production increases from 5 mL at 20 weeks to approximately 50 mL at 40 weeks [4]. After 20 weeks, the kidneys provide over 90% of the amniotic fluid volume [5]. Severe oligohydramnios due to abnormal fetal renal function in the second trimester can result in pulmonary hypoplasia and in severe cases, Potter's syndrome [6].

At birth, the newborn consists largely of water, with total body water comprising 75% of body weight at full term and about 80–85% in preterm infants [7]. Adaptation to the extrauterine environment involves an increase in glomerular filtration with an immediate postnatal natriuresis. High circulating levels of atrial natriuretic peptide (ANP) in the newborn are responsible for the postnatal physiological natriuresis [8]. In addition, maturation of tubular function occurs postnatally [9]. Changes include an increase in resorptive surface area, transporter number and function, together with further modification of paracrine regulatory mechanisms [9]. In the following section, we will discuss the developmental changes in the neonatal kidney that are necessary for extrauterine adaptation. Regulatory mechanisms of the mature kidney will be discussed elsewhere.

Glomerular Function in the Fetal, Perinatal, and Postnatal Period

Glomerular filtration is the transudation of plasma across the glomerular filtration barrier (GFB) and is the first step in the formation of urine (Fig. 6.2). Filtration depends both on Starling's forces and an adequate RBF [10]. The total glomerular filtration rate (GFR) is the sum of the GFR of each single functioning nephron, SNGFR [where SNGFR = $(k \times S) \times (\Delta P - \Delta\pi)$]. ΔP, is the hydrostatic pressure difference between the glomerular capillary pressure (P_{GC}) and the hydrostatic pressure in Bowman's space (P_{BS}). $\Delta\pi$ is the oncotic pressure difference between the glomerular capillary pressure (π_{GC}) and the oncotic pressure in Bowman's space (π_{BS}). K_f is the product of the hydraulic permeability of glomerular capillary walls (k) and the surface area available for filtration, (S), ($K_f = k \times S$). In an adult, the rate of glomerular filtration is about 100–120 mL/min/1.73 m^2. Even though the term neonate has the full number of glomeruli, its

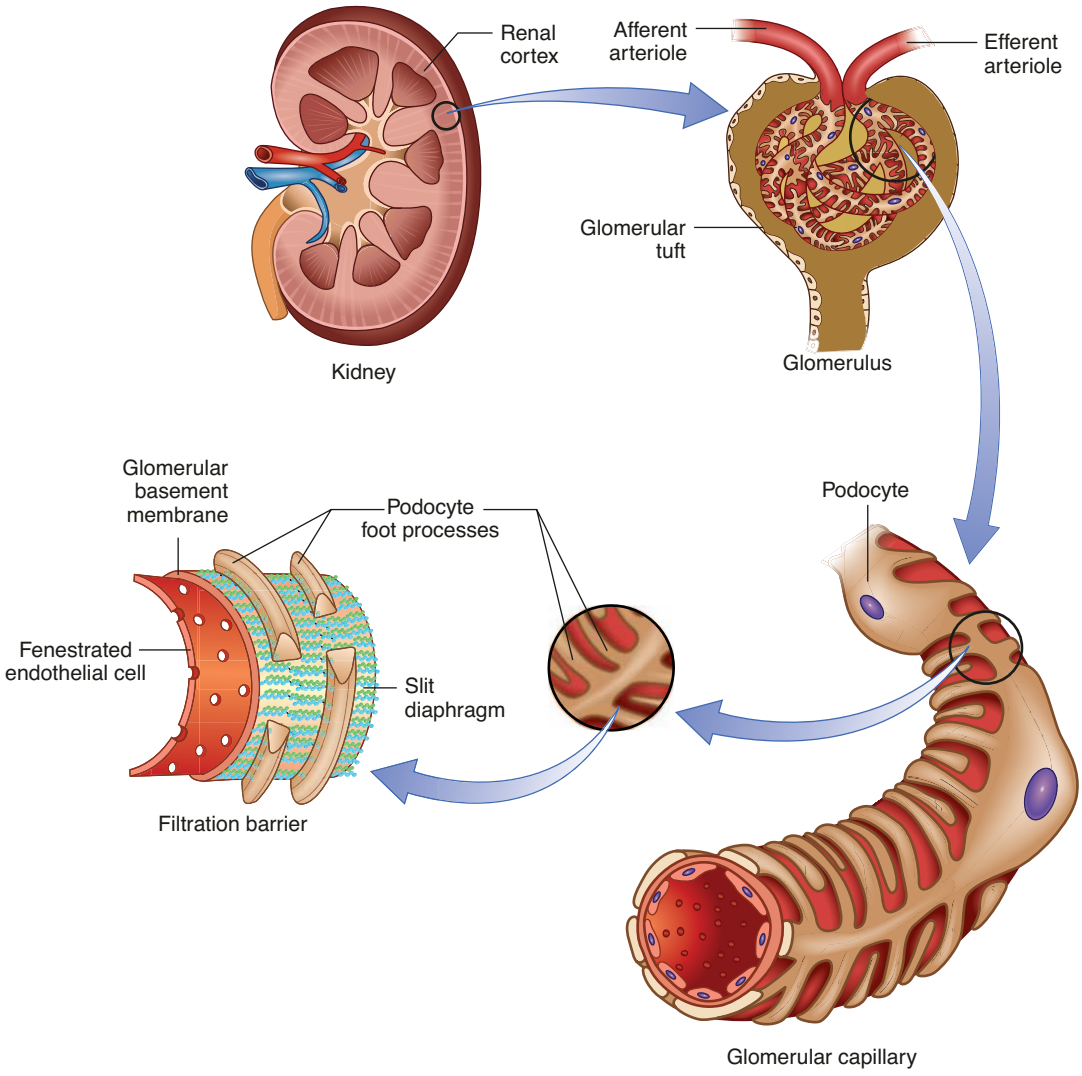

Fig. 6.2 Schematic of the structure and function of the glomerular filtration barrier. It consists of layers that block the passage of plasma macromolecules and also maintain plasma oncotic pressure. A fenestrated capillary endothelium lines each capillary loop. A porous glomerular basement membrane attached to highly dynamic epithelial cells (podocytes) is on the other side of this lining. The slit diaphragm is the prime barrier to filtration of plasma macromolecules. This slit diaphragm consists of podocytes that have interdigitating foot processes with neighbor podocytes and are connected to each other by a platform of signaling molecules

GFR is about 30 mL/min/1.73 m^2. In this section, we will discuss the functional development of glomerular filtration in the perinatal period.

Fetal GFR

During nephrogenesis, the increase in renal mass parallels an increase in fetal GFR [11]. Indeed, fetal GFR correlates well with both GA and body weight [11]. Preterm infants of 30 week GA have a creatinine clearance of less than 10 mL/min/1.73 m^2 within the first 24–40 h of birth [12], whereas creatinine clearance in term infants is higher and ranges between 10 and 40 mL/min/1.73 m^2 [13]. A study involving 275 neonates between 27 and 31 weeks GA reported GFR reference values as 3rd, 10th, 50th, 90th,

Table 6.1 Glomerular filtration rate reference values in premature infants

	Glomerular filtration rate, mL/min/1.73 m^2								
	Day 7			Day 14			Day 21		
Gestational age at birth	10th	Median	90th	10th	Median	90th	10th	Median	90th
27 weeks	8.7	13.4	18.1	11.5	16.2	20.9	13.3	18	22.7
28 weeks	11.5	16.2	20.9	14.4	19.1	23.8	16.1	20.8	25.5
29 weeks	14.4	19.1	23.8	17.2	21.9	26.6	19	23.7	28.4
30 weeks	17.2	21.9	26.6	20.1	24.8	29.4	21.8	26.5	31.2
31 weeks	20.1	24.8	29.5	22.9	27.6	32.3	24.7	29.4	34.1

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and 97th percentiles and provide useful reference ranges of the various GAs (Table 6.1) [3].

All four determinants of SNGFR contribute to the maturational increase in GFR to varying degrees [14]. Mean arterial pressure increases during fetal development and an increase in P_{GC} occurs as a result [15]. An increase in renal plasma flow leads to a further increase in SNGFR [16]. In addition, the oncotic pressure also rises with advancing GA [17]. However, the increase in P_{GC} is greater than that observed for π_{GC}, favoring ultrafiltration.

Fetal RBF can be measured by Doppler ultrasound techniques and increases from 20 mL/min at 25 weeks of gestation to more than 60 mL/min at 40 weeks [12]. Fetal RBF is low due to the high renal vascular resistance (RVR) [1, 18]. RVR depends on arteriolar tone and on the number of resistance vessels. As nephrogenesis proceeds, there is an increase in the number of glomerular vessels and in preterm infants born before 36 weeks gestation, the postnatal fall in RVR can, in part be attributable to new nephron formation [18]. Concomitantly, a re-distribution of RBF occurs from the inner medullary nephrons to the more superficial cortical nephrons. The superficial cortex is the site of more recent glomerulogenesis and the increase in SNGFR of the superficial nephrons significantly contributes to the increase in total GFR [14, 19].

Assessment of fetal glomerular function is possible by measurement of fetal serum cystatin C, α1-microglobulin and β2-microglobulin [20]. Cystatin C is a proteinase inhibitor involved in intracellular catabolism of proteins, produced by all nucleated cells, freely filtered across glomeruli and completely catabolized and reabsorbed in the proximal tubule. Fetal serum cystatin C is independent of GA and has been shown to have a high specificity (92%) for the prediction of postnatal kidney dysfunction. Reference intervals were calculated in a study of 129 cordocentesis involving 54 fetuses without renal disease [21]. Mean serum cystatin C levels were 1.6 mg/L with 2.0 mg/L being the upper limit of normal. In the same study, the authors showed that fetal serum β2-microglobulin decreased significantly with GA. In the same study, serum β2-microglobulin was demonstrated to have a higher sensitivity (87%) than cystatin C in predicting postnatal renal dysfunction. Both tests, therefore, may be used to assess fetal glomerular function in antenatally diagnosed renal malformations.

Neonatal GFR

A rapid rise in GFR occurs in term infants over the first 4 days of life. Preterm infants also experience a rise in GFR, but the rise occurs more slowly than that in term neonates [22]. Overall, a doubling of GFR is seen over the first 2 weeks of life in term infants and reaches almost 50 mL/min/1.73 m^2 between 2 and 4 weeks after birth and adult values by 2 years of age (Fig. 6.1b) [23, 24]. Postnatally, the mean arterial pressure increases and consequently an increase in glomerular hydraulic pressure occurs, resulting in an increase in GFR. A dramatic postnatal fall in RVR with redistribution of intrarenal blood flow from the juxtamedullary nephrons to the superficial cortical nephrons also contributes to the increased GFR. The fraction of cardiac output supplying the neonatal kidneys increases to

15–18% over the first 6 weeks of life [25]. In addition, an increase in the area available for glomerular filtration also contributes to the increase in GFR seen postnatally [14]. Glomerular size, glomerular basement membrane surface area and capillary permeability to macromolecules all contribute to the increase in GFR seen from the neonatal period to adulthood [26]. Maturation of glomerular filtration also occurs, as result of changes in both afferent and efferent arteriolar tone. A decrease in renal vasoconstrictors and activation of renal vasodilators occurs over the first 2 weeks of life and will be discussed in the following section.

Neonatal renal function is often assessed by measurement of serum creatinine, which is derived from creatinine and phosphocreatine of muscles and therefore reflects muscle mass. The serum creatinine concentration in the neonate is determined by maturation of the renal tubules, the total muscle mass of the body, GFR and tubular secretion. Several studies have now shown that the plasma creatinine in most infants actually increases after birth (Table 6.2) [27–30]. This is consistent with the idea that the tubules in the neonate are reabsorbing creatinine and not secreting it. Clearly, this has important implications for misinterpreting a rising creatinine in many neonatal ICU (NICU) patients as renal impairment.

Emerging evidence suggests that cystatin C is a more reliable marker of GFR in the neonatal period. A recent report involving 60 preterm (<37 weeks' GA) and 40 term infants studied from birth demonstrated that creatinine-based equations consistently underestimated GFR, whereas cystatin C and combined equations were more consistent with referenced inulin clearance studies [31].

Table 6.2 Reference values for serum creatinine levels in term neonates

	Serum creatinine, mg/dL		
Age	10th	Median	90th
Day 1	0.49	0.62	0.79
Day 3	0.37	0.48	0.61
Week 1	0.31	0.38	0.50
Week 2	0.27	0.35	0.45
Week 4	0.23	0.28	0.36

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Vasoregulatory Mechanisms of the Neonatal Kidney

Renal Vasoconstrictors in the Developing Nephron

The Renin-Angiotensin System (RAS)

The renin-angiotensin system (RAS) plays an important role in the regulation of RBF and glomerular filtration. Angiotensin II is a potent vasoconstrictor of the efferent arteriole, causing a resultant increase in P_{GC} and, therefore, GFR. Both plasma renin activity and angiotensin II levels are high in the neonate. Renal angiotensin converting enzyme (ACE) levels are higher than adult levels during first 2 weeks of life and expression is localized to the proximal tubules and capillaries in the developing human kidney [32, 33]. Expression of angiotensinogen and ACE increases during late gestation and peaks after birth [34, 35]. In addition, the number of angiotensin type 1 receptors (AT1) are also twice that of adult levels at 2 weeks of age [35, 36]. Angiotensin II receptors, on the other hand, are more abundant in the fetal kidney with progressive downregulation during fetal maturation. In contrast, AT1 receptors undergo upregulation as the fetal kidney matures [36, 37].

Animal studies have shown that angiotensin II constricts the fetal renal arteries via the AT1 receptor and during fetal life plays an important role in controlling the resistance of the umbilical arteries and, therefore, the total fetal peripheral vascular resistance [38]. Maintenance of arterial pressure and baroreceptor control of heart rate and renal sympathetic nerve activity is controlled by circulating and endogenous angiotensin II in newborn lambs [39]. Therefore, the RAS plays a significant role in maintaining blood pressure as well as vascular resistance in the developing fetus.

Indeed, the importance of the fetal RAS is highlighted by studies reporting cases of ACE fetopathy with the use of ACE inhibitors (ACEI) in pregnancy. Maternal ACEI use can result in decreased placental perfusion, fetal hypotension, oligohydramnios and neonatal renal failure [40]. Recently, mutations in genes coding for renin,

angiotensinogen, ACE and AT1 have been described in association with autosomal recessive renal tubular dysgenesis with fetal hypotension [41]. Both inherited and acquired defects of the RAS, therefore, can alter fetal renal haemodynamics, with deleterious effects on renal development.

Renal Nerves and Catecholamines

The high RVR in the perinatal period can in part be due to increased renal sympathetic nerve activity (through α1 receptor stimulation) and rising circulating catecholamine levels [42]. The renal sympathetic nerves cause renal vasoconstriction, primarily of the afferent arteriole, which results in a decrease in P_{GC} and GFR (Fig. 6.3)

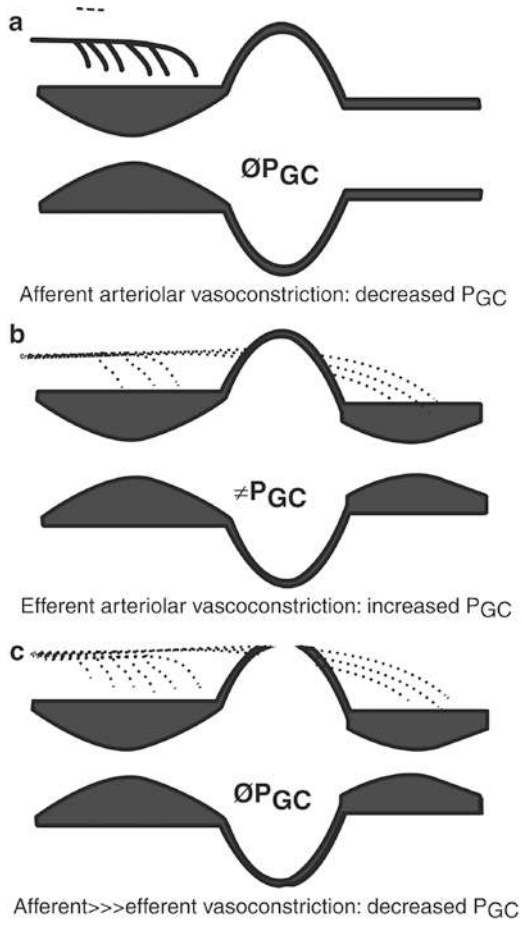

Fig. 6.3 (**a**–**c**) The renal nerves constrict the renal vasculature, causing decreases in renal blood flow and glomerular filtration. (Used with permission of John Wiley and Sons from Denton et al. [43])

[44]. Renal sympathetic nerve activity increases immediately after birth in sheep and plasma epinephrine and norepinephrine increase several fold immediately following birth [45, 46]. A fall in catecholamine levels subsequently occurs over the first few days of life [47]. Renal denervation in maturing piglets has been shown to increase RBF, demonstrating the role of renal nerves in maintaining high RVR. The sympathetic nervous system also has an important secondary role by stimulating the release of renin. Rodent studies have shown that renin-containing cells and nerve fibers are detected at 17 days of gestation, in close spatial relationship along the main branches of the renal artery [48]. Innervation of renin-containing cells follows the centrifugal pattern of renin distribution and nephrovascular development. The density and organization of nerve fibers increases with age along the arterial vascular tree [48]. Therefore, an interplay between increased sympathetic nerve activity and high plasma renin is likely necessary for the high RVR during the perinatal period.

Endothelin

Endothelin (ET) is a potent vasoconstrictor secreted by the endothelial cells of renal vessels, mesangial cells, and distal tubular cells in response to angiotensin II, bradykinin, epinephrine and shear stress [48]. Renal vasomotor tone is exquisitely sensitive to ET. An increase in RVR occurs following ET-induced contraction of glomerular arterioles (afferent > efferent) with a subsequent reduction in GFR [49]. In the first days of life, ET is elevated both in term and preterm neonates. A subsequent reduction in ET levels occur after the first week of life [50]. Newborn rat kidneys have a higher number of ET receptors than adult rat kidneys. A comparable binding affinity for ET has also been shown [51]. In addition, ET can also cause vasodilatation. Activation of ET_B receptors on the vascular endothelium evokes the release of vasodilators. The renal vasculature of fetal renal lambs reacts with vasodilatation to low doses of ET which may be due to the secondary release of nitric oxide (NO) which blunts the vasoconstrictor effects of ET [52, 53]. ET, therefore, may have both vasoconstrictor effects and vasodilatory effects on the neonatal kidney.

Renal Vasodilators in the Developing Nephron

Prostaglandins

The major prostaglandins (PGs), PGE_2, PGD_2, and PGI_2, increase RBF by stimulating afferent arteriolar vasodilatation, free water clearance, urine flow, and natriuresis. PGs are synthesized by the fetal and neonatal kidney [54]. Alterations in the synthetic and catabolic activity of renal prostaglandins occur with advancing gestational and postnatal age. Concomitant alterations in RBF, GFR, water and electrolyte excretion occur, suggesting an important role for PGs in renal functional development.

Newborns have high circulating levels of PGs that counteract the highly activated vasoconstrictor state of the neonatal microcirculation [55]. The deleterious renal vasoconstrictor effect of PG synthesis inhibitors illustrates the protective role of PGs in the immature kidney. Long-term maternal indomethacin treatment may decrease fetal urine output enough to alter amniotic fluid volume [56]. Severe renal impairment leading to fetal or neonatal death has been reported with the use of PG synthesis inhibitors which include indomethacin [57]. Neonatal indomethacin therapy may cause transient dose-related renal dysfunction characterized by a decrease in urine output. Renal dysfunction depends in part, on dosage, timing of therapy, and the cardiovascular and renal status of the infant prior to treatment [58]. In addition, recent data from studies in rodents with targeted gene disruption have shown that cyclooxygenase type-2 (COX-2) are necessary for late stages of kidney development and lack of COX-2 activity leads to pathological change in cortical architecture and eventually to renal failure [59]. Therefore, both the RAS and PGs are not only important for renal hemodynamics but are also necessary for kidney development.

Nitric Oxide

Nitric oxide (NO) plays a major role in maintaining basal renal vascular tone in the mature kidney. Through activation of its second messenger, cGMP, NO results in vasodilatation, modification of renin release and change in GFR [60]. Nitric oxide plays an important role in the maintenance of glomerular filtration in the developing kidney. Animal studies have shown that inhibition of NO synthesis by infusion with L-arginine analogues significantly decreases GFR in the developing kidney but not in the adult [61–63]. Treatment with angiotensin receptor blockers abolishes the decrease in GFR observed in the developing kidneys treated with L-arginine analogues [63]. Therefore, NO plays a critical role in the developing kidney by counter-regulating the vasoconstricting effects of angiotensin II and protecting the immature kidney.

The Kallikrein-Kinin System

Bradykinin (BK) is a vasodilator and diuretic peptide, produced by the action of kallikrein (KK), an enzyme produced by the collecting duct (CD) epithelial cells. Activation of the BK-2 receptor by BK stimulates NO and PG production, resulting in vasodilation and natriuresis. An endogenous kallikrein-kinin system is expressed in the developing kidney with higher neonatal expression than that found in adult kidneys [64, 65]. Renal expression and urinary excretion of KK rapidly rises in the postnatal period with excretion of KK correlating well with the rise in RBF [66, 67]. Blockade of the BK-2 receptor results in renal vasoconstriction in newborn rabbits, demonstrating the renal vasodilatory action of BK in the neonatal kidney [68].

Disordered Vasoregulatory Mechanisms

Vasomotor Nephropathy (VMNP)

Vasomotor nephropathy (VMNP) is defined as renal dysfunction due to reduced renal perfusion, and the preterm infant is particularly vulnerable to VMNP [69]. The main causes of neonatal acute renal failure are prerenal mechanisms and include hypotension, hypovolemia, hypoxemia and neonatal septicemia. Hypotension can stimulate vasoconstrictive mediators such as angiotensin II, causing renal vasoconstriction and hypoperfusion and thus further reduce the GFR in the newborn. The treatment of neonatal

hypotension can involve inotropic support and dopamine is usually considered as the first line agent. Dopamine has a direct effect on renal function via renal dopaminergic receptors located in the renal arteries, glomeruli, and proximal and distal tubules [70]. At low doses (0.5–2 μg/kg/min), dopamine causes renal vasodilatation and increases GFR and electrolyte excretion. In neonatal intensive care units, higher doses of dopamine (6–10 μg/kg/min) are needed to achieve systemic cardiovascular effects. Such doses have an opposite effect on renal function, causing renal vasoconstriction and reduction in sodium and water excretion [71]. Hypoxemia reduces RBF and GFR. In a study of severely asphyxiated neonates, 61% developed acute renal failure [72]. Hypoxemia stimulates ET, ANP and PG release. In addition, mechanical ventilation can reduce venous return and cardiac output and can thus cause renal hypoperfusion and impair renal function [69]. Therefore, in the neonate, VMNP can result from disturbances of glomerular hemodynamics through complex interplay of the renal vasoregulatory mechanisms.

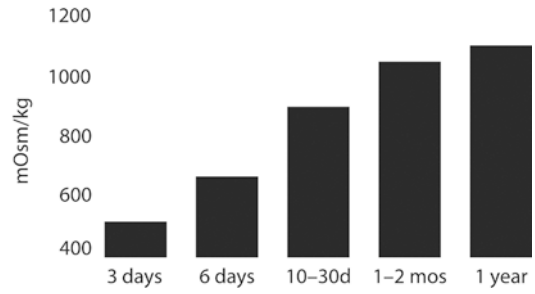

Fig. 6.4 Renal concentrating capacity increases in the postnatal period in the term infant, reaching adult values by the first month of life. (Used with permission of BMJ Publishing Group from Polacek et al. [75])

Water Transport in the Developing Kidney

Term neonates can dilute their urine to an osmolality as low 50 mOsm/L which is similar to adults [73]. However, the ability to excrete a water load is limited by the neonate's low GFR. As a result, the newborn infant is largely water, with total body water comprising 75% of body weight at full term and about 80–85% in babies between 26 and 31 weeks gestation [7]. Under normal physiological conditions, the kidneys have to excrete this water load during the first week of life [74]. Therefore, maximal concentrating abilities are not necessary at birth and, in fact, are low in the neonatal period. A progressive increase in concentrating capacity occurs postnatally and in term infants reaches adult levels by the first month of life (Fig. 6.4) [75]. In the premature neonate, maximal concentrating capacity is about 500 mOsm/L for a more prolonged period [76], which places the sick premature infant at greater risk for serious disturbances in water and electrolyte homeostasis [77]. The reasons for the limited concentrating capacity include diminished responsiveness of the CDs to antidiuretic hormone (ADH), anatomical immaturity of the renal medulla and decreased medullary concentration of sodium chloride (NaCl) and urea [78, 79]. In the following section, we will discuss each of these components in detail.

Antidiuretic Hormone in the Development of Water Transport

Normal ADH Physiology

ADH exerts its antidiuretic effect in the CD via the V_2 receptor on the basolateral membrane of the principal and inner medullary CD cells [80, 81]. Binding of ADH to the V_2 receptor results in activation of adenyl cyclase, increased cAMP and activation of protein kinase A. Subsequent phosphorylation of the cytoplasmic COOH terminus at serine 256 of the aquaporin-2 water channel (AQ2) occurs and results in the insertion of AQ2 into the apical membrane of the CD cells [82–84]. Water enters the cells via AQ2 and exits the cell via the AQ3 and AQ4 water channels located on the basolateral membrane of the CD cells [85]. Water reabsorption depends on a hypertonic medullary interstitium, which drives water from the luminal fluid across the tubular epithelium [86].

Development of Water Transport in the Collecting Duct

Neonatal low urine concentrating capacity is not attributable to low ADH levels. During labour, ADH levels are elevated, which is consistent with the raised intracranial pressure and hypoxemia acting as stimuli for ADH release [87]. Despite adequate ability to secrete ADH, no correlation exists between ADH levels and urine osmolality in the first 3 weeks of life [88]. ADH stimulation of the neonatal cortical CD (CCD) results in a lower permeability response to water than that seen in the adult [89, 90]. The response to ADH does, however, improve with age [91]. Similarly, studies have shown that the concentrating capacity is even lower in infants who have sustained neonatal asphyxia [92]. V_2 receptor mRNA expression is observed in rodents as early as day 16 of gestation in cells of the developing medullary and cortical CD [93]. During the first 2 weeks of life in rats, the number of receptors does not change. By the fifth week of life, the number of receptors reaches adult levels [94]. However, the low response of the immature kidney to ADH is more likely due to immaturity of the intracellular second messenger systems rather than inadequate receptor number. ADH binding sites precede the onset of adenyl cyclase responsiveness [95]. In addition, ADH stimulation of adenyl cyclase generation is markedly lower in the neonatal period and is only about one-third that of the cAMP response seen in the adult CCD [96]. However, even when cAMP generation is rescued using cAMP analogs, the hydraulic permeability of isolated, microperfused rabbit CCD remains low [97]. Intracellular phosphodiesterases degrade cAMP. Indeed, an increase in phosphodiesterase IV and inhibition of the production of cAMP by PGE2 acting through EP3 receptors has been shown to inhibit adenyl cyclase generation on ADH stimulation. Therefore, cAMP inhibition likely accounts for the immature kidney's reduced response to ADH [98, 99].

AQ-2 levels (mRNA and protein) are lower in early postnatal life and reach maximal expression at 10 weeks of age (Fig. 6.5) [100]. AQ2 trafficking can be appropriately stimulated by dehydration and vasopressin in the immature kidney but the urine osmolality remains low [100]. Glucocorticoids regulate the AQ2 expression in the infant and not in the adult by increasing expression of both AQ2 protein and mRNA [101]. The expression of AQ3 and AQ4 does not change significantly after birth and they do not seem to play a role in the maturation of water transport in the CD [102].

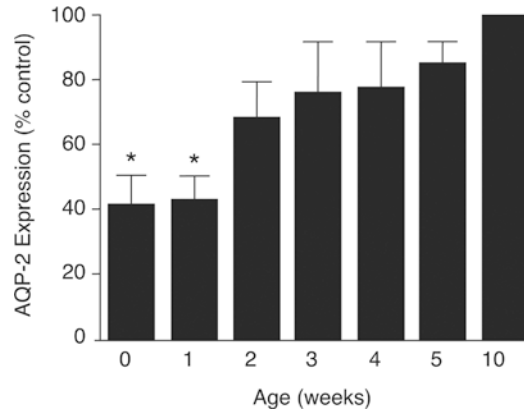

Fig. 6.5 Aquaporin-2 (AQ2) expression between 0 and 10 weeks of age. An increase in protein expression occurs in the postnatal period. (Used with permission from Bonilla-Felix [78])

Tonicity of the Developing Medullary Interstitium

In addition to low CD responsiveness to ADH, two other factors are responsible for the low concentrating capacity of the neonatal nephron. The medullary interstitium of the neonate has a low tonicity due to a low concentration of NaCl and urea [103]. Factors such as low protein intake, low sodium transport by the thick ascending limb of loop of Henle (TALH) [104], immaturity of the medullary architecture with shorter loops of Henle [105, 106] and alterations in urea transport [107] all contribute to the lower tonicity of the medullary interstitium. The activity of the Na-K-ATPase in the TALH increases after birth, with the most pronounced increase in activity between the second and third week of life, correlating well with the increase in urine concentrating capacity [108]. The loops of Henle elongate and penetrate

the medulla, forming tubulovascular units that are completed by the fourth postnatal week in rodents [109].

The medulla/cortex urea ratio increases over the first 3 weeks of life in newborn rabbits [103]. Rodent studies have shown that there is a striking increase in the number of urea transporters during the first 2 weeks of life [107]. The urea transporters prevent the loss of urea from the medulla into the circulation thereby ensuring a high concentration of urea in the medullary interstitium. Renal concentrating capacity is dependent on dietary protein intake [103] and infants fed high protein diets show a significant improvement in urinary concentrating capacity [92, 110].

In summary, the neonatal kidney's ability to concentrate urine is dependent on a number of steps involving the ADH-signal transduction pathway (Fig. 6.6), the maturation of Henle's loop and tonicity of the medullary interstitium.

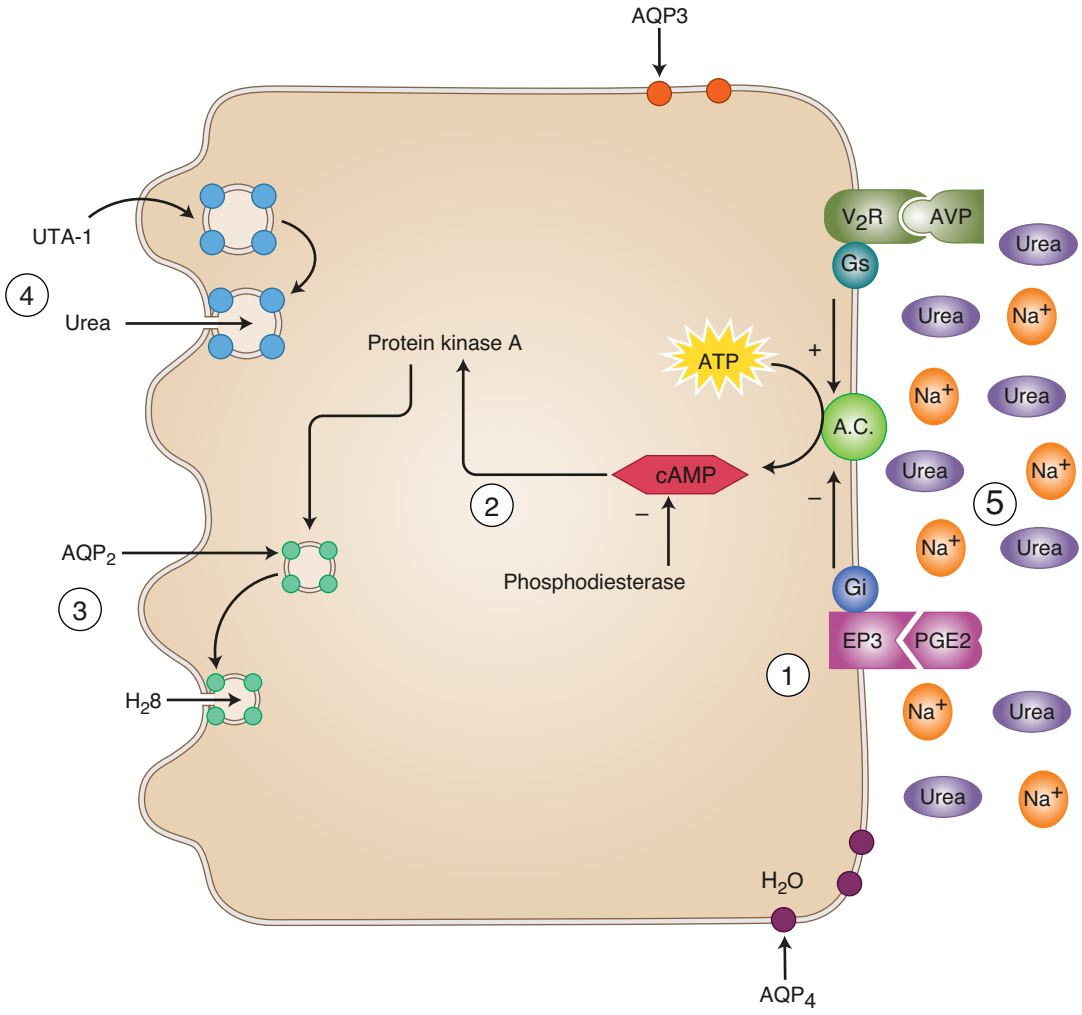

Fig. 6.6 The immature kidney's response to ADH: Responsible mechanisms are illustrated as follows: (1) Inhibition of cAMP generation by PGE_2 through EP3 receptor, (2) rapid degradation of formed cAMP resulting from increased phosphodiesterase activity, (3) low expression of AQP2 during early postnatal life, (4) low expression of UTA-1 during early postnatal life, (5) low concentration of urea and sodium in the medullary interstitium resulting from low rates of sodium transport, low dietary protein intake, and low expression of urea transporters

Postnatal Urine Flow

Oliguria is the most helpful sign of renal impairment in the neonate and a delay in the first void in a newborn may signal a renal disorder. Preterm neonates void earlier than term or post-term neonates [111] and the majority of normal newborns void within the first 24 h of life regardless of GA. Therefore, any neonate who remains anuric beyond the first day of life should be evaluated for renal insufficiency. The factors determining urine output include water balance, solute load and renal concentrating ability.

Minimum urine volume (L) = Urine solutes to be excreted/urine osmolality (max). As a result, a neonate receiving the usual renal solute load (7–15 mOsm/kg daily) with a maximal renal concentrating capacity of 500 mOsm/kg would require a minimal urine output of approximately 1 mL/kg/h to remain in solute balance. Since acute renal failure results in progressively positive solute balance, a urine flow rate less than 1 mL/kg/h has become an accepted criterion for the definition of oliguria in the neonate.

Sodium Transport in the Developing Kidney

Adaptation to the extrauterine environment involves a physiological natriuresis in the immediate postnatal period with preterm infants losing up to 16% of their birth weight in the first 3 days of life and term infants losing slightly less [112]. Human neonates remain in negative sodium balance for the first 4 days of life and then shift to a positive sodium balance by the second and third weeks of life (Fig. 6.7) [113]. Sodium conservation occurs because sodium is essential for growth in the neonate. In contrast to term neonates, preterm infants less than 35 weeks of gestation do not tolerate sodium deprivation, and hyponatremia may develop due to tubular immaturity and sodium wasting [114]. For this reason, sodium supplementation is important. Thus, the sodium requirements for a term newborn range from 1 to 1.5 mEq/kg daily, whereas the requirements for a preterm neonate range from 3 to 5 mEq/kg daily. Sodium supplementation in the preterm infant enhances the cumulative weight gain following the initial postnatal diuresis [113]. In the following section, we will discuss the mechanisms involved in the postnatal natriuresis and then the factors involved in the neonatal transition from negative to positive sodium balance.

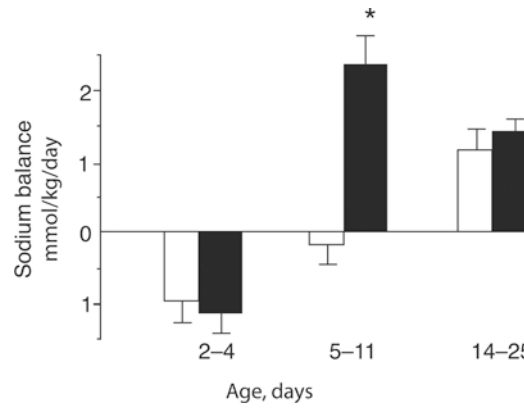

Fig. 6.7 Net external sodium balance for preterm infants in the first 3 weeks of life. Symbols are: (□) control infants; (■) sodium-supplemented infants, 4–5 mEq/kg/day; *$P < 0.0005$ vs. controls. In the first 2–4 days after birth, infants undergo natriuresis regardless of sodium intake, whereas by 1 week, supplemented infants achieve positive sodium balance sooner than controls. (Used with permission of BMJ Publishing Group from Al-Dahhan et al. [113])

Early Postnatal Natriuresis

High perinatal circulating levels of ANP have been implicated in the immediate postnatal natriuresis seen in both term and preterm infants [8]. ANP is a natriuretic hormone produced within the cardiac myocytes and released by stretch of the atrial wall. At birth, pulmonary vascular resistance falls and left atrial venous return increases, stimulating the release of ANP. ANP exerts a number of physiological effects, including an increase in GFR, natriuresis, diuresis, inhibition of renin and aldosterone release, vasorelaxation and an increase in vascular permeability [115].

ANP modulates sodium homeostasis by binding to physiologically active receptors, increasing intracellular cGMP [116]. Inhibition of sodium transport occurs through inhibition of apical sodium channels in renal tubular epithelial

cells, leading to natriuresis. Plasma ANP concentration decreases with maturation [117, 118]. A fall in right atrial volume occurs over the first 4 days of fetal life with parallel reductions in ANP concentration and urinary cGMP excretion [119]. In addition, a decrease in cGMP production per ANP binding site has been shown to occur rapidly in the suckling period in neonatal rats [120]. Therefore, sodium excretion is reduced after the first few postnatal days and the neonatal kidney subsequently aims to conserve sodium.

Neonatal Transition to Positive Sodium Balance

A reduction in the fractional excretion of sodium occurs after the first week of life, with fractions <1% in the majority of infants (Fig. 6.8) [122]. Factors contributing to the decrease in the fractional excretion of sodium include maturation of the sodium transport mechanisms in the postnatal nephron, in addition to high circulating levels of angiotensin II, catecholamines, glucocorticoids and a reduction in ANP. Each of these factors will be discussed in the following section.

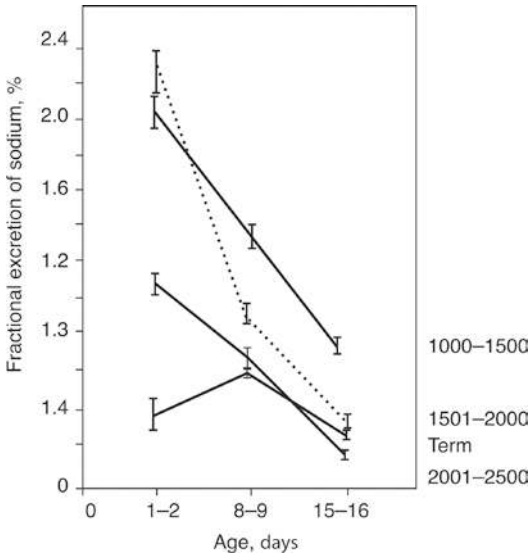

Fig. 6.8 Fractional excretion of sodium during the first 2 weeks of life in preterm and term infants. Sodium is conserved despite an increase in GFR. (Used with permission of Elsevier from Chevalier [121])

Maturation of Sodium Transport Mechanisms in the Developing Nephron

A progressive maturation of each tubular segment occurs in the postnatal kidney [123]. Each tubular segment will be discussed separately in the following section.

Proximal Tubule

Solute transport in the neonatal proximal tubule is similar to that in the adult and follows both chloride and bicarbonate reabsorption. Several animal studies have shown an increase in the activity of the Na^+/H^+ exchanger as the neonate matures [124, 125]. In addition, an increase in activity of the chloride/formate exchanger has also been shown to occur [126]. The Na-K-ATPase transporter plays a key role in sodium reabsorption in the proximal tubule and slower transport has been shown in neonates compared to adults, with a progressive maturation occurring from birth (Fig. 6.9) [127, 128]. In guinea pigs, posttranslational increase in the α1 and β1 subunits of the Na-K-ATPase transporter occurs immediately after birth [129].

Loop of Henle

NaCl transport in the TALH occurs by paracellular and transcellular pathways via the apical

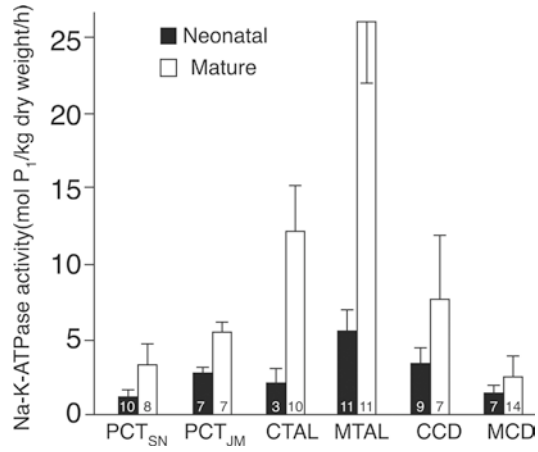

Fig. 6.9 Na^+/K^+ ATPase activity in the neonatal and adult nephron. The Na^+/K^+/ATPase activity is lower in the neonate compared to the adult. (Used with permission of Schmidt and Horster [127])

sodium-potassium-chloride cotransporter (NKCC2) and sodium-hydrogen antiporter 3 (NHE3) exchangers. The basolateral cell membrane utilizes the Na-K-ATPase to extrude sodium. Transcription of NKCC2 is observed early in development, prior to the onset of filtration in the descending loop of Henle. Physiological studies show, however, that the NKCC2 is unlikely to be functional until postnatally, as a low reabsorptive capacity has been shown for this segment in early postnatal life [109]. Compared to the adult, the expression of all of these transporters is lower in the neonate [130–133]. A postnatal five to tenfold increase in activity of the Na-K-ATPase co-transporter occurs and is greater than that seen in other tubular segments (Fig. 6.9) [127, 134]. Na-K-ATPase consists of a catalytic (α subunit) and a regulatory (β subunit). Both the α1 and β1 isoforms are present in the mature kidney. On the other hand, the α1 subunit is detected early in fetal life whereas the β1 subunit is detected only after birth. Interestingly, the β2 isoform is expressed in the fetal kidney and, in contrast to the adult, Na-K-ATPase is expressed on both the apical and basolateral cell membranes. After birth, the β2 isoform is downregulated and the α1 and β1 isoforms are upregulated [135]. Heterodimerization of the α1 and β1 isoforms is essential for the function of the Na-K-ATPase. Of note, treatment with glucocorticoids increases the synthesis of mRNA for both the catalytic and regulatory subunits of the Na-K-ATPase. During postnatal life, there is a 20% increase in the amount of sodium reabsorbed along this segment, which reflects functional maturation of transporters, an increase in the resorptive surface area and maturation mechanisms of hormonal control.

Distal Tubule

The Na^+–Cl^- co-transporter (NCC) is the major sodium influx co-transporter in the distal tubule. In the mature nephron, NCC is expressed along the entire distal tubule, starting beyond the NKCC2-expressing post-macular segment and ending at the transition into the collecting tubules [136]. During development, NCC mRNA is detected in distal tubule segments before the expression of NKCC2 mRNA and sodium-phosphate type 2 co-transporter mRNA. Later in development, NCC expression proceeds gradually into the post-macula segment of the TALH [132].

Cortical Collecting Duct

Fine-tuning of sodium reabsorption occurs in the CCD where the amiloride-sensitive epithelial sodium channel (ENaC) plays an important role. ENaC is located on the apical membrane of distal tubular, cortical and outer medullary CD cells [137]. ENaC is comprised of three subunits, α,β and γ. Rodent studies show that the amount of total renal embryonic rat ENaC subunit mRNA is low but increases from murine gestational day 16–19 [138]. A sharp rise to almost adult levels occurs in the first three postnatal days [139]. After birth, the mRNA for α ENaC increases, whereas that for β- and γ-decreases. In the immature kidney, the greatest expression is seen in the terminal CD for all three subunits. As the kidney matures, the expression in the cortical distal nephron increases and in rodents is complete by the ninth postnatal day [140]. Endogenous glucocorticoids do not appear to have any effect on the prenatal maturation of ENaC in the kidney [141]. Although this response has long been assumed to be solely the result of liganded nuclear hormone receptors transactivating αENaC, epigenetic controls of basal and aldosterone-induced transcription of αENaC in the CD were recently described [142].

The Na-K-ATPase is also present in the CCD on the basolateral cell membrane. Tracer uptake assays of individual CCDs have shown that the activity of the Na-K-ATPase increases within the same time interval as it takes for maturation of the net transepithelial reabsorption of sodium and potassium [143]. The capacity of the CCD to reabsorb sodium increases immediately after birth [144] and reflects the increase in expression of the aforementioned sodium channels.

Developmental Paracrine Regulation of Renal Sodium Excretion

Renin: Angiotensin: Aldosterone System (RAS)

Studies have shown that the RAS is involved in renal tubular sodium reabsorption in the neonate. Acute volume expansion in neonatal rat pups results in natriuresis and AT1 blockade attenuates the natriuretic response, demonstrating that angiotensin II mediates sodium reabsorption via the ATI receptor [145]. The proximal tubule is the likely site of sodium reabsorption because angiotensin II augments sodium reabsorption in the proximal tubule in adult rats during volume contraction [146]. In addition, angiotensin II stimulates aldosterone, which stimulates sodium reabsorption in the TALH as well as the distal tubule and the CD. Preterm neonates without sodium supplementation demonstrate markedly increased plasma renin and aldosterone activity compared to their sodium supplemented counterparts, indicating that the neonatal RAS is involved in sodium homeostatic mechanisms [147].

Catecholamines

Catecholamines stimulate NaCl and water reabsorption by the proximal tubule, ascending limb of Henle's loop, distal tubule and CD. Circulating plasma catecholamines are high in the neonatal period and then fall over the first few days of life as discussed earlier (see section on "Glomerular Function in the Fetal, Perinatal, and Postnatal Period"). Catecholamines stimulate an increase in renin release, which promotes sodium reabsorption. In addition, dopamine acting via the D2 receptor in preterm neonates enhances sodium reabsorption in the proximal tubule [148].

Glucocorticoids and Thyroid Hormone

Plasma cortisol levels increase markedly after birth [149]. Maturation of the Na^+/H^+ exchanger occurs under the influence of glucocorticoids, as demonstrated by the attenuated postnatal increase of Na^+/H^+ exchanger activity and protein and mRNA abundance in the brush border of proximal tubular cells of adrenalectomized newborn rodents [150]. Glucocorticoids also play a role in the maturation of transporters along the entire nephron [151, 152]. Thyroid hormone plays a role in the maturation of the paracellular pathways of sodium reabsorption [153] and in the regulation of the Na^+/K^+ ATPase activity [154].

Fractional Excretion of Sodium

In the oliguric term neonate (urine flow less than 1 mL/kg/h), fractional excretion of sodium of less than 2.5% suggests a pre-renal cause, such as volume depletion, hypoalbuminemia or reduced cardiac output [155]. The criterion of 2.5% is valid after the first 10 days of life in the low birth weight newborn after the period of postnatal natriuresis [122]. In addition, very low birth weight infants have greater fractional sodium excretion due to immaturity of the sodium reabsorptive capacity [156].

Potassium Transport in the Developing Kidney

Like sodium, potassium is critical for somatic growth and an increase in total body potassium content is associated with growth [157]. In contrast to the adult, neonates greater than 30 weeks GA must maintain a positive potassium balance [157, 158]. Premature newborns, as a result, tend to have higher plasma potassium concentrations than children [158]. In utero, the placenta transports potassium from the mother to the fetus [159]. Interestingly, potassium levels >6.5 mmol/L are observed in 30–50% of very low birth weight infants in the first 48 h in the absence of potassium intake and not after 72 h [160]. A shift from the intracellular to the extracellular fluid compartment, as a result of either Na/K ATPase pump failure and/or a limited renal potassium excretory capacity have been postulated to account for this increase [161, 162].

Renal potassium excretion is determined by the rate of potassium excretion by the principal cells of the distal tubule and CD. Net potassium secretion cannot be detected in microperfused CCD of newborn rabbits until after the third week of life (Fig. 6.10) [164] and flow-stimulated transport is not detected until after the first postnatal month [165].

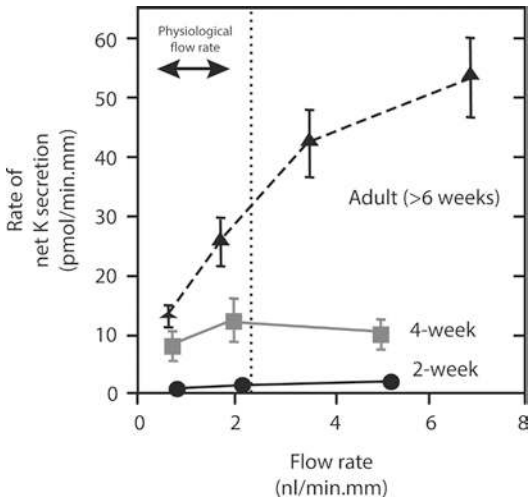

Fig. 6.10 Net potassium secretion in maturing rabbits. (Used with permission of Elsevier from Zhou and Satlin [163])

Maturation of Potassium Transport Mechanisms in the Developing Nephron

Maturation of tubular transport mechanisms will be discussed for each tubular segment in this section.

Proximal Tubule and Loop of Henle

In the mature nephron, 65% of the filtered potassium load is reabsorbed passively in the proximal tubule [166] and only 10% reaches the early distal tubule. In contrast, 35% of the filtered potassium load reaches the distal tubule of the newborn rat [167]. Therefore, postnatal maturation of the TALH is required for further potassium reabsorption. Indeed, both the diluting capacity and Na-K-ATPase activity increase after birth [108, 127]. As discussed earlier, transcription of NKCC2 is observed early in development, prior to the onset of filtration in the descending loop of Henle, but is unlikely to be functional until postnatally in view of the low reabsorptive capacity shown for this segment in early postnatal life [104]. Apical renal outer medullary potassium channel (ROMK) has been detected in the TALH at an earlier developmental stage compared to the CCD. Functional analyses on ROMK in the developing TALH have not been performed.

Cortical Collecting Duct

In the fully differentiated CCD, two types of potassium channels are involved in potassium secretion: (1) the ROMK channel mediates potassium secretion under baseline conditions [168, 169] and (2) the maxi-K^+ channel, which mediates flow-stimulated potassium secretion [170]. ROMK has only been shown in principal cells of the CCD. Maxi-K^+ exists in both the principal and intercalated cells of the CCD [170]. In isolated CCDs of neonatal rabbits, apical ROMK channels are not detected in the first 7 days of life and, subsequently, a threefold increase in the number of ROMK channels is seen in the principal cells of CCD between the third and fifth week of life [171]. The initial increase in expression follows 1 week after an increase in ENaC activity is detected [172]. Also, expression of the ROMK protein on apical cell membranes of the TALH and occasional CCD in the inner cortex and outer medulla is detected in 1-week old animals by indirect immunofluorescence studies [173]. By 3 weeks of age, expression has increased to involve the mid and outer CCDs [173]. Maxi-K^+ channels mediate flow stimulated K^+ secretion and do not appear to be functional in 4-week old rabbits subjected to a sixfold increase in tubular flow rate [165]. However, a small but significant increase in net potassium secretion after 5 weeks of age is observed. An associated increase in the mRNA and protein expression of the α subunit of the maxi-K^+ channel is seen on the apical surface of the intercalated cells of the CCD [165]. In addition to potassium excretion, potassium reabsorption also occurs in the distal nephron via the apical H^+/K^+ ATPase. Fluorescent functional assays identify significant H^+/K^+ ATPase activity on the apical cell membranes of neonatal intercalated cells [174], which suggests that neonatal CDs have a capacity to retain potassium. Indeed, a longitudinal prospective study of fractional potassium excretion in 23–31 week GA infants demonstrated that despite a threefold increase of

filtered potassium, the renal excretion fell by half between 26 and 30 weeks [157]. This study supports the idea that the developing kidney has the capacity for potassium reabsorption.

Regulation of Potassium Balance in the Neonate

Table 6.3 illustrates the factors which acutely regulate plasma potassium in the neonate. The immature kidney displays an insensitivity to aldosterone despite high circulating levels of aldosterone [158]. The number of mineralocorticoid receptors, the receptor affinity and degree of nuclear binding of hormone-receptor is similar in adult and neonatal rats [175]. Aldosterone insensitivity may result from immature intracellular signal transduction mechanisms. Aldosterone insensitivity in the immature kidney is supported by the low transtubular potassium gradients (TTKG) reported in 27 week GA infants compared to 30-week infants followed over the first 5 days of postnatal life [176]. However, the low TTKG may also reflect a low secretory ability. Glucocorticoids have a significant effect on potassium balance in extremely low birth weight infants during the first week of life. Infants whose mothers received a full course of prenatal steroids had no hyperkalemia (>6.5 mmol/L) and a less negative potassium balance at the end of the first week of life [177]. Several studies have shown that glucocorticoids upregulate the expression of the Na^+/K^+ ATPase [178], resulting in a decrease of the intracellular to extracellular potassium shift.

Table 6.3 Neonatal potassium regulation

	Effect on cell uptake of K^+
Physiologic	
Plasma K concentration	
↑	↑
↓	↓
Insulin	↑
Catecholamines	
α-Agonists	↓
β-Agonists	↑
Pathologic	
Acid-base balance	
Acidosis	↓
Alkalosis	↑
Hyperosmolality	↑ cell efflux
Cell breakdown	↑ cell efflux

Used with permission of Elsevier from Zhou and Satlin [163]

Acid-Base Regulation in the Developing Kidney

The term neonate has a lower bicarbonate concentration than the adult (Fig. 6.11) [179, 180]. In the low birth weight newborn, total bicarbonate may be as low as 15 mmol/L during the early postnatal period and is within normal limits [179]. Bicarbonate gradually increases with increasing GFR. Misdiagnosis of renal tubular acidosis may occur if one does not take into account the physiologically low plasma bicarbonate in neonates. In addition, neonates need to excrete 2–3 mEq/kg/day of acid due to their high protein intake and formation of new bone. The neonate has a reduced ability to respond to an acid load while ammoniagenesis and titratable acidity mature after 4–6 weeks in the low birth weight newborn [181]. Renal regulation of acid-base balance undergoes complex changes during development and will be discussed in detail.

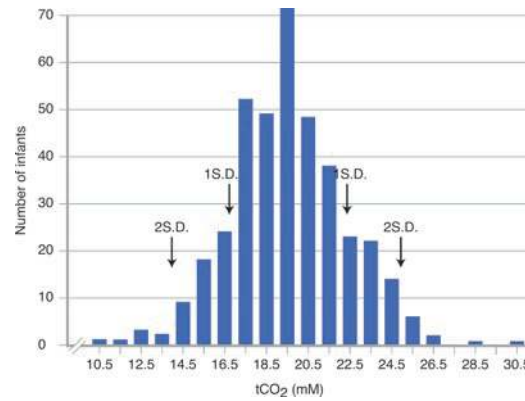

Fig. 6.11 Frequency distribution of serum total bicarbonate (tCO_2) in low birth weight neonates during first month of life. Mean is approximately 20 mM and normal range ±2 standard deviations (S.D.) is 14.5–24.5 mM. (Used with permission of Elsevier from Schwartz et al. [179])

Proximal Tubule Handling of Bicarbonate in the Neonate

Neonatal bicarbonate reabsorption in the proximal tubule is one third that of the adult [182] and is due to lower activity of the transporters present in the adult [183]. The number of apical NHE3 antiporters is lower than that in the mature nephron and has about one-third the activity [124]. The H^+/ATPase does not appear to be active in the neonate while the basolateral Na-K-ATPase has about one-half of activity compared to the adult [184]. The carbonic anhydrase (CA) type IV isoform which is expressed on the brush border of proximal tubular epithelial cells has a lower activity in the developing nephron of rabbits, but activity does increase during maturation and parallels the increase in bicarbonate reabsorption occurring in the proximal tubule [185]. Ammoniagenesis does occur in the neonatal kidney, but at a much lower rate compared to the adult [186, 187]. Glutamine and activity of the deaminating enzyme, glutaminase, is lower in the neonatal kidney while glutaminate, an inhibitor of glutaminase, is higher. Neonates, as a result, cannot generate the same amount of ammonia during an acid load and take a longer time to recover their acid-base balance.

Thick Ascending Limb of Henle's Loop

While transcription of NKCC2 is observed early in development, NKCC2 is unlikely to be functional until postnatally as a low reabsorptive capacity has been shown for this segment in early postnatal life [104]. As discussed earlier, the expression of all of the TALH transporters is lower in the neonate [130–133]. As a result, bicarbonate and ammonium reabsorption occur at a lower rate compared to the adult. A postnatal five to tenfold increase in activity of the Na-K-ATPase co-transporter has been shown and is greater than that seen in other tubular segments (Fig. 6.9) [127, 134].

Cortical Collecting Duct

Microperfusion studies of neonatal rabbit kidneys demonstrate a lower capacity to secrete acid compared to adult controls [188]. The neonatal number of intercalated cells is half that of the adult [189–191]. In addition, lower levels of the CA II isoform have been shown in neonatal rat kidneys [191, 192]. The CA II isoform is important for the function of the α-intercalated cell and may be indicative of the increase in acid secreting capability of the developing CCD.

Regulation of Maturational Acid-Base Homeostatic Mechanisms

Glucocorticoids

Glucocorticoids can stimulate bicarbonate reabsorption and a developmental increase in circulating cortisol levels precedes the increase in bicarbonate reabsorption [193]. Pregnant rabbits injected with glucocorticoids give birth to neonatal rabbits with proximal tubular bicarbonate reabsorption rates similar to that of adults [193]. An increase in NHE3 antiporters occurs with prenatal glucocorticoids [194]. Adrenalectomy prevents this maturational increase in NHE3 antiporter expression at both the level of protein and mRNA. Therefore, the maturational increase in glucocorticoids is responsible for the postnatal increase seen in proximal tubule acidification.

Renal Calcium Handling in the Developing Kidney

Higher calcium and phosphate levels are required for the growing skeleton in the fetus to ensure a positive calcium balance when bone calcium deposition rate is at its highest [195, 196]. A high geomaterial calcium ratio is maintained during pregnancy and is mediated by active transport in the placenta [197]. The elevated fetal calcium suppresses PTH release [198]. PTH is the main regulator of calcium metabolism after birth.

Circulating fetal calcium levels increase with advancing GA and at term the fetus is hypercalcemic relative to the maternal levels [199]. Serum calcium levels fall over the first 24 h in the absence of the placenta. As a result, PTH secretion is stimulated [200], but the response to the falling calcium is not sufficient such that a physiological nadir of serum calcium occurs in the first 2 days of life. This nadir is still within the adult range but represents a significant decrease compared to fetal levels [198]. Term infants typically achieve normal serum calcium levels by the second week of life, with typical circulating concentrations of ionized calcium in neonates being in the range of 1–1.5 mmol/L (2–3 mEq/L) [201].

After birth, the kidney plays an important role in calcium and phosphate homeostasis (Table 6.4). Calcium filtered by the kidney is reabsorbed along the nephron to maintain the serum concentration [202]. Only 1–2% of filtered calcium is excreted in urine by the mature nephron [203]. Most reabsorption of filtered calcium, about 70% in the proximal tubule and 20% in the thick ascending limb, is paracellular, a passive process occurring down an electrochemical gradient. Calcium diffuses across the apical cell membrane into the cell. Sodium-driven water absorption plays a significant role in this process as highlighted by a two-fold increase in the fractional excretion of calcium (FECa) in mice null for the *Nhe3* transporter, which mediates the majority of sodium reabsorption from the proximal tubule [204]. Paracellular movement of calcium is mediated by tight junction proteins called claudins (Cldn) [205]. Claudin-2 is responsible for calcium permeability in the proximal tubule. *Cldn2* deficient mice have a three-fold increase in the fractional excretion of calcium (FECa) associated with decreased proximal tubular monovalent cation permeability [206]. Little is known about the role of other claudins in paracellular calcium permeability across the proximal tubule.

Table 6.4 Summarises the developmental expression of the proteins involved in renal handling of calcium

Age	<3 weeks	3–8 weeks	>8 weeks
Paracellular			
Cldn 2	Yes	Yes	Yes
Cldn 6	Yes	NK	No
Cldn 9	Yes	NK	No
Cldn 14	NK	Yes	Yes
Cldn 16	Yes	Yes	Yes
Cldn 19	NK	Yes	Yes
Transcellular			
TRPV5	Yes	Yes	Yes
TRPV6	Yes	NK	Yes
CaBP9K	Yes	Yes	Yes
CaBP28K	Yes	Yes	Yes
Pmca1	NK	Yes	Yes
Pmca4	NK	Yes	Yes
Ncx1	NK	NK	Yes

NK not known
Modified from Beggs and Alexander [202]

Claudins 16 and 19, in the TALH, form a cation permeable pore [207]. Upregulation of Claudin-14 occurs in hypercalcaemia and is associated with increased transepithelial resistance (TER) and decreased absolute calcium permeability, similar to the effect of pharmacological inhibition of Claudin-14. Activating mutations in the calcium sensing receptor (CaSR) mediate downregulation of Claudin-14 transcript, supporting the role of claudin-14 as an important negative regulator of calcium reabsorption in the TALH [202]. The regulatory role of the CaSR during postnatal development has yet to be determined. Autosomal dominant hypocalcaemia is associated with gain of function mutations, whilst loss of function mutations cause hypercalcemia, ranging from benign familial hypocalciuric hypercalcemia (autosomal dominant) to potentially fatal neonatal severe hyperparathyroidism (autosomal recessive).

Eighty percent of filtered calcium is reabsorbed by the proximal tubule whilst the remainder of calcium is reabsorbed in the distal nephron and, in particular, the distal convoluted tubule and connecting tubule via an active transcellular process. Active calcium reabsorption occurs through the highly Ca^{2+}-selective TRPV5 channel and binds to calbindin/D28K. The calbindin/D28K ferries Ca^{2+} to the basolateral $3Na^{+}/Ca^{2+}$ exchanger (NCX1) and the plasma membrane calcium ATPase 1b which extrude calcium into the blood compartment. Animal studies have shown that TRPV6, calbindin/

D9K, TRPV5 and calbindin/D28K are expressed in the kidneys of fetal mice at GA 18 days [208]. TRPV6 reaches a maximum level at 1 week of age and then decreases to <10% of TRPV5 expression, suggesting a possible role for TRPV6 during developmental regulation of calcium homeostasis. The expression of TRPV5 and calbindin/D28K peaks at the third postnatal week and then falls [208].

Renal Calcium Handling in Postnatal Period

The amount of calcium excreted increases over the first 2 weeks of life [209], and normal calcium:creatinine ratios are accordingly higher in infants. Premature and low birth weight infants have a high incidence of nephrocalcinosis on ultrasound that is most likely secondary to a three-fold higher urine calcium excretion than the upper limit of normal for term infants.

During childhood, urine calcium excretion declines by 50% between 1 month and 2 years of age. By 10 years of age, a further reduction of 30% occurs [210].

Renal Phosphate Handling in the Developing Kidney

Phosphate is of critical importance to body functions, particularly during periods of growth. Neonates excrete only 60% of intestinally absorbed phosphate and have a higher phosphate concentration than adults [211]. In neonates, the transtubular reabsorption of phosphate is high. Neonates reabsorb 99% of the filtered load of phosphate on the first day of life and 90% by the end of the first week [212]. Micropuncture studies performed on guinea pig neonatal proximal tubules demonstrated a higher phosphate reabsorption rate than adult guinea pigs [213]. Reabsorption does not occur through the $2Na^+$/Pi IIa antiporter but rather through its developmental isoform, the $2Na^+$/Pi IIc antiporter, the expression of which is higher in weaning animals and has a reduced function in adults [214].

Regulation of Renal Phosphate Handling in the Developing Kidney

The increased phosphate reabsorption in the early postnatal period is thought to be multifactorial. Parathyroidectomy in immature rats results in a greater increase in the maximal tubular phosphate reabsorption than in mature rats, suggesting a role for PTH in neonatal phosphaturia [215]. However, a decline in resorptive capacity is also observed with age in the presence of parathyroid glands, suggesting that there is an enhanced capacity of the immature tubule to reabsorb phosphate. In addition, responsiveness to PTH increases threefold during the first few weeks of life, suggesting a maturation of second messenger systems [216]. In the mature nephron, Klotho and PTH both increase the expression and activity of the $2Na^+$/Pi symporter [217] resulting in phosphaturia. Future research will provide interesting insights into the ontogeny of Klotho and fibroblast growth factor 23 (FGF-23), a phosphaturic hormone, during maturation of the renal phosphate transport systems.

Growth hormone (GH) has also been shown to upregulate $2Na^+$/Pi symporter in micropuncture studies performed on 4 week old rat proximal tubules, an effect that is independent of PTH [218, 219]. As GH levels in rodents peak in the first week of life, high serum GH in the neonate may contribute to the elevated phosphate reabsorption observed in the kidneys of neonates [220].

The mechanism enhancing the GH effect is unknown as developmental differences in the expression of GH receptors have not been shown. Both GH and IGF-1 mRNA have been localized to the apical membrane of proximal tubular epithelial cells suggesting a role of the GH/IGF-1 axis in phosphate reabsorption [221].

Magnesium Handling in the Developing Kidney

In the adult kidney, 80% of total serum magnesium is filtered and >95% is reabsorbed along the nephron [222]. The proximal tubule reabsorbs 15–20% in the adult kidney but interestingly 70%

in the developing proximal tubule [222]. A maturational decrease in the paracellular permeability at the level of the tight junction has been suggested as a reason for the decline in proximal magnesium reabsorption. From early childhood on, the majority of magnesium transport (70% of the filtered load) occurs in the loop of Henle. The distal convoluted tubule reabsorbs 5–10%. Transport in the TALH is passive and paracellular, driven by the lumen positive transepithelial voltage and involves paracellin-1, a member of the claudin family involved in tight junction formation [223]. Active and transcellular reabsorption of magnesium occurs in the distal convoluted tubule and probably through the apical TRPM6 channel [224]. Ontogeny of the TRPM6 channel and its family members and paracellin-1 requires further research.

Renal Glucose Handling in the Developing Kidney

In the mature nephron, more than 99% of the filtered glucose is reabsorbed [225]. Glucosuria is more common among neonates, with the highest levels in preterm infants [226]. The maximum tubular reabsorption of glucose is lower in preterm and term infants than in adults [227]. Age-related differences in glucose transport activity correlate with differences in sodium conductance. Changes in membrane permeability to sodium affect membrane potential, a factor which modifies glucose reabsorption. Therefore, factors such as an increase in cell membrane surface area and in basolateral Na^+/K^+-ATPase, increased density of transporter proteins and the development of new nephrons are implicated in the increase in glucose resorptive capacity observed as the fetus matures [228–230].

Renal Amino Acid Handling in the Developing Kidney

Amino acids are reabsorbed in the proximal one third of the proximal tubule in an active, sodium-dependent process [231]. Specific amino acid transport systems on the luminal cell membrane reabsorb the amino acids by secondary active transport against an uphill concentration gradient along with sodium. Aminoaciduria is frequently observed in the neonate. Factors include decreased activity of the amino acid-sodium cotransporter, increased Na^+/H^+ exchange at the luminal membrane and decreased activity of the Na^+/K^+/ATPase at the basolateral membrane [232]. Of note, not all of the amino acids are wasted to the same degree [233]. Developmental differences have been shown for the amino acid system and the glycine transporter systems [234, 235].

Assessment of Renal Functional Maturation

Renal functional maturation can be measured by either glomerular or tubular indicators. Glomerular function is assessed by serum creatinine and cystatin C levels, urinary microalbumin and immunoglobulin G and GFR. Tubular function can be assessed by the fractional excretion of sodium or urinary α1-microglobulin and urinary levels of other tubular proteins normally reabsorbed by the proximal tubule such as *N*-acetyl-β-D-glucosaminidase or β2-microglobulin [236]. All markers have been closely associated with GA. A decrease in urinary tubular proteins occurs with increasing GA [237].

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Structural Development of the Kidney

7

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Abbreviations

Agt	Angiotensinogen
Agtr1	Angiotensinogen receptor 1
Agtr2	Angiotensinogen receptor 2
Alk3	Bone morphogenetic protein receptor, type 1A
Alk6	Bone morphogenetic protein receptor, type 1B
Ang1	Angiopoietin-1
Ap-2	Transcription factor AP-2
Bcl2	B-cell lymphoma 2
Bmp4	Bone morphogenetic protein 4
Bmp5	Bone morphogenetic protein 5
Bmp7	Bone morphogenetic protein 7
Brn1	Brain specific homeobox 1
Cited1	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 1
Ctnnb1	β-Catenin
Cxcr4	Chemokine (C-X-C motif) receptor 4
Dsch1/2	Dachsous ½
Ecm1	Extracellular matrix protein 1
Egf	Epidermal growth factor
Emx2	Empty spiracles homolog 2
Etv4	ETS transcription factor 4
Etv5	ETS transcription factor 5
Eya1	Eyes absent homolog 1
Fat4	Fat atypical cadherin 4
Fgf20	Fibroblast growth factor 20
Fgf8	Fibroblast growth factor 8
Fgf9	Fibroblast growth factor 9
Fgfr1	Fibroblast growth factor receptor 1
Fgfr2	Fibroblast growth factor receptor 2
Foxc1	Forkhead box C1
Foxc2	Forkhead box C2
Foxd1	Forkhead box D1
FoxF1	Forkhead box F1
Frs2α	Fibroblast growth factor receptor substrate 2α
Gata3	Gata binding protein 3
Gdf11	Growth/differentiation factor-11
Gdnf	Glial-derived neurotrophic factor
Gfrα-1	Glial-derived neurotrophic factor receptor alpha-1
Gli3	Gli family zinc finger 3
Gpc3	Glypican 3
Grem1	Gremlin 1
Hgf	Hepatocyte growth factor
Hnf1β	Hepatic nuclear factor 1β
Hnf4a	Hepatic nuclear factor 4a
Hoxa11	Homeobox A11
Hoxc11	Homeobox C11
Hoxd11	Homeobox D11
Hs2st	Heparan sulfate 2-sulfotransferase
Igf2	Insulin-like growth factor 2
Lhx1	Lim homeobox 1
Lmx1b	Lim homeobox 1b

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Met	Met proto-oncogene
Mmp14	Matrix metallopeptidase 14
mTor	Mechanistic target of rapamycin
Myb	Myb proto-oncogene
Osr1	Odd-skipped related1
Pax2	Paired box gene 2
Pax8	Paired box gene 8
Pdgfβ	Platelet derived growth factor beta
Pod1	Podocyte expressed 1
Psen1	Presenilin 1
Psen2	Presenilin 2
Ptch1	Patched1
Raldh2	Aldehyde dehydrogenase 1 family, member A2
Rarα	Retinoic acid receptor α
Rarβ2	Retinoic acid receptor β2
Rbpsuh	Recombining binding protein suppressor of hairless
Ret	Ret proto-oncogene
Robo2	Roundabout, axon guidance receptor, homolog 2
Ror1	Receptor tyrosine kinase like orphan receptor 1
Ror2	Receptor tyrosine kinase like orphan receptor 2
Sall1	Sal-like 1
sFrp	secreted Frizzled-related protein
Shh	Sonic hedgehog
Six1	Sine oculis homeobox homolog 1
Six2	Sine oculis homeobox homolog 2
Slit2	Slit homolog 2
Spry1	Sprouty1
Tak1	TGF-β-activated kinase
Tbx18	T box transcription factor 18
Tbx2	T box transcription factor 2
Tbx3	T box transcription factor 3
TGFα	Transforming growth factor alpha
TGFβ2	Transforming growth factor beta2
Tie2	Angiopoietin-1 receptor
Timp	Tissue inhibitor of metalloproteinase
Tsc1	Hamartin
Tshz3	Teashirt zinc finger homeobox 3
Vegf	Vascular endothelial growth factor
Vegfr2	Vascular endothelial growth factor receptor 2
Wnt11	Wingless-type MMTV integration site family 11
Wnt4	Wingless-type MMTV integration site family 4
Wnt5a	Wingless-type MMTV integration site family 5a
Wnt7b	Wingless-type MMTV integration site family 7b
Wnt9b	Wingless-type MMTV integration site family 9b
Wt1	Wilms tumour 1

Overview of Human Kidney Development

Human kidney development begins in the fifth week of gestation, with the first functioning nephrons making urine by the ninth week [1]. The formation of new nephrons continues until approximately 32–34 weeks gestation [2, 3]. Further renal growth is the result of growth and maturation of already formed nephrons, rather than the generation of new nephrons. Remarkably, there exists wide variability in the number of nephrons that occur naturally in humans, from 200,000 to 1.8 million per person [4]. In humans that suffer fetal or perinatal renal injury, the developing kidney is incapable of compensating for irreversible nephron loss by either accelerating the rate of nephron formation *ex utero* in infants born prematurely, or by *de novo* generation of nephrons once nephrogenesis is completed [2, 5]. Thus, the number of nephrons formed at birth is thought to be an important determinant of renal function later in life.

This concept is supported by the association of renal failure in humans with oligomeganephronia [6, 7], and by the demonstration of reduced glomerular number in humans with primary hypertension and chronic kidney disease [8, 9]. Quantitative analyses in humans and rodents using stereological methods of glomerular counting in renal autopsy specimens have revealed a relationship between birth weight and glomerular number [4, 10]. The latter data are consistent with the "Barker Hypothesis", which proposes that adult disease has fetal origins and is based on

epidemiological studies showing a correlation between birth weight and the incidence of cardiovascular disease [11, 12]. Equally important is the normal structural development of each nephron (or, nephron pattern), which is critical for nephron function. Abnormal nephron pattern results in renal dysplasia. Consequently, mechanisms that control congenital nephron endowment and nephron pattern are likely to be crucial factors in determining long-term as well as short-term renal function.

Our understanding of human kidney development historically began with histological descriptions of microdissected human fetal kidney autopsy specimens performed by Edith Potter and Vitoon Osathanondh [1, 13, 14]. Their seminal work was complemented by analyses of mouse kidney development performed by Lauri Saxen [15]. The mammalian kidney derives from two parts of the metanephros, its embryonic precursor. The first part is the ureteric bud, which gives rise to the collecting duct system, including the cortical and medullary collecting ducts, the renal calyces, the renal pelvis, the ureter, and the trigone of the bladder [2, 15]. The second part is the metanephric mesenchyme, which differentiates into all the epithelial cell types comprising the mature nephron, including the visceral and parietal epithelium of the glomerulus, the proximal convoluted tubule, the ascending and descending limbs of the Loops of Henle, and the distal convoluted tubule [2, 15]. Reciprocal signals between these two tissues are critical for normal kidney development.

The molecular and genetic control of kidney morphogenesis is the subject of several comprehensive reviews [16–20]. Mutational analyses in mice have yielded important insights into the molecular pathways that regulate key events during the formation of nephrons, including the specification and differentiation of the metanephric mesenchyme, ureteric bud induction, renal branching morphogenesis, nephron segmentation and glomerulogenesis. The phenotypes resulting from murine gene mutations also serve as paradigms for renal malformations (*viz.* renal agenesis, duplex kidney) that predict roles for corresponding human gene mutations in the pathogenesis of these conditions. Thus, advances in human genomics and mammalian developmental genetics have accelerated the tempo of discovery in the field of developmental nephrology, providing novel insights into the genetic, epigenetic and environmental factors that impact nephron number and pattern. In the following sections, the morphologic events and molecular underpinnings of these developmental processes will be described.

Origin of the Mammalian Kidney

The mammalian kidney is derived from the intermediate mesoderm of the urogenital ridge, which develops along the posterior abdominal wall of the developing fetus between the dorsal somites and the lateral plate mesoderm. The Wolffian (also known as the mesonephric or nephric duct) is a paired embryonic epithelial tubule extending in an anterior-posterior orientation on either side of the midline, which arises from the intermediate mesoderm. The Wolffian duct is divided into three segments—the pronephros, mesonephros, and metanephros (Fig. 7.1). At its anterior end, the pronephros forms the renal anlage in fish [21] and frogs [22], but degenerates in mammals. The mid-portion of the Wolffian duct, the mesonephros, gives rise to male reproductive organs including the rete testis, efferent ducts, epididymis, vas deferens, seminal vesicle, and prostate [23]. In females, the mesonephric portion of the Wolffian duct degenerates. The caudal portion of the Wolffian duct, the metanephros, becomes the mature mammalian kidney. The posterior segment of the Wolffian duct ultimately communicates with the cloaca to form the trigone of the bladder [23].

Several molecules have been identified as necessary in establishing the immediate precursors to the ureteric bud and metanephric mesenchyme of the developing metanephros in the intermediate mesoderm. The regional specification of metanephric mesenchyme at the posterior intermediate mesoderm next to the Wolffian duct requires the transcription factor, *Odd-skipped related 1* (*Osr1*) [24]. Cell-fate tracing studies

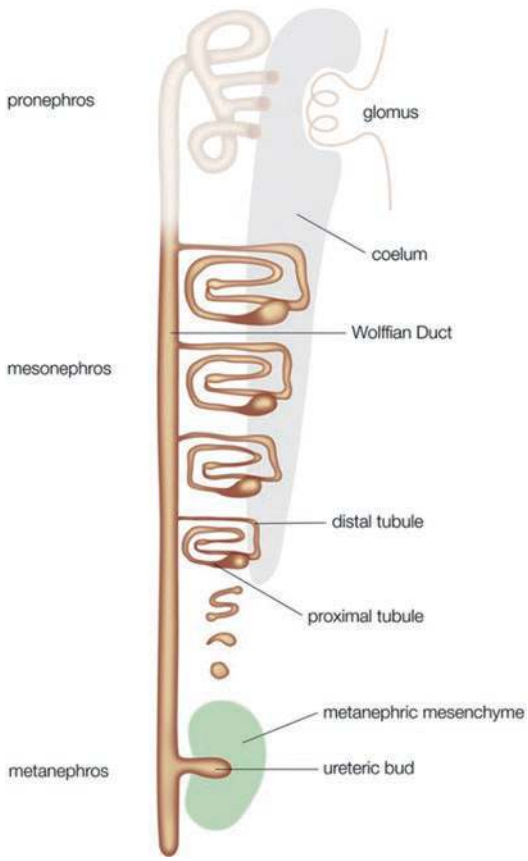


Fig. 7.1 Schematic overview of kidney development. Mammalian kidney development begins with the formation of the Wolffian duct, which is divided into three segments: pronephros, mesonephros and metanephros. The pronephros degenerates in mammals, whereas the mesonephros forms the male reproductive organs (rete testis, efferent ducts, epididymis, vas deferens, seminal vesicles and prostate). The metanephros becomes the mature mammalian kidney, and forms via inductive interactions between the metanephric mesenchyme and the ureteric bud. (Reproduced with kind permission from Springer Science+Business Media: Factors Influencing Mammalian Kidney Development: Implications for Health in Adult Life, Morphological Development of the Kidney, Advances in Anatomy and Cell Biology, Volume 196, 2008, pp. 9–16, Moritz K, et al., Figure 1)

have identified *Osr1*-expressing cells of the intermediate mesoderm as the origin of the principal cellular components of the metanephric kidney: the main body of the nephron, vascular and interstitial cell types, and the Wolffian duct [25]. The establishment and development of the Wolffian duct requires the function of the transcription factors, *Paired box gene 2* (*Pax2*) [26], *Pax8* [27], *Lim homeobox 1* (*Lhx1*) [28] and *Gata binding protein 3* (*Gata3*) [29], along with signaling through the tyrosine kinase receptor, *Ret* [30], and *β-catenin* (*Ctnnb1*) [31].

The ureteric bud forms as an outgrowth of the Wolffian duct in response to external cues provided by the surrounding metanephric mesenchyme. Signals that promote and direct ureteric bud branching morphogenesis originate from all derivative cell types of the metanephric mesenchyme, including induced and uninduced mesenchyme [32–34], stromal cells [35–39], and angioblasts [40, 41], as well as the ureteric bud itself [42]. The metanephric mesenchyme, in turn, originates from undifferentiated cells in the intermediate mesoderm adjacent to the Wolffian duct, to form nephrons and the renal stroma [25]. Similarly, the metanephric mesenchyme responds to inductive cues supplied by the ureteric bud and renal stroma to initiate nephron formation [43–47]. Subsequent patterning and differentiation of the cell types of the nephron is highly dependent on factors secreted by developing epithelial and stromal cells [38, 48, 49].

Induction of Nephrons from the Metanephric Mesenchyme

Once induced by the ureteric bud, the metanephric mesenchyme condenses around the ureteric bud tip, resulting in formation of the cap mesenchyme (Fig. 7.2). The cap mesenchyme is thought to represent a population of nephron progenitors, as these cells have the capacity to self-renew to generate an appropriate number of nephrons at the end of kidney development, and to differentiate into the multiple cell types required to form a mature nephron [50, 51]. The molecules that regulate the specification, proliferation, survival and differentiation of nephron progenitors are a focus of many studies, because an improved understanding of these processes may guide the development of novel cell-based therapies for chronic kidney disease [52].

The differentiation of the cap mesenchyme involves a process termed mesenchymal-

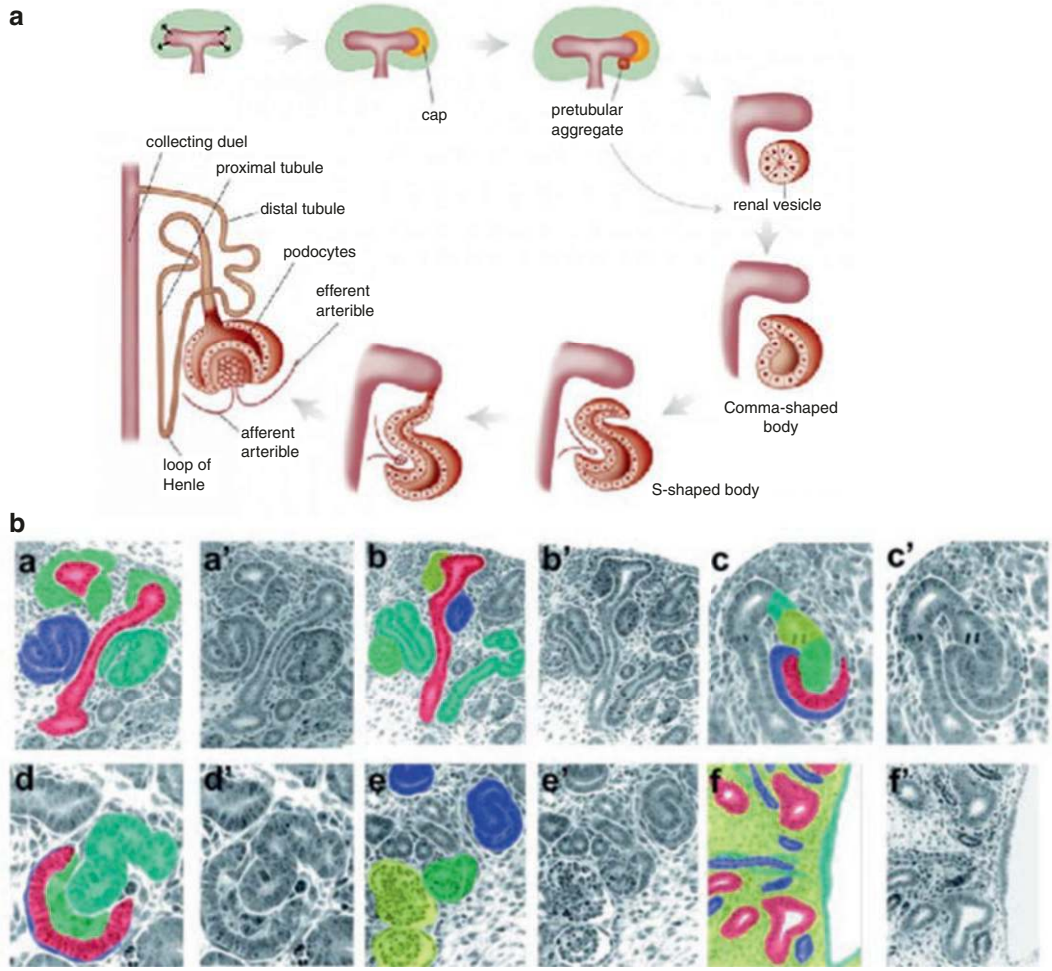


Fig. 7.2 Stages of nephrogenesis. (**A**) Induction of the metanephric mesenchyme by the ureteric bud promotes aggregation of the cap mesenchyme around the tip of the ureteric bud. The cap mesenchyme subsequently undergoes a mesenchymal to epithelial transition to form the pre-tubular aggregate, followed by a polarized renal vesicle. A cleft forms in the renal vesicle giving rise to the comma-shaped body. The development of the S-shaped body involves the formation of a proximal cleft which is subsequently invaded by angioblasts and starts the process of glomerulogenesis. Fusion of S-shaped body occurs with the collecting ducts (reproduced with kind permission from Springer Science+Business Media: Factors Influencing Mammalian Kidney Development: Implications for Health in Adult Life, Morphological Development of the Kidney, Advances in Anatomy and Cell Biology, Volume 196, 2008, pp. 9–16, Moritz, K., et al.). (**B**) Series of matched-pair histological images from a developing metanephric kidney with coloring for specific developmental stages. (a, a′) The ureteric tip and renal cortical collecting duct are in red, with the cap mesenchyme in green. Derivatives of the cap mesenchyme include the comma-shaped body in light blue and S-shaped body in dark blue. (b, b′) Next to the ureteric bud and renal cortical collecting duct (red) is a pre-tubular aggregate (yellow), a renal vesicle (dark blue), capillary loop stage developing glomerulus (green) and renal tubules (light blue). (c, c′) Segments of the S-shaped body include the visceral epithelium (red), parietal epithelium (dark blue), medial segment (green), distal segment (yellow) and renal junctional tubule (light blue). (d, d′) Segments of the capillary loop stage developing glomerulus include the visceral epithelium (red), parietal epithelium (dark blue), presumptive mesangium (green) and renal tubule (light blue). (e, e′) Image of the cortex of the metanephros showing different stages of glomerular development: an S-shaped body (dark blue), capillary loop stage (green) and maturing glomeruli (yellow). (f, f′) Image of the renal medulla and pelvis of the metanephros showing the renal medullary interstitium (yellow), medullary collecting ducts (red), immature loop of Henle (dark blue), renal medullary vasculature (green) and renal pelvic urothelial lining (blue) (reprinted from Gene Expression Patterns, Vol. 7(6), Little MH et al., A high-resolution anatomical ontology of the developing murine genitourinary tract, p. 688, 2007, Figure 3C, with permission from Elsevier)

epithelial transformation (MET). A localized cluster of cells separates from the cap mesenchyme under the ureteric bud tip, and acquires epithelial characteristics, becoming a "pre-tubular aggregate" (Fig. 7.2) [53]. Simultaneous with epithelialization, an internal cavity forms within the pre-tubular aggregate, at which point the structure is termed a renal vesicle. The renal vesicle subsequently forms a connection with its neighboring ureteric bud ampulla, permitting the ureteric bud lumen to communicate with the internal cavity of the renal vesicle. Further differentiation of the renal vesicle in a spatially organized proximal-distal pattern results in formation of the glomerular and tubular segments of the mature nephron (discussed in section "Formation of Nephrons").

Specification of the Metanephric Mesenchyme

The formation of the metanephric mesenchyme is molecularly marked by expression of the transcription factors *Wilms tumour 1* (*Wt1*) [54], *Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 1* (*Cited1*) [55], *Eyes absent homolog 1* (*Eya1*) [56, 57], *sine oculis homeobox homolog 1* (*Six1*) [58], *Six2* [59], *Sal-like 1* (*Sall1*) [60], *Pax2* [26], and *Lhx1* [61], as well as by expression of transmembrane molecules *cadherin-11* (*Cdh11*) [62] and *α8 integrin* (Fig. 7.3A, B) [63]. *Sall1* [60], *Six1* [58], *Eya1* [56, 57] and the secreted peptide growth factor, *Glial-derived neurotrophic factor* (*Gdnf*) [64], are expressed in intermediate mesoderm in the presumptive metanephric mesenchyme. Indeed, the cap mesenchyme is now understood to represent a heterologous population of nephron progenitors, which differ in their relative response to cues for self-renewal and differentiation. Molecular evidence of this is the differing expression patterns of key transcription factors. Thus, *Wt1, Cited1* and *Six2* expression is induced in the cap mesenchyme at the onset of ureteric bud outgrowth [54, 55, 59]. *Pax2* [65] and *Lhx1* [61] are also expressed in cap mesenchyme and its early epithelial derivatives. Recent single cell RNA-sequencing studies in developing mouse [66–71] and human kidneys [72–74] have contributed to our understanding of these subpopulations of nephron progenitors as well as the differences between mouse and human kidney development (Table 7.1).

Phenotypic analyses of mice with targeted gene deletions or tissue-specific inactivation of conditional alleles for these transcription factors have been informative regarding their role in specification of the cap mesenchyme, and subsequent induction of ureteric bud outgrowth. Homozygous deletion in many of these genes (including: *Eya1* [57], *Six1* [58], *Pax2* [75, 76], *Wt1* [54], *Sall1* [60], *Six2* [59] and *Lhx1* [61, 77]) causes ureteric bud outgrowth failure, and results in bilateral renal agenesis or severe renal dysgenesis with variable penetrance depending on the gene involved (Fig. 7.3E, F). However, the underlying molecular mechanism responsible for renal agenesis/severe dysgenesis in each of these mutants varies. For example, *Pax2* mutants fail to form the posterior Wolffian duct from which derives the ureteric bud [76]. In contrast, *Lhx1* [61, 78] and *Sall1* [60] mutants initiate, but do not complete, ureteric bud induction. *Wt1* mutants also exhibit failed ureteric bud induction and show apoptosis of the metanephric mesenchyme [54] (Fig. 7.3E, F). Tissue recombination experiments show that isolated metanephric mesenchyme explants from *Wt1* knock-out mice are neither competent to respond to signals from wild-type ureteric buds, nor able to induce growth and branching of isolated wild-type ureteric bud explants [54, 79]. In contrast, *Sall1*-deficient mesenchyme, which expresses *Wt1*, responds to a heterologous inducer in *ex vivo* tissue recombination experiments [60], suggesting that *Sall1* functions downstream of *Wt1* in a genetic regulatory cascade. Taken together, these data present an emerging image of intricate interplay between transcription factors that are required for the establishment of the nephric duct, subsequent specification of the cap mesenchyme, induction of *Gdnf* expression, and regulation of the differentiation capacity of the cap mesenchyme in response to inductive cues.

Indeed, insights into this interplay come from recent genome-wide studies of transcription fac-

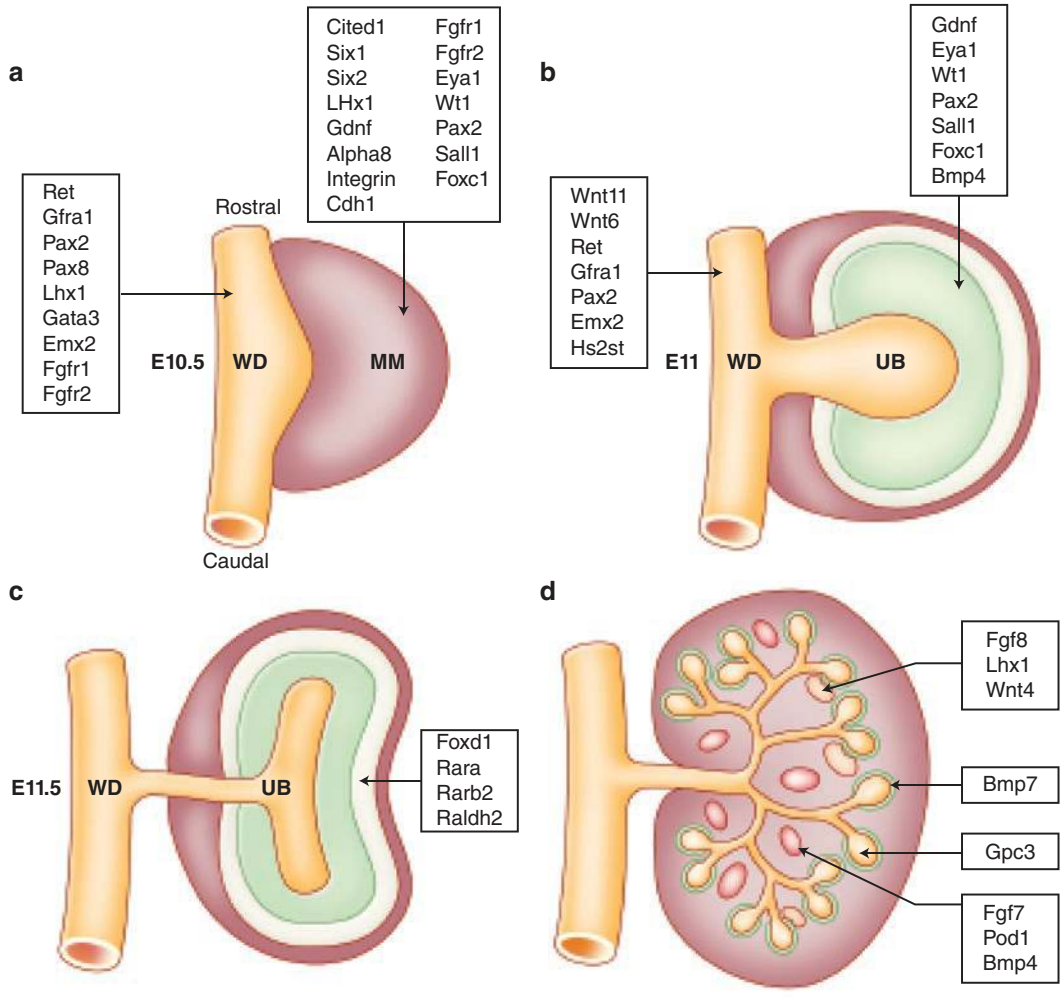


Fig. 7.3 Molecules involved in specification of the metanephric mesenchyme and nephron induction. (**A**) The mammalian kidney starts to develop when the ureteric bud forms at the caudal end of the Wolffian duct (WD) at about embryonic day (E)10.5 in mice. The ureteric bud grows into the metanephric mesenchyme (MM) and (**B**) induces the mesenchyme that is adjacent to the tips of the ureteric bud (UB) to condense to form the cap mesenchyme, as well as the stromal cells that are peripheral to the cap. (**C**) The cap mesenchyme induces the ureteric bud to branch from E11.5 onwards. (**D**) In association with ureteric branching morphogenesis, mesenchymal cells are induced at each ureteric bud tip to undergo a mesenchymal-to-epithelial transformation to form the nephron. *Bmp7* bone morphogenetic protein 7, *Emx2* empty spiracles 2, *Eya1* Eya1, eyes absent 1, *Fgf7* fibroblast growth factor 7, *Foxc1* forkhead box C1, *Foxd1* forkhead box D1, *Gdnf1* glial cell-line-derived neurotrophic factor 1, *Gfra1* glial cell-line derived neurotrophic factor receptor-α1, *Gpc3* glypican-3, *Hs2st* heparan sulfate 2-*O*-sulphotransferase 1, *Pax1* paired-box gene 1, *Rara* retinoic acid receptor-α, *Ret* Ret proto-oncogene, *Sall1* sal-like 1, *Wnt* Wingless-related, *Wt1* Wilms tumour 1 (adapted with permission from Macmillan Publishers Ltd.: Nature Reviews Genetics, Coordinating early kidney development: lessons from gene targeting, Vol. 4(7), p. 535, 2002, Vainio S and Lin Y, Figure 1). Representative kidney phenotypes of mice with targeted deletions affecting nephron progenitors and nephron induction. (**E**–**F′**) Histological transverse sections from E11.5 embryos showing a wild-type (**E**) and *Wt1* mutant embryo (**F**) with the ureteric bud (U), Wolffian duct (W) and metanephric mesenchyme (M) labeled. (**E′**–**F′**) At higher magnification, a cluster of apoptotic cells with dark nuclear fragments is visualized in the Wt1 mutant kidney, resulting in loss of nephron progenitors (arrow) (reprinted from Cell, Vol. 74(4), Kreidberg et al., WT-1 is required for early kidney development, p. 682, 1993, with permission from Elsevier). (**G**–**H′**) Loss of *Wnt4* results in renal hypoplasia and failure of nephron induction, with no epithelial to mesenchymal transition when comparing control (**G**–**G′**) to control kidneys (**H**–**H′**) (reprinted with permission from Macmillan Publishers Ltd.: Nature, Epithelial transformation of metanephric mesenchyme in the developing kidney regulated by Wnt-4, 372(6507), p. 682, 1994, Stark et al., Figure 3)

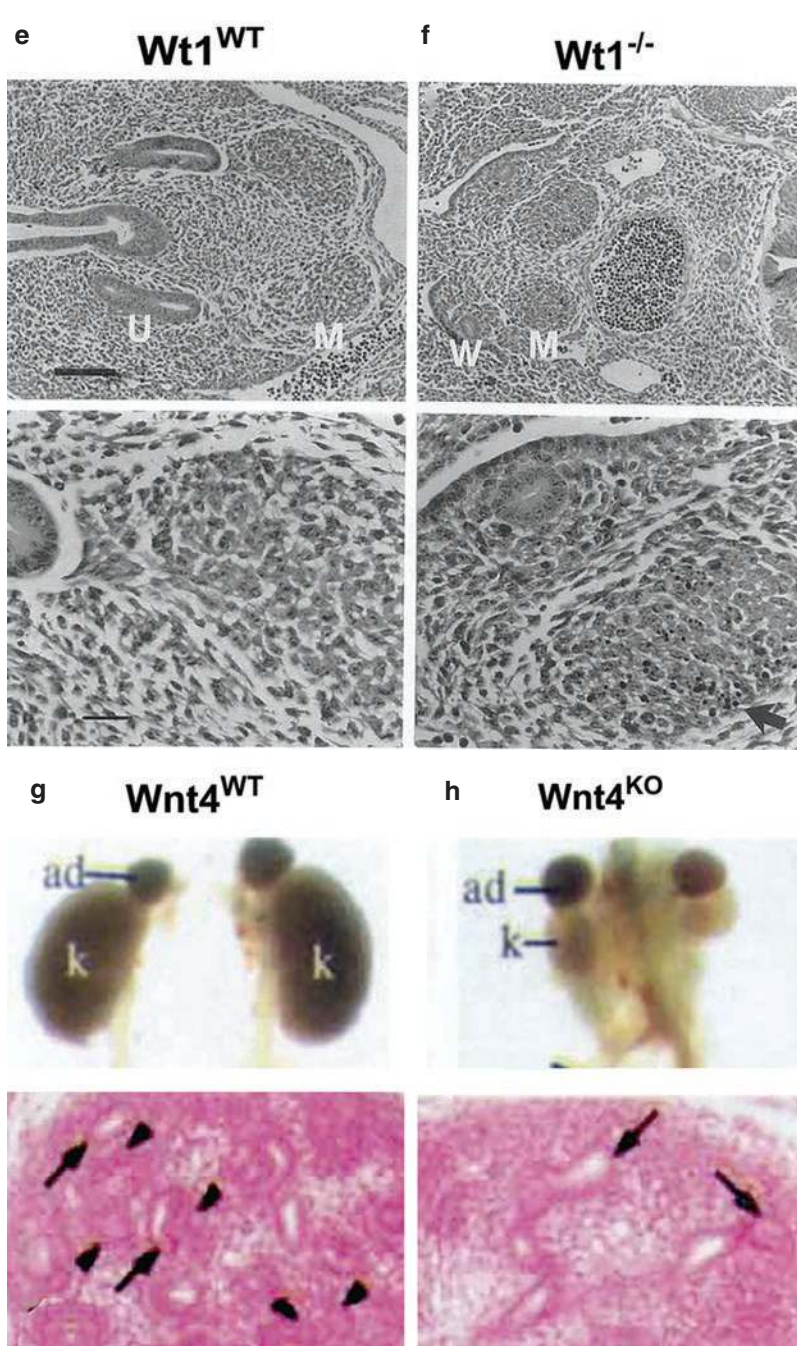


Fig.7.3 (continued)

tor binding using chromatin immunoprecipitation followed by sequencing (ChIP-seq). The transcription factors Six2, Hoxd11, Osr1, and Wt1 have been shown to bind enhancer sequences located in "regulatory hot-spots" in the genome in nephron progenitors [80]. In addition, Six2 co-binds enhancers with β-catenin to drive expression of genes associated with nephron progenitor self-renewal and differentiation [81]. These studies have also identified differences in the transcriptional regulation of mouse and human nephron progenitors mediated by Six1 and Six2 [82].

There is a growing body of work defining the signals required to induce and regulate nephron progenitors, to enable the generation of induced pluripotent stem cell (iPSC)-derived nephron progenitors and optimize the culture of kidney organoids (reviewed in [83]). Of these, the growth factor, *Wingless-type MMTV integration site family 9b* (*Wnt9b*), is required in the ureteric bud for induction of the cap mesenchyme (Fig. 7.3B). The loss of *Wnt9b* activity results in failure of the cap mesenchyme to undergo MET [84]. Moreover, *Wnt9b* plays an important role in regulating the balance between nephron progenitor differentiation and self-renewal, in cooperation with signals mediated by stromal cells [47, 85].

Fibroblast growth factor (FGF) signaling has also been shown to be critical in regulating nephron progenitors. The FGF ligands belong to a large family of secreted peptides that signal

Table 7.1 Mouse mutations exhibiting defects in kidney morphogenesis and predominant accompanying renal phenotypes

Mutant gene	Morphogenetic defect	Predominant mutant renal malformation phenotype
Failed ureteric bud outgrowth		
Metanephric mesenchyme-derived		Renal aplasia
Eya1 *Fgf9/20* *Fgfr1/2* *Frs2α* *Gdnf* *Grem1* *Lhx1* *Osr1* *Pax2* *Sall1* *Six1* *Wt1*		
Ureteric bud-derived		
Emx2 *Etv4/5* *Gata3* *Gfrα1* *Hoxa11/Hoxd11/Hoxc11* *Hs2st* *Itgα8* *Lhx1* *Pax2/8* *Ret* *Wnt9b*		
Ectopic ureteric bud outgrowth		
Bmp4 *Foxc1/c2* *Robo2* *Slit2* *Spry1*		Duplex collecting system
Decreased ureteric bud branching		

Table 7.1 (continued)

Mutant gene	Morphogenetic defect	Predominant mutant renal malformation phenotype
Alk3 *Alk6* *Dsch1/2* *Ecm1* *Fat4* *Fgfr2* *Foxd1* *Hnf1β* *Pod1* *Raldh2* *Rarα/Rarβ2* *Spry2* *Wnt11*		Renal hypoplasia, renal dysplasia
Defective renal medulla formation		
Fgf7 *Fgf10* *Gpc3* *Hnf1β* *p57^{KIP2}*		Medullary dysplasia
Agt *Agtr1* *Agtr2* *Bmp4* *Bmp5*		Hydronephrosis
Defective tubulogenesis		
Bmp7 *Brn1* *Fgf8* *Hnf4a* *Lhx1* *Notch1* *Notch2* *Pod1* *Psen1/Psen2* *Rbpsuh* *Six2* *Wnt4* *Wnt9b*		Renal hypoplasia, renal dysplasia
Defective glomerulogenesis		
Jag1 *Notch2* *Pdgfβ* *Pdgfrβ* *Pod1* *Vegf*		Glomerular malformation
Col4α3/Col4α4/Col4α5 *Lamb* *Lmx1β* *Mafβ* *Wt1*		Loss of glomerular filtration selectivity

Adapted with permission from Comprehensive Pediatric Nephrology, Structural and Functional Development of the Kidney, 1st edition, p. 98, 2008, Tino Piscione

through their cognate receptor tyrosine kinases, FGF receptors (FGFRs). Several FGFs are expressed in the developing kidney, including FGF2, FGF7, FGF8, and FGF10 [39, 86, 87] (Fig. 7.3). Two FGF ligands, *Fgf9* and *Fgf20*, have been shown to be critical in regulating

nephron progenitor survival, proliferation and competence to respond to inductive signals in mice and humans, with loss of these signals resulting in renal agenesis [88]. These *in vivo* findings were supported by *in vitro* studies showing that the addition of FGF1, 2, 9 and 20 protein results in the ability to maintain early nephron progenitor cells in culture [89]. In addition, *in vivo* data is consistent with a functionally important role for *Fgfr1* and *Fgfr2* in the metanephric mesenchyme during kidney development, with loss of *Fgr1* and *Fgfr2* function in the metanephric mesenchyme resulting in renal agenesis [90–92].

Bone morphogenetic protein (BMP) signaling interacts with FGF signaling to balance the self-renewal and differentiation of nephron progenitors. Bmp7 induces the initial exit of nephron progenitors into a state "primed" for differentiation by ureteric bud-derived Wnt 9/β-catenin signaling (Fig. 7.3). Conversely, remaining nephron progenitors are kept in an undifferentiated and self-renewing state in response to FGF9, Wnt and BMP7 signals [81, 85, 88, 89, 93–97]. Mice deficient for *Bmp7* demonstrate developmental arrest and rudimentary kidneys without the inhibition of differentiation of already induced cells, which is likely to due to failure to expand and renew the epithelial precursor population [98, 99]. Together, these data have now informed several *in vitro* protocols to propagate nephron progenitors that rely on a combination of FGF ligands and manipulation of BMP signaling, amongst other growth factors in the culture media [100–103].

The regulation of nephron progenitor survival also contributes to determining nephron number. Archetypal organ culture experiments have demonstrated that isolated metanephric mesenchyme undergoes apoptosis unless induced by co-culture with ureteric buds or a heterologous inducer (e.g. spinal cord) [15, 33]. Several soluble factors have been described to be essential to prevent mesenchymal apoptosis, e.g. epidermal growth factor (EGF), FGF2, and BMP7 [43, 104, 105]. In addition, microRNAs (miRNAs) have been implicated in regulating nephron progenitor survival [106, 107]. However, inhibition of apoptosis by pharmacologic or genetic manipulation causes defects in ureteric bud branching and nephrogenesis [108, 109], suggesting that alterations in cell survival disrupt important functional interactions between mesenchymal and epithelial cells. Possible roles for apoptosis in the metanephric mesenchyme include regulation of nephron number or establishment of tissue boundaries between cells destined to become epithelial or stromal [110, 111].

Recent studies have also shed light on the molecular mechanisms that regulate the cessation of nephrogenesis. Transcriptional changes in nephron progenitors occur as kidney development proceeds, and they are associated with genes and pathways that influence stem cell aging, in particular *Mechanistic target of rapamycin* (*mTor*) and its repressor *Hamartin* (*Tsc1*) [112, 113]. These changes are thought to desensitize nephron progenitors to signals for self-renewal, contributing to the cessation of nephrogenesis [113]. Furthermore, miRNA in the *let-7* family are thought to play an important role in the timing of cessation of nephrogenesis. Ectopic expression of *Lin28b*, a known let-7 family repressor, is sufficient to prolong nephrogenesis [114].

Nephron Induction

Seminal experiments involving isolated kidney rudiments cultured *ex vivo* established the role of ureteric bud-derived secreted factors in providing inductive cues for cap mesenchyme to undergo epithelial differentiation to initiate tubulogenesis [33]. The isolation of ureteric bud cell lines has subsequently facilitated the identification of several secreted factors that function individually or in combination to cause mesenchymal-epithelial conversion and tubulogenesis, including FGF2 [115], leukemia inhibitory factor (LIF) [44, 116, 117], transforming growth factor-β2 (TGFβ2) [117, 118], and growth/differentiation factor-11 (GDF-11) [117, 119].

Wnt genes also play a key role in epithelial conversion, as suggested by the observation that cells expressing WNT proteins are potent inducers of tubulogenesis in isolated metanephric mesenchyme [120, 121]. A genetic requirement for *Wnt4* in MET is revealed by the demonstration of the arrest of the cap mesenchyme, and the inabil-

ity to form pre-tubular aggregates in *Wnt4* mutant mice [84] (Fig. 7.3D, G, H). The effects of Wnt signals are modulated by mesenchymal-derived secreted Wnt binding proteins, including secreted Frizzled-related protein (sFrp). sFrp antagonizes the actions of Wnt4 and Wnt9b by binding secreted Wnt proteins *in vitro* and preventing them from activating membrane-bound Wnt receptors [122]. Similar to the induction of nephron progenitors, FGF signaling is also critical for MET. Mice with deletions in *Fgf8* fail to undergo tubulogenesis, and the data suggests that *Wnt4* and *Fgf8* cooperate in the conversion of mesenchymal to epithelial cells, possibly by upregulating *Lhx1* expression [123, 124]. The transcription factor, *Lhx1*, is uniformly expressed in renal vesicles [61], and conditional knockout of *Lhx1* in metanephric mesenchyme causes developmental arrest at the renal vesicle stage [61].

Ureteric Bud Induction and Branching

Ureteric bud formation begins at week 5 of human fetal gestation and at embryonic day 10.5 (E10.5) in mice. Signaling from the metanephric mesenchyme induces the ureteric bud from the Wolffian duct (Fig. 7.4A). The ureteric bud then elongates and branches into the surrounding mesenchyme, a process termed *branching morphogenesis*, to ultimately pattern the collecting ducts and pelvicaliceal system [125, 126]. Renal branching morphogenesis occurs as a sequence of tightly regulated events: (1) ureteric bud outgrowth; (2) ureteric bud branching and derivation of collecting ducts; (3) cortical and medullary patterning of the collect ducts; and (4) formation of the renal pelvises and calyces.

Ureteric Bud Induction and Outgrowth

Genes encoding *Gdnf* [127], its tyrosine kinase receptor *Ret* [128], and its co-receptor *Gfrα1* [129] are crucial regulators of ureteric bud outgrowth. Targeted *Gdnf* mutations in mice cause failure of ureteric bud induction and bilateral renal aplasia [130, 131] (Fig. 7.4B). Homozygous deletion of *Ret* or *Gfrα1* cause the same defect [128, 132–135] (Fig. 7.4C). However, initial ureteric bud outgrowth was evident in 20–40% of $Gdnf^{-/-}$ or $Ret^{-/-}$ mutants [128, 130], indicating that GDNF-RET signaling is not the only molecular pathway involved in ureteric bud induction and outgrowth. Mice with homozygous null mutations in *heparan sulfate 2-sulfotransferase* (*Hs2st*), which is involved in proteoglycan synthesis, show bilateral renal agenesis and induction of the ureteric bud, but no further outgrowth [136]. Mice with a mutation in integrin *α8* show a similar phenotype in 50% of the mutant mouse embryos [137], implicating integrin signaling as an important molecular pathway for ureteric bud outgrowth.

The downstream effect of GDNF-RET signaling is ureteric bud proliferation, cell survival, and epithelial branching. Downstream genes of GDNF-RET signaling include *ETS transcription factor 4* (*Etv4*) and *Etv5*, which are upregulated in response to RET signaling, and loss of both transcription factors results in bilateral renal agenesis due to ureteric bud formation defects [138]. Expression of several genes in the ureteric bud tip depend on *Etv4* and *Etv5*, including *Chemokine (C-X-C motif) receptor 4* (*Cxcr4*), *matrix metallopeptidase 14* (*Mmp14*), *Myb proto-oncogene* (*Myb*), *Met proto-oncogene* (*Met*), and *Wnt11* [138]. Loss of *Wnt11* in mice leads to abnormal ureteric branching with resultant renal hypoplasia and decreased levels of GDNF in the metanephric mesenchyme; conversely, *Wnt11* expression is reduced in the absence of GDNF, suggesting a feedback loop that coordinates ureteric branching [97]. Furthermore, *hepatocyte nuclear factor-1 beta* (*Hnf1β*) has been implicated as a transcriptional regulator of *Etv5*, and mice lacking *Hnf1β* also show abnormal ureteric bud outgrowth and branching [139, 140]. Thus, these studies highlight that improper ureteric bud induction and outgrowth lead to renal agenesis or renal hypoplasia.

GDNF in organ cultures induce ectopic ureteric buds along the Wolffian duct [127, 141], indicating that additional signals are required to

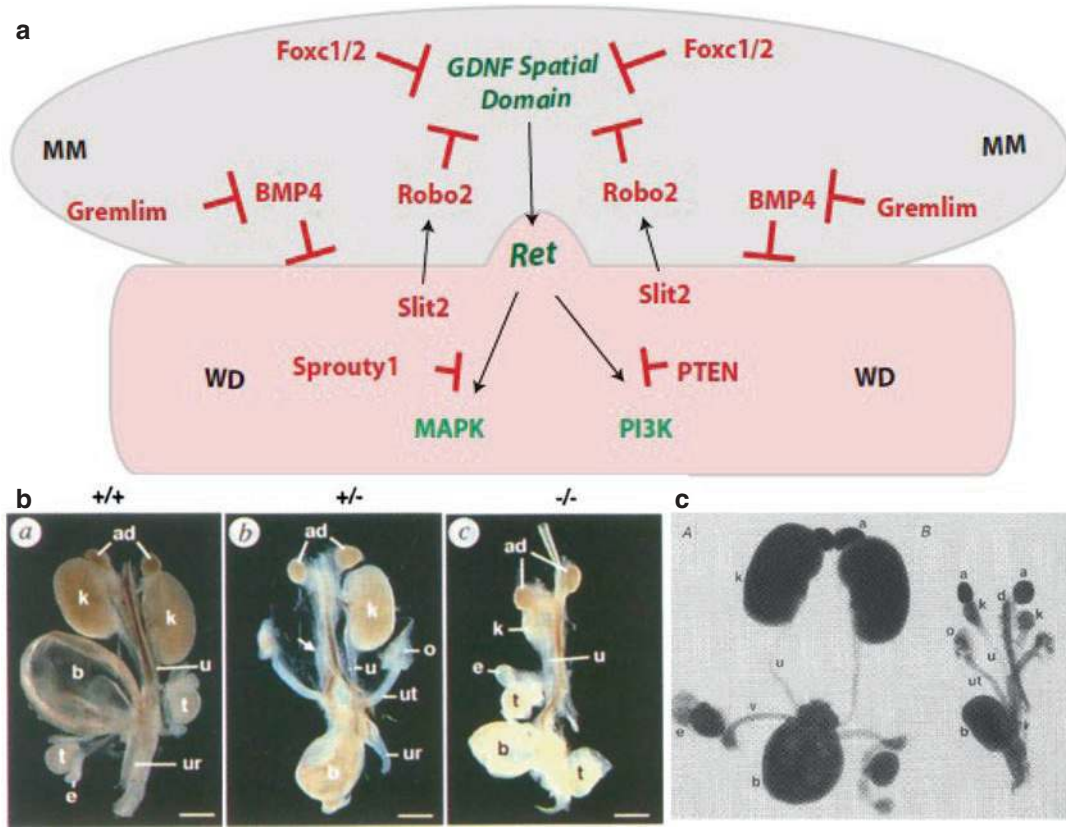


Fig. 7.4 Schematic demonstrating the molecular control of ureteric bud branching. (**A**) Glial cell-line derived neurotrophic factor (GDNF) is secreted from the mesenchyme (grey) and binds to the Ret receptor to induce a thickening of the WD (pink); the WD will then elongate to form the UB. Binding of GDNF to RET leads to activation of the MAPK and PI3K signal transduction pathways. These pathways are negatively regulated by SPROUTY1 and PTEN, respectively. SLIT2/ROBO2, FOXC1 and FOXC2 inhibit the spatial GDNF expression domain and limit UB outpouching to a single site. BMP4 inhibits branching, in a manner that is opposed by Gremlin. RED Inhibitory, Green stimulatory, Grey MM and GDNF spatial domain, Pink WD (with kind permission from Springer Science+Business Media: Pediatric Nephrology, Stimulatory and inhibitory signaling molecules that regulate renal branching morphogenesis, Vol. 24, p. 1616, 2009, Bridgewater D and Rosenblum ND, Figure 4). Representative kidney phenotypes of mice having targeted mutations affecting branching morphogenesis. (**B**) Bilateral renal agenesis in *GDNF* mutant mice (reprinted with permission from Macmillan Publishers Ltd.: Nature, Defects in enteric innervation and kidney development in mice lacking GDNF, 382(6586), p. 74, 1996, Pichel et al., Figure 2). (**C**) Severe bilateral renal hypoplasia in *Ret* null mutants. A, adrenal; K, kidney; U, ureter; B, bladder (reprinted with permission from Macmillan Publishers Ltd.: Nature, Defects in the kidney and enteric nervous system of mice lacking the tyrosine kinase receptor Ret, 367(6461), p. 382, 1994, Schuchardt et al., Figure 2)

restrict the number of ureteric buds induced, as well as the position of ureteric bud induction. When multiple ureteric buds form from a single Wolffian duct, renal and urogenital malformations occur (e.g. duplex kidney) [142]. The position of ureteric bud outgrowth from the Wolffian duct is also critical for the formation of a single ureter and competent vesicoureteral junction. Outgrowth of the ureteric bud at an ectopic site leads to ectopic insertion of the ureter into the bladder [143]. Mice with mutations in *Bmp4* [144], *Slit homologue 2* (*Slit2*) or its receptor *Roundabout guidance receptor 2* (*Robo2*) [145], components of the renin-angiotensin cascade [109], *Forkhead box C1* (*Foxc1*), *Foxc2* [146], *Fgfr2* [147, 148], *FGF receptor substrate 2α* (*Frs2α*) [149], *Dicer1* [150–152], *Ret* [153], and *Pax2* [154] exhibit abnormal ureteric bud induc-

tion sites and/or ureteric branching with subsequent ureteral duplications, abnormal ureter insertion into the bladder, and/or vesicoureteral reflux. These studies underscore the importance of tightly regulated GDNF-RET signaling activity to establish normal ureteric bud induction site and subsequent branching.

Positive Regulation of GDNF-RET Signaling

Prior to ureteric bud induction, *Gdnf* is expressed along the entire length of the intermediate mesoderm parallel to the Wolffian duct [127] and *Ret* is expressed throughout the Wolffian duct [155]. By the time of ureteric bud induction, *Gdnf* expression is restricted to the posterior intermediate mesoderm, marking the location of the presumptive metanephric mesenchyme close to the site of ureteric bud outgrowth. Once the ureteric bud enters the metanephric mesenchyme, *Ret* expression becomes restricted to the epithelial cells of the branching ureteric bud [155]. As noted above, mice that ectopically express *Gdnf* or *Ret* during ureteric bud induction and branching have renal malformations, which underscores the importance of regulation of the spatial activity of GDNF-RET signaling [156, 157].

Three transcription factor genes expressed in the intermediate mesoderm—*Eya1*, *Six1*, and *Pax2*—promote *Gdnf* expression during kidney development (Fig. 7.3A). Genetic mutations in *Eya1* or *Pax2* in mouse models both result in no *Gdnf* expression [57, 158]. *Eya1* knock-out mice fail to undergo ureteric bud induction due to loss of *Gdnf* expression and the result is renal agenesis [57]. *Eya1* mutant mice also show a loss of *Six1* expression, suggesting an Eya-Six-Gdnf signaling cascade [57, 159]. *Six1* null mice fail ureteric bud induction into the mesenchyme, which subsequently undergoes apoptosis [58]. *Eya1* expression in the *Six1* null mice is normal, further supporting that it is upstream of *Six1* [58]. *Pax2* is expressed in the intermediate mesoderm and homozygous null mice do not undergo ureteric bud outgrowth and *Gdnf* expression is absent [158]. *Gdnf* transcription is directly activated by *Pax2* [158].

Other activators of *Gdnf* expression have been identified, including the paralogous genes *Homeobox A11* (*Hoxa11*), *Hoxc11*, and *Hoxd11* [160]. Compound inactivation of at least two of these genes causes renal aplasia and loss of *Gdnf* and *Six2* expression, with normal *Eya1* and *Pax2* [160]. It has been suggested that Hox genes maintain *Gdnf* expression by cooperating with *Eya1* to induce *Six1* and *Six2* expression [159]. An additional positive regulator of *Gdnf* is *Empty spiracles homolog 2* (*Emx2*), which is expressed in the Wolffian duct [161]. Homozygous *Emx2* mutant mice exhibit renal agenesis with failure of ureteric bud branching after it is induced [161]. There is down-regulation of ureteric bud markers *Pax2*, *c-Ret*, and *Lim1* as well as reduced *Gdnf* expression in the metanephric mesenchyme [161]. In explant culture experiments, branching of the *Emx2* mutant ureteric bud was not induced by wild-type mesenchyme; whereas, mutant mesenchyme was able to induce wild-type ureteric bud branching [161]. These data suggest that *Emx2* may be important in the ureteric bud to provide cues to maintain *Gdnf* expression in the mesenchyme and sustain ureteric bud branching.

Negative Regulation of GDNF-RET Signaling

Negative regulation of Gdnf expression is equally important to control the location and number of ureteric buds induced. *Foxc1*, *Slit2*, and *Robo2* are involved in restricting *Gdnf* expression to the posterior intermediate mesoderm (Fig. 7.4A). Mice lacking either *Slit2* or its receptor *Robo2* develop supernumerary ureteric buds and multiple ureters, with *Gdnf* expression inappropriately maintained in the anterior metanephric mesenchyme [145]. The anterior expansion of *Gdnf* occurs without alterations in expression of *Foxc1*, *Eya1*, or *Pax2* [145], suggesting that SLIT2 and ROBO2 function in a parallel pathway to restrict GDNF-RET activity during ureteric bud outgrowth. The secreted protein SLIT2 and its receptor ROBO2 are encoded by genes best known for their role in axon guidance, functioning as chemorepellents that cause axons or migrating cells to move away from the source of SLIT2 [162],

which may be the mechanism by which they regulate *Gdnf* expression domain. Their expression pattern supports this: *Slit2* is primary expressed at the tips of the branching ureteric buds and *Robo2* is expressed in a complementary pattern in the metanephric mesenchyme [163]. More recently, further evaluation of *Robo2* null mice shows failure of the nephrogenic cord to separate from the Wolffian duct, leading to increased proliferation signals, which suggests a possible second mechanism by which *Gdnf* domain expands and aberrant ureteric buds form [164]. *Foxc1* homozygous null mutant mice have anterior expansion of *Gdnf* and *Eya1*, leading to ectopic anterior ureteric buds that result in ureter and renal abnormalities [146]. *Foxc1* encodes a transcription factor that overlaps in domain with *Gdnf* in the intermediate mesoderm and these data suggest that *Foxc1* regulates either *Eya1* or its upstream genes [146].

The gene *Sprouty1* (*Spry1*), a receptor tyrosine kinase agonist, is implicated in a regulatory feedback loop involving GDNF-RET signaling and downstream effector, *Wnt11*. *Spry1* is expressed along the Wolffian duct, with highest levels of expression in the posterior aspect [165] (Fig. 7.4A). Loss of *Spry1* function in mice results in ectopic ureteric bud induction and urogenital malformations such as multiple ureters and kidneys [165, 166]. Recently, tyrosine 53 of *Spry1* is implicated as essential for *Spry1* function, showing the same phenotype as *Spry1* null mice [167]. *Gdnf* and *Wnt11* expression extends more anteriorly in *Spry1* null mice [165, 166]. Metanephric cultures showed more sensitivity to GDNF in *Spry1* null mutants, inducing supernumerary ureteric buds at low concentrations compared to wildtype [165]. Further, *Ret* is required to maintain *Spry1* expression in the Wolffian duct [165]. Ectopic expression of human *SPRY2*, a related homologue to *Spry1*, in the ureteric bud in transgenic mice leads to decreased ureteric branching [168]. Together, these data suggest that *Spry1* acts as a negative regulator of a GDNF/RET/*Wnt11* feedback loop to prevent supernumerary ureteric budding and guide branching.

Another example of negative feedback in GDNF-RET signaling and branching morphogenesis involves secreted growth factors belonging to the Bone Morphogenetic Protein (BMP) family. Kidney organ culture studies reveal inhibitory roles for BMP-2, -4, and -7 in ureteric bud branching morphogenesis [169–172]. However, only *Bmp4* shows convincing evidence *in vivo* for modulating ureteric bud outgrowth [144] (Fig. 7.4A). *Bmp4* is expressed in stromal cells adjacent to the Wolffian duct and the early ureteric bud and its receptors are expressed in the Wolffian duct [144, 173]. Mice heterozygous for *Bmp4* exhibit ectopic or multiple ureteric buds, shortened ureteric trunk and first branch stem, and a spectrum of urinary tract and renal malformations [144]. The abnormal sites of ureteric bud outgrowth in *Bmp4* heterozygous mice suggests that BMP4 inhibits the local effect of GDNF-RET signaling to constrain ureteric bud induction at the appropriate site and promotes elongation at the stalk of the branching ureteric bud [144]. BMP4 can block the ability of GDNF to induce ureteric bud outgrowth from the Wolffian duct *in vitro* [158], and also inhibits further ureteric bud branching *in vitro* in an asymmetric manner [169, 170]. *Gremlin1* (*Grem1*) inhibits BMP4 activity, enabling ureteric bud outgrowth [174].

Other signaling factors have been implicated in regulating the appropriate position of the ureteric bud. For example, *Receptor tyrosine kinase like orphan receptor-1* (*Ror1*) and *-2* (*Ror2*) bind Wnt5a, and have recently been implicated in ureteric bud formation. *Ror2* and *Wnt5a* deficient mice exhibit ectopic rostral ureteric buds and abnormal *Gdnf* expression domain [175, 176].

Renal Branching Morphogenesis

Renal branching morphogenesis begins between the fifth and sixth week of gestation in humans [2], and at E11.5 in mice [177] when the ureteric bud invades the metanephric mesenchyme and forms a T-shaped, branched structure (Fig. 7.3C). The T-shaped structure then undergoes further iterative branching events to generate approximately 15 generations of branches. In human kidney development, the first 9 generations of branching are complete by approximately

15 weeks gestation [2]. During this time, new nephrons are induced through reciprocal inductive interactions between the newly formed tips of the ureteric bud and the surrounding metanephric mesenchyme. By the 20th–22nd week of gestation, ureteric bud branching is completed, and the remainder of collecting duct development occurs by extension of peripheral (or cortical) segments and remodeling of central (or medullary) segments [2]. During these final stages, new nephrons form predominantly through the induction of approximately four to seven nephrons around the tips of terminal collecting duct branches that have completed their branching program, while retaining the capacity to induce new nephron formation [2, 177].

Analysis of renal branching morphogenesis in organ culture systems employing kidneys of transgenic mice expressing the fluorescent reporter, enhanced green fluorescent protein (EGFP), in the ureteric bud lineage have been informative regarding the sequence and pattern of branching events that occur following the formation of the initial T structure [178, 179]. Throughout renal branching morphogenesis, the branching ureteric bud recapitulates a patterned, morphogenetic sequence. This sequence includes: (1) expansion of the advancing ureteric bud branch at its leading tip (called the ampulla); (2) division of the ampulla causing the formation of new ureteric bud branches; and (3) elongation of the newly formed branch segment.

Proliferation and Apoptosis in Branching Morphogenesis

During branching morphogenesis, there is a zone of high proliferation at the site of ureteric bud and then at the tips of the branching ureteric buds/collecting ducts [180]. Proliferation rates are highest at and localized to sites undergoing active branching morphogenesis [180] and as new branches form, proliferation rates are higher in branch tips than trunks [42, 180, 181], which suggests that localized cell proliferation contributes to evagination of the ureteric bud from the Wolffian duct and formation of ampullae. A time-lapse imaging study traced individual ureteric bud tip cells to find that most tip cells are self-renewing progenitors whose progeny either populate the growing ureteric bud trunk or remain at the tips [182].

Apoptotic cells are rarely seen in the ureteric branches [183], and cultured ureteric bud cells do not demonstrate apoptosis *ex vivo* [184]. This suggests that ureteric bud-derived cells may have an intrinsic survival tendency. Further evidence that cell survival is tightly regulated during branching morphogenesis is provided by several studies in which dysregulated apoptosis and cell proliferation are associated with defective collecting duct development. *Glypican-3*-(*Gpc3*) deficient mice exhibit increased cell proliferation with increased ureteric bud branching, increased cortical collecting duct proliferation, and increased medullary collecting duct system apoptosis leading to cystic and dysplastic kidneys [185, 186]. Additionally, increased apoptosis was associated with collecting duct cyst formation in mice with mutated genes associated with cell survival including *B-cell lymphoma 2* (*bcl2*) [187] and *transcription factor AP2* (*AP-2*) [188]. Also, apoptosis is prominent in dilated collecting ducts in experimental models of fetal and neonatal urinary tract obstruction [189, 190]. These data suggest a relationship between collecting duct apoptosis and two frequent features of renal dysplasia—cystogenesis and urinary tract dilation.

Signaling Molecules in Branching Morphogenesis

In addition to stimulating ureteric bud induction, GDNF is also a stimulus for subsequent ureteric bud branching [157, 191]. Transgenic mice that express *Gdnf* ectopically in the Wolffian duct and ureteric buds develop multiple, ectopic ureteric buds that branch independently of the metanephric mesenchyme [157]. However, *in vitro* studies demonstrate that GDNF is not sufficient to induce robust branching in isolated ureteric bud culture [127, 192], which suggests that other factors cooperate with GDNF-RET to control ureteric branching.

Retinoic acid (RA) signaling is also essential for ureteric bud induction and branching. Inactivation of retinoic acid receptors RARα and RARβ2 leads to decreased ureteric bud branch-

ing and downregulation of *Ret* in the ureteric bud [36]. Forced expression of *Ret* in the ureteric bud lineage in *Rarα* and *Rarβ2* double mutant mice is sufficient to restore ureteric bud outgrowth [37]. Retinoic acid activates RA-receptor signaling in the ureteric bud and regulates *Ret* expression in the ureteric bud tips and subsequent proper branching morphogenesis [37, 193]. More recently, *in vitro* studies suggest that retinoic acid-regulated *Extracellular matrix 1* (*Ecm1*) restricts *Ret* expression to the ureteric bud tip for proper branching morphogenesis [194].

WNT signaling acts in concert with GDNF-RET signaling to regulate branching morphogenesis. *Wnt11* is expressed at the tips of the ureteric bud and mice that are deficient in *Wnt11* have defects in branching morphogenesis [97, 195] (Fig. 7.3B). This data suggests that *Wnt11* functions at least in part to maintain GDNF expression; conversely, *Wnt11* expression is reduced in the absence of GDNF-RET signaling [97]. β-catenin is an effector that regulates downstream transcriptional targets of the canonical WNT pathway. Mice with β-Catenin deficiency in the ureteric bud cell lineage [196] and the Wolffian duct epithelium [31] display abnormal ureteric branching, loss of gene expression in the ureteric bud tip, and premature expression of differentiated collecting duct epithelial genes. However, β-Catenin overexpression in the metanephric mesenchyme also leads to ectopic and abnormal branching as well as overexpression of *Gdnf* [197], which highlights the complex nature of interacting compartmental signals that coordinate for proper branching. The Prorenin receptor also appears to control branching morphogenesis via the Wnt/β-Catenin pathway [198]. Together, these data suggest that WNT/β-catenin and GDNF-RET signaling pathways act to regulate branching morphogenesis.

The FGF family is also involved in growth and cell proliferation of the ureteric bud [86]. FGF family members exert unique spatial effects on ureteric bud cell proliferation *in vitro*, suggesting that they may coordinate control of three-dimensional growth. For example, FGF10 preferentially simulates cell proliferation at the ureteric bud tips, whereas FGF7 induces proliferation in a non-selective manner throughout the developing collecting duct system [86]. Displaced ureteric buds and aberrant ureteric branching occur in mice with conditional inactivation of the FGF receptor gene, *Fgfr2*, or the docking protein, fibroblast receptor substrate 2α (*Frs2α*) [91, 147, 149, 199, 200]. Increased ureteric bud apoptosis and reduced proliferation are present when *Fgfr2* is conditionally inactivated in the ureteric bud tissue or metanephric mesenchyme [91, 200]. *Fgfr2* appears to function downstream of *Eya1* and *Six1,* but upstream of *Six2*, *Sall1*, and *Pax2* [91]. FGF7 induces expression of the Sprouty gene, *Spry2*, *in vitro,* so FGF7 may also participate with *Spry2* in the feedback loop that controls ureteric bud branching by regulating *Gdnf* and *Wnt11* expression [168]. Further, *Fgf-7* null mice had smaller developing ureteric bud and collecting systems than wild-type [201].

Hepatocyte nuclear factor-1 beta (*Hnf1β*) inactivation in the ureteric bud cell lineage leads to decreased ureteric branching morphogenesis and defective collecting duct differentiation, with subsequent collecting duct system cystic dilations, renal dysplasia, hypoplasia, or agenesis [139]. Importantly, *HNF1β* mutations in humans present with various renal (and extra-renal) manifestations [202]. *Ex vivo* kidney culture studies in the *Hnf1β* null mice suggest a role in both the ureteric bud tip domain and ureteric bud stalks to organize the epithelium and cell polarity of the ureteric bud branches [139]. HNF1β is believed to directly regulate *Gfrα1* and *Etv5*, so may be involved in regulation both upstream and downstream of GDNF-RET pathway to regulate ureteric bud branching [139].

GDNF, FGF7, and TGF-β also promote expression of *Tissue inhibitors of metalloproteinases* (*TIMPs*) from cultured ureteric bud cells [203, 204]. TIMPS regulate the local activity of extracellular matrix metalloproteases (MMPs), which are implicated in altering the composition of the extracellular matrix to facilitate branch initiation. This concept is supported by demonstration that TIMPs block ureteric bud branching *in vitro* [203, 205]. Consequently, growth factors may play an important role in regulating the local

activity of matrix-degrading proteases by controlling TIMP expression.

Various other signaling factors also stimulate ureteric branching, including *Vascular endothelial growth factor* (*VEGF*) [206]. *VEGF receptor 2* (*VEGFR2*) (critical for endothelial cell development) blockade or deletion both *in vitro* and *in vivo* leads to reduced ureteric branching [40, 207], which further highlights a role for endothelial cell involvement in renal branching morphogenesis. Finally, components of the tight junction such as claudin proteins have also been implicated in branching morphogenesis [208].

Formation of Nephrons

Proximal-distal patterning of nephron epithelial cell fate, as reflected by the formation of tubular and glomerular cell fate domains, is a crucial step in nephron segmentation. Nephron segmentation begins with the sequential formation of two clefts in the renal vesicle, the earliest epithelial derivative of nephron progenitors [2] (Fig. 7.2). Creation of a lower cleft, termed the vascular cleft, heralds formation of the comma-shaped body. The comma-shaped body is a transient structure that rapidly undergoes morphogenetic conversion into an S-shaped structure (termed S-shaped body) upon generation of an upper cleft. The S-shaped body is characterized by three segments or limbs. The middle and upper limbs give rise to the tubular segments of the mature nephron. While the middle limb of the S-shaped body gives rise to the proximal convoluted tubule [2], the descending and ascending limbs of the loops of Henle and the distal convoluted tubule originate from the upper limb of the S-shaped body [2, 15] (Fig. 7.5A). As the vascular cleft broadens and deepens, the lower limb of the S-shaped body forms a cup-shaped unit (Fig. 7.5B). Epithelial cells lining the inner wall of this cup will comprise the visceral glomerular epithelium, or podocytes. Cells lining the outer wall of the cup will form parietal glomerular epithelium, or Bowman's capsule.

All parts of the developing nephron increase in size as they become mature. However, the most striking changes consist of increased tortuosity of the proximal convoluted tubule, and elongation of the loop of Henle [2]. Cellular maturation of the proximal tubule involves transition from columnar to cuboidal epithelium, elaboration of apical and basal microvilli, and gradual increase in tubular diameter and length [209]. The human kidney at birth shows marked heterogeneity in proximal tubule length as one progresses from the outer cortex to the inner cortex [210]. Uniformity in proximal tubule length is achieved by 1 month of life, and the proximal tubule subsequently lengthens at a uniform rate. Prospective cells of the loop of Henle are thought to be first positioned at the junctional region of the middle and upper limbs of the S-shaped body near the vascular pole of the glomerulus, where it will form the macula densa [211]. The descending and ascending limbs of the presumptive loop of Henle are first recognizable as a U-shaped structure in the periphery of the developing renal cortex, termed the nephrogenic zone [211, 212]. Maturation of the primitive loop involves elongation of both ascending and descending limbs through the corticomedullary boundary. Continued maturation involves differentiation of descending and ascending limb epithelia [213]. Development of the presumptive distal tubule involves elongation of the connecting segment, which joins with the ureteric bud/collecting duct.

Longitudinal growth of the medulla contributes to lengthening of the loops of Henle such that all but a small percentage of the loops of Henle extend below the corticomedullary junction in full term newborn infants [2]. As the kidney increases in size postnatally, the loops of Henle further elongate and reach the inner two-thirds of the renal medulla in the mature kidney. Functional development of the kidney's urine concentrating mechanism is dependent on elongation of the loops of Henle during nephrogenesis since longer loops favor urine concentrating capacity. In the extremely premature fetus, the loops of Henle are short owing to the relative distance between the renal capsule and the renal papilla. Consequently, the urine concentrating capacity of the premature kidney is limited by generation of a shallow medullary tonicity gradient.

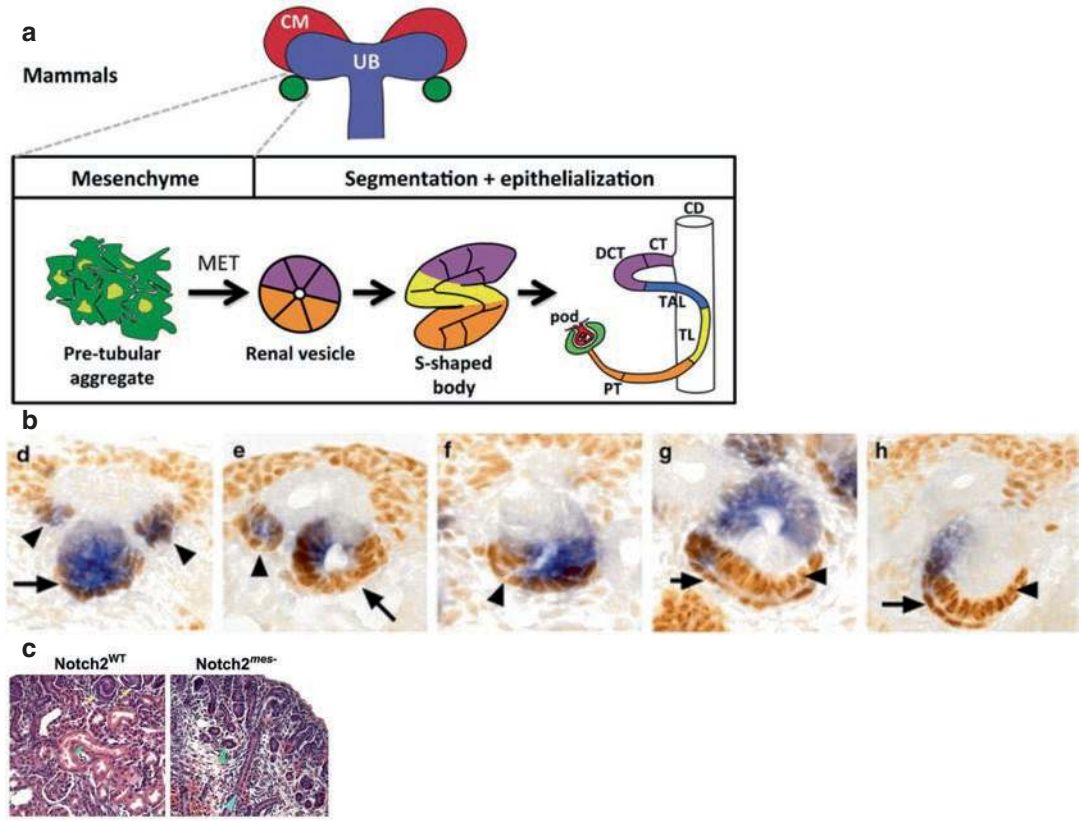


Fig. 7.5 Schematic of nephron segmentation. (**A**) The stages of mammalian metanephric nephron developed are shown, with colors denoting the segmentation of the renal vesicle, S-shaped body and mature nephron (with kind permission from Springer Science+Business Media: Pediatric Nephrology, Hnf1beta and nephron segmentation, published online Nov 5, 2013, Naylor RW and Davidson AJ, Figure 1). (**B**) Dual section in situ hybridization and immunohistochemistry for Wt1 (brown) and Wnt4 (purple) in the mouse kidney at embryonic day 15.5. (d) Wt1 and Wnt4 expression in a pre-tubular aggregate. (e) Wt1 in a pre-tubular aggregate (arrowhead) and the proximal portion of the renal vesicle (arrow). (f) Wt1 expression in the lower limb of the comma-shaped body. (g) Wt1 in the podocytes and parietal epithelium of the proximal segment of an early S-shape body. (h) Wt1 in podocyte and parietal epithelium of the proximal segment of an S-shape body (with kind permission from Springer Science+Business Media: Histochemistry and Cell Biology, Use of dual section mRNA in situ hybridization/immunohistochemistry to clarify gene expression patterns during the early stages of nephron development in the embryo and in the mature nephron of the adult mouse kidney, Vol. 130, p. 937, 2008, Georgas et al., Figure 5). (**C**) Representative kidney phenotype of mice with a conditional mutation affecting nephron segmentation. Loss of Notch2 in the metanephric mesenchyme results in loss of proximal nephron elements (glomeruli, proximal tubules and S-shaped bodies). Red arrows, glomeruli; green, proximal tubule; yellow, S-shaped bodies; turquoise, collecting duct (reprinted with permission from: Company of Biologists: Development, Notch2, but not Notch1, is required for proximal cell fate acquisition in the mammalian nephron, 134(4), p. 803, 2007, Cheng et al., Figure 1)

Nephron Segmentation

Proximal-distal patterning of nephron epithelial cell fate, as reflected by the formation of glomerular and tubular cell fate domains, is a crucial step in nephron segmentation. Recently, elegant studies have provided a high resolution view of comparative histology, RNA expression and protein localization of mouse and human developing kidneys throughout nephrogenesis [214, 215]. These studies offer insights into when proximal-distal patterning is established in renal vesicles, along with differences in marker genes in the mouse and human.

One mechanism for patterning glomerular and tubular cell fates in the S-shaped body appears to be dependent on negative feedback between *Wt1* and *Pax2* [216–218] (Fig. 7.5B). During early kidney development, the expression patterns of *Pax2* and *Wt1* become restricted in S-shaped bodies such that the expression domain of *Pax2* is complementary to the corresponding domain for *Wt1*. *Wt1* expression is restricted to glomerular epithelial precursors, which give rise to podocytes later in glomerular development [219]. In contrast, *Pax2* expression is restricted to that portion which gives rise to tubular epithelial precursors of the proximal and distal nephron segments and is later repressed in differentiated tubular epithelium [123, 220]. The precise roles for *Wt1* or *Pax2* in nephron differentiation is not evident from the analyses of renal phenotypes in mice with targeted *Wt1* or *Pax2* mutations since these mutants fail to form kidneys [54, 76]. However, evidence from transgenic mice that over-express PAX2 in all nephrogenic cell types illustrates the importance of spatially restricting *Pax2* expression during early nephrogenesis since these mice exhibit dysplastic kidneys with defective differentiation of both tubular and glomerular epithelia [221].

Two other transcription factors expressed at the renal vesicle stage, *Lhx1* and *Brn1*, also appear to be involved in initiating proximal-distal nephron epithelial cell fate patterning. While *Lhx1* is uniformly expressed in renal vesicles [61], *Brain specific homeobox 1* (*Brn1*) expression occurs in a more spatially restricted pattern in renal vesicles [212]. Conditional knockout of *Lhx1* in the metanephric mesenchyme causes developmental arrest at the renal vesicle stage, and results in loss of *Brn1* expression [61]. In contrast, targeted deletion of *Brn1* in the metanephric mesenchyme does not prevent the early stages of nephron morphogenesis, but blocks formation of the loop of Henle, and suppresses terminal differentiation of distal nephron epithelia [212]. Taken together, these data suggest that *Brn1* functions downstream of *Lhx1* in a genetic hierarchy which establishes distal cell fates. An additional role for *Lhx1* in specifying podocyte cell fate is revealed by the analysis of *Lhx1* chimeric mutant mice [61].

Genetic evidence in mice suggests that the process for selecting which nephrogenic progenitors will comprise the proximal portion of the developing nephron (i.e. the podocytes and proximal convoluted tubule) is dependent on Notch signaling [222–224]. Conditional knock-out of *Notch2* and *Recombining binding protein suppressor of hairless* (*Rbpsuh*), but not *Notch1*, in metanephric mesenchyme prior to nephron segmentation results in complete lack of both proximal tubule and glomerular epithelia [223] (Fig. 7.5C). Similar effects were observed in mutant mice when presenilin-mediated Notch activation was abrogated by mutagenesis of *Psen1* and *Psen2* [224]. Moreover, ectopic Notch activation in nephron progenitors results in premature differentiation and MET, with a preference towards proximal tubule cell fate [225].

There is limited information regarding the subsequent specification of cell types in the proximal tubule, loop of Henle and distal tubule. Mutations in the transcription factor, *Hepatic nuclear factor 4a* (*Hnf4a*), are associated with Fanconi syndrome, and *Hnf4a* has been shown to be required for the formation of differentiated proximal tubules [226].

Glomerulogenesis

During embryonic development, formation of the lower limb of the S-shaped body heralds the onset of glomerulogenesis [2, 227] (Fig. 7.6A). The vascular cleft provides an entry point to which progenitor endothelial and mesangial cells are recruited [228]. Cells residing along the inner surface of the lower S-shaped body limb represent nascent podocytes. At this stage, immature podocytes are proliferative and exhibit a columnar shape, apical cell attachments and a single-layer basement membrane [227]. Development of the glomerular capillary tuft is a dynamic process involving recruitment and proliferation of endothelial and mesangial cell precursors, formation of a capillary plexus, and concomitant assembly of podocytes and mesangial cells distributed around the newly formed capillary loops [227].

Recruitment of angioblasts and mesangial precursors into the vascular cleft results in formation of the lower S-shaped body limb into a cup-like structure [2] (Fig. 7.6A). Formation of a primitive vascular plexus occurs at this so-called capillary loop stage. Podocytes of capillary loop stage glomeruli lose mitotic capacity [229] and begin to demonstrate complex cellular architecture, including the formation of actin-based cytoplasmic extensions, or foot processes, and the formation of specialized intercellular junctions, termed slit diaphragms [230, 231] (Fig. 7.6B). Subsequent development of the glomerular capillary tuft involves extensive branching of capillaries and formation of endothelial fenestrae [2]. Mesangial cells, in turn, populate the core of the tuft and provide structural support to capillary loops through the deposition of extracellular matrix [232, 233]. The full complement of glomeruli in the fetal human kidney is attained by 32–34 weeks when nephrogenesis ceases [2]. At birth, superficial glomeruli, which are chronologically the last to be formed, are significantly smaller than juxtamedullary glomeruli, which are the earliest formed glomeruli [210]. Subsequent glomerular development involves hypertrophy, and glomeruli reach adult size by 3½ years of age [210].

Podocyte Terminal Differentiation

Functional and genetic evidence support the role of Notch signaling in the determination of podocyte cell fate early in nephron development [222, 224]. Additional roles for the Notch receptor gene, *Notch2*, and its ligand, *Jagged1*, at later stages of glomerular capillary tuft assembly are revealed by the analysis of compound mutant mice which show avascular glomeruli or aneurysmal defects and absent mesangial cells in glomerular capillary tuft formation [234].

Following podocyte cell fate determination, the transcription factors *Wt1*, *podocyte expressed 1* (*Pod1*), *Lim homeobox 1b* (*Lmx1b*), *Foxc2*, *Mafb*, and *Osr1* [235, 236] have been shown to have important roles in podocyte terminal differentiation. Loss of function mutations in *Foxc2*, *Pod1*, *Lmx1b*, and *Mafb* cause podocyte defects in mice which become evident at the capillary loop stage (in the case of *Pod1*) or later (in the case of *Foxc2*, *Lmx1b*, *Mafb*) [237–240]. Analysis of chimeric mice reveal that normal glomerular epithelial differentiation requires the function of *Pod1* in neighboring stromal cells, suggesting that *Pod1* regulates stromal factors that act to promote podocyte cell fate [49]. In humans, *LMX1b* mutations are identified in patients with Nail-Patella Syndrome, which is associated with focal segmental glomerulosclerosis [241]. A role for *Wt1* in podocyte differentiation is suggested by the identification of *WT1* mutations in humans with Denys-Drash and Frasier syndromes [242–244], which are inherited disorders associated with mesangial sclerosis, a form of glomerular disease characterized by defects in podocyte differentiation [245]. The demonstration of an identical glomerular phenotype in mice with targeted *Wt1* mutations, genetically similar to the *WT1* mutation in humans with Denys-Drash and Frasier syndromes, [246–249] serves as additional support that *Wt1* has important roles in podocyte differentiation. *TGF-β-activated kinase* (*Tak1*) targeted deletion in podocytes results in disrupted podocyte architecture associated with decreased *Wt1* expression [250].

Podocyte maturation coincides with a loss of mitotic activity and cell cycle blockade [229]. The limited capacity of mature podocytes to undergo cell proliferation has important implications on the glomerular response to injury since damaged podocytes are not capable of compensating for their loss of function by way of regeneration. Moreover, escape from cell cycle blockade in mature podocytes has been associated with severe changes in glomerular cytoarchitecture and a rapidly progressive decline in renal function, as demonstrated by the deleterious course of idiopathic collapsing and human immunodeficiency virus (HIV) nephropathies [251].

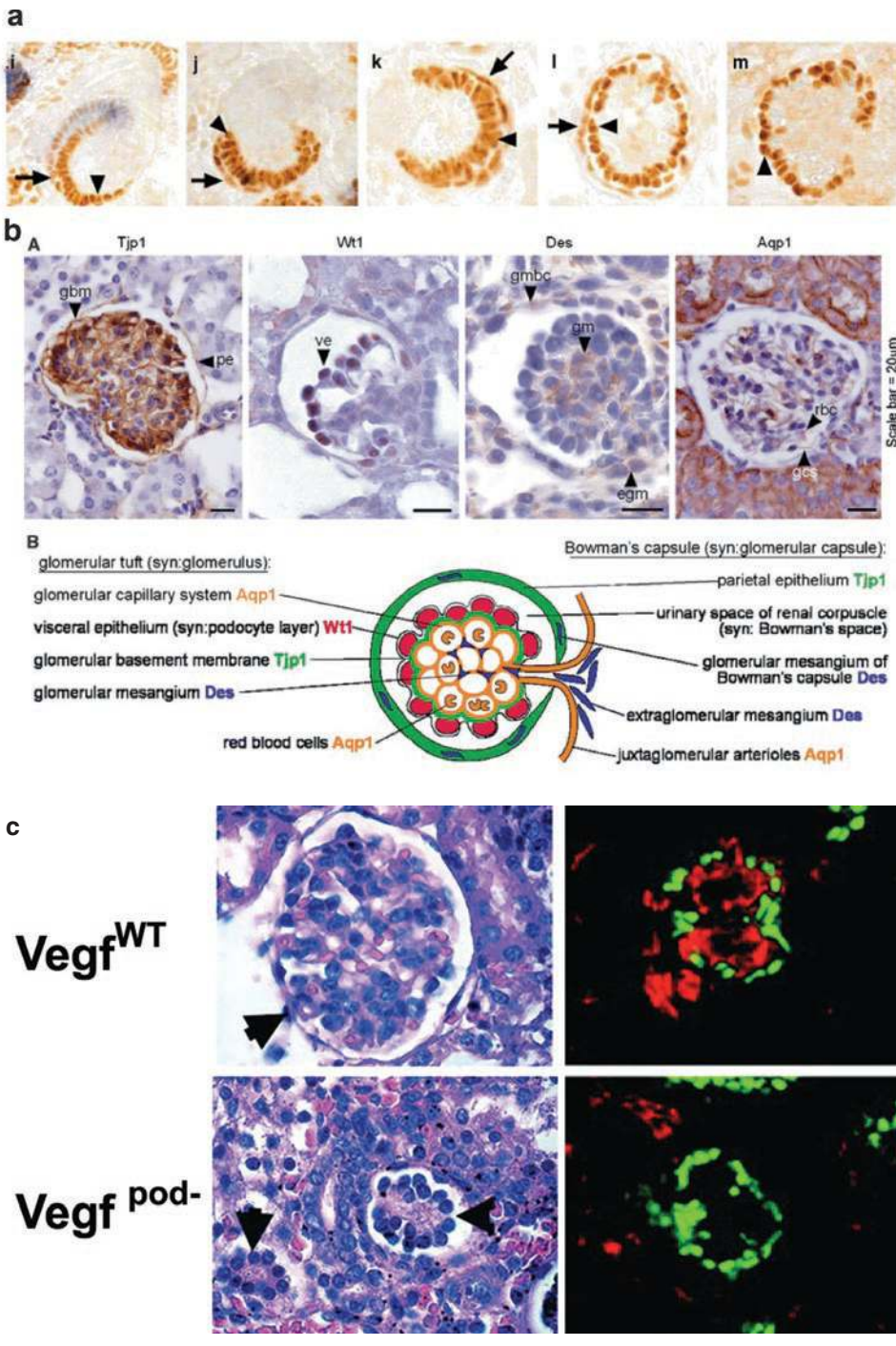
Glomerular Capillary Tuft Development

Lineage tracing studies show that the precursors of most glomerular endothelial cells (angioblasts) are Foxd1-positive stromal cells [252, 253]. Studies of autologous transplantation of embryonic kidney rudiments into adult renal cortex suggests that angioblasts originate from a unique subpopulation of induced metanephric mesenchyme that do not differentiate along epi-

thelial lineages (vasculogenesis) [228, 254, 255]. An alternate theory is provided by evidence that glomerular capillaries originate from ingrowth of primitive sprouts from external vessels through experiments involving engraftment of rodent fetal kidneys onto avian chorioallantoic membrane [256], suggesting that angiogenesis also plays a role in glomerular endothelial development.

Several signaling systems are involved in the recruitment of endothelial and mesangial precursors during the formation and assembly of the glomerular capillary tuft. Vascular endothelial growth factor (VEGF) is secreted by podocyte precursors of early S-shaped bodies [257]. VEGF promotes recruitment of VEGFR2-expressing angioblasts into the vascular cleft to initiate glomerular vasculogenesis [258] and is required to induce apoptosis for lumen capillary formation [259, 260]. This process is under tight regulatory control, as suggested by the demonstration of severe glomerular defects in mice when the gene dosage of *Vegf* is genetically manipulated [261, 262] (Fig. 7.6C). Appropriate development of the glomerular mesangial cells is also important to provide structural and functional support to the glomerular capillary network. Recruitment of mesangial cells is under the guidance of platelet-derived growth factor (PDGF)–β, expressed by endothelial cells, which binds to its receptor, PDGF receptor-β (PDGFRβ) [263]. This axis is required for proliferation and assembly of glomerular capillaries and mesangium as revealed by the absence of glomerular capillary tufts in mice deficient for either *Pdgfβ* or *Pdgfrβ* [264, 265]. Other regulators required for normal renal vasculogenesis include the renin angiotensin system genes [266–270], *Angiopoietin1* and its tyrosine receptor *Angiopoietin-1 receptor* (*Tie2*) [271–273], *Dicer* [274, 275], Notch signaling [276, 277] and TGFβ signaling [278–280]. Other

Fig. 7.6 Development of the glomerulus. (**A**) Immunohistochemical staining for Wt1 (brown) in the developing glomerulus. (i) Wt1 in podocytes and parietal epithelium in a late S-shaped body. Endothelial cells are recruited into the cup-shaped glomerular precursor region of the S-shaped body forming a primitive vascular tuft. (j, k) Wt1 in podocytes and parietal epithelium in a capillary loop stage glomerulus. Podocyte precursors contact invading endothelial cells and begin to differentiate. In turn, endothelial cells form a primitive capillary plexus (capillary loop stage). (l, m) Wt1 is strongly expressed in podocytes in maturing glomeruli. Parietal epithelial cells encapsulate the developing glomerulus. (**B**) (a) Immunohistochemistry of maturing glomeruli using antibodies to Tjp1, Wt1, Des and Aqp1. Tjp1 expression in the glomerular basement membrane (gbm) and parietal epithelium (pe); Wt1 in podocytes or visceral epithelium (ve); Desmin in the extraglomerular mesangium (egm), glomerular mesangium of Bowman's capsule (gmbc) and the glomerular mesangium (gm); and Aqp1 in endothelial cells of the glomerular capillary system (gcs) and red blood cells (rbc). (b) Schematic of a developing glomerulus showing the structures present in both the adult and embryonic kidney. The developing glomerulus is composed of a central glomerular tuft, which contains a capillary loop network arising from the afferent arteriole termed the glomerular capillary system. Forming a tight association with the endothelial cells of the capillaries is the visceral epithelium (or podocytes), a layer of highly specialized epithelial cells specific to the nephron. The fused basal lamina of the endothelial and visceral epithelial cells forms the glomerular basement membrane, an extracellular component of the renal corpuscle. In the interstitial spaces between the capillaries is the glomerular mesangium, a complex of mesangial cells and extracellular matrix. The glomerular tuft is surrounded by the Bowman's capsule, which is composed of the parietal epithelium, mesangium and the urinary space of the renal corpuscle. Extraglomerular mesangium located outside the renal corpuscle is a component of the juxtaglomerular complex and is associated with the afferent arteriole. The antibodies used to identify each of the structures are shown; Aqp1 (orange), Wt1 (red), Tjp1 (green) and Des (blue) (with kind permission from Springer Science+Business Media: Histochemistry and Cell Biology, Use of dual section mRNA in situ hybridization/immunohistochemistry to clarify gene expression patterns during the early stages of nephron development in the embryo and in the mature nephron of the adult mouse kidney, Vol. 130, p. 932 and 937, 2008, Georgas et al., Figure 2 and 5). (**C**) Representative kidney phenotype of mice with a conditional deletion affecting glomerulogenesis. Loss of VEGFA in the podocyte results in a failure of the glomerular endothelial cells to undergo fenestration and progressive loss of endothelial cells. +/+, wild-type glomerulus; −/−, VEGF-null glomerulus; green, Wt1 staining; red PECAM staining (reprinted with permission from: American Society for Clinical Investigation: Journal of Clinical Investigation, Glomerular-specific alterations of VEGF-A lead to distinct congenital and acquired renal diseases, 111(5), p. 712, 2003, Eremina et al., Figure 5)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glomerular tuft (syn:glomerulus):
glomerular capillary system Aqp1
visceral epithelium (syn:podocyte layer) Wt1
glomerular basement membrane Tjp1
glomerular mesangium Des
red blood cells Aqp1
Bowman's capsule (syn:glomerular capsule):
parietal epithelium Tjp1
urinary space of renal corpuscle (syn: Bowman's space)
glomerular mesangium of Bowman's capsule Des
extraglomerular mesangium Des
juxtaglomerular arterioles Aqp1
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molecules implicated in exerting attraction and repulsion guidance for glomerular capillary formation include the chemokine Stromal cell derived factor 1 (SDF1) [281], which acts on CXCR3 and CXCR7, the transmembrane protein EphrinB2 [282–284] and chemorepellent glycoprotein Semaphorin 3a [285].

During the S-shaped stage, podocyte progenitors express a primitive glomerular basement membrane which is composed predominantly of laminin-1, and α-1 and α-2 subchains of type IV collagen [286]. During glomerular development, composition of the glomerular basement membrane undergoes transition as laminin-1 is replaced by laminin-11, and α-1 and α-2 type IV collagen chains are replaced by α-3, α-4, and α-5 subchains [286]. As demonstrated in several mouse models, failure of these changes result in severe structural and functional defects [287–289].

Formation of the Collecting System

Between the 22nd–34th week of human fetal gestation [2], or E15.5-birth in mice [15], morphologic changes result in the establishment of peripheral (i.e. cortical) and central (i.e. medullary) domains in the developing kidney. The renal cortex, which represents 70% of total kidney volume at birth [290], becomes organized as a relatively compact, circumferential rim of tissue surrounding the periphery of the kidney. The renal medulla, which represents 30% of total kidney volume at birth [290], has a modified cone shape with a broad base contiguous with cortical tissue. The apex of the cone is formed by convergence of collecting ducts in the inner medulla and is termed the papilla.

Distinct morphologic differences emerge between collecting ducts located in the medulla compared to those located in the renal cortex during this stage of kidney development. Medullary collecting ducts are organized into elongated, relatively unbranched linear arrays which converge centrally in a region devoid of glomeruli. In contrast, collecting ducts located in the renal cortex continue to induce metanephric mesenchyme. The specification of cortical and medullary domains is essential to the eventual function of the mature collecting duct system. The most central segments of the collecting duct system formed from the first five generations of ureteric bud branching undergo remodeling by increased growth and dilatation of these tubules to form the pelvis and calyces (reviewed in [291]).

The developing renal cortex and medulla exhibit distinct axes of growth. The renal cortex grows along a circumferential axis, resulting in a ten-fold increase in volume while preserving compact organization of cortical tissue around the developing kidney [290]. In this manner, differentiating glomeruli and tubules maintain their relative position in the renal cortex with respect to the external surface of the kidney, or renal capsule. In contrast to the circumferential pattern of growth exhibited by the developing renal cortex, the developing renal medulla expands 4.5-fold in thickness along a longitudinal axis perpendicular to the axis of cortical growth [290]. This pattern of renal medulla growth is largely due to elongation of outer medullary collecting ducts [290]. The development of a medullary zone coincides with the appearance of stromal cells between the seventh and eighth generations of ureteric bud branches [290]. It has been suggested that stromal cells provide stimulatory cues to promote the growth of medullary collecting ducts [290]. Additional support for this hypothesis is provided by analyses of mutant mice lacking functional expression of the stromal transcription factors *Pod1* and *Forkhead box d1* (*Foxd1*) [35, 49, 292], which demonstrate defects in medullary collecting duct patterning.

In the developing collecting system, apoptosis is infrequently detected in the tips and trunks of the branching ureteric bud [110]. At later stages of embryonic and post-natal kidney development, apoptosis is prominent in the medullary regions of the rat collecting duct system that give rise to the calyces, renal pelvis and renal papilla [110]. The prominence of apoptosis in these regions suggests a potential role for apoptosis in remodeling the first 3–5 generations of the branched ureteric bud/developing collecting duct system. The extent to which apoptosis contributes to this morphogenetic process is, however,

unknown. Other suggested roles for medullary apoptosis include elimination of medullary interstitial cells as a mechanism for making room for new blood vessel ingrowth [293].

Medullary Patterning and Formation of the Pelvicaliceal System

Regional specification of cortical and medullary domains of the renal collecting duct system is a relatively late event in kidney development. At least five soluble growth factor genes (*Fgf7*, *Fgf10*, *Bmp4*, *Bmp5 and Wnt7b*), one proteoglycan gene (*Gpc3*), one cell cycle regulatory gene ($p57^{KIP2}$), and molecular components of the renin-angiotensin axis (*Angiotensinogen* (*Agt)*, *Angiotensinogen receptor* 1 and *2* (*Agtr1*, *Agtr2*)) are implicated in medullary collecting duct morphogenesis as revealed by the demonstration of defects in renal medulla development in mutant mice.

The kidneys of *Fgf7* null mice are characterized by marked underdevelopment of the papilla [39]. Similarly, *Fgf10* null kidneys exhibit modest medullary dysplasia with reduced numbers of loops of Henle and medullary collecting ducts, increased medullary stromal cells, and enlargement of the renal calyx [294]. Cellular responses to FGFs are modulated through interactions with cell surface proteoglycans [295]. Syndecans and glypicans are heparan sulfate proteoglycans expressed in developing collecting ducts [186, 296], and their expression is required for normal collecting duct growth and branching [136, 297]. Moreover, treatment of embryonic kidney explants with pharmacologic inhibitors of sulfated proteoglycan synthesis leads to loss of *Wnt11* expression at the ureteric bud branch tips [298], suggesting that sulfated proteoglycans interact with multiple mechanisms that control ureteric bud branching.

Functional and genetic evidence in humans and mice demonstrate that GPC3, a glycosylphosphatidylinositol (GPI)-linked cell surface heparan sulfate proteoglycan, is required for normal patterning of the medulla [186, 299]. Medullary dysplasia in the *Gpc3* deficient mouse arises from overgrowth of the ureteric bud and collecting ducts due to increased cell proliferation in the ureteric bud lineage [299], with subsequent destruction of these elements due to apoptosis [186]. The defect is thought to be caused by an altered cellular response of GPC3-deficient collecting duct cells to growth factors such as FGFs [186, 300, 301]. The defective renal medulla formation in *Gpc3* null mutant mice illustrates the importance of tightly regulated cell proliferation and apoptosis in this process. Other signals that promote collecting duct cell survival include Wnt7b and Egf [302, 303].

Additional support for this concept is provided by the phenotypic analysis of mice carrying a null mutation for $p57^{KIP2}$, a cell cycle regulatory gene. $p57^{KIP2}$ knock-out mice show medullary dysplasia characterized by a decreased number of inner medullary collecting ducts, in addition to abdominal, skeletal, and adrenal defects [304]. Genetic studies in humans and mice suggest a potential functional interaction between $p57^{KIP2}$ and the *Insulin-like growth factor-2* (*Igf2*) gene in the formation of the renal medulla. For example, phenotypic features of mice with $p57^{KIP2}$ null mutations are exhibited by approximately 15% of individuals with Beckwith-Wiedemann Syndrome, a heterogeneous disorder characterized by somatic overgrowth and renal dysplasia [305]. Genetic linkage studies in humans with this syndrome have mapped the disease to chromosome 11p15.5, which harbors loci for $p57^{KIP2}$ as well as for *IGF2* and *H19*. Murine *H19* mutations result in enhanced *Igf2* expression, but do not cause renal dysplasia [306]. However, $H19^{-/-}$; $p57^{KIP2-/-}$ double knock-out mice exhibit elevated serum levels of IGF2, and more severe renal dysplasia than that observed in $p57^{KIP2}$ single knock-out mice [307]. These findings support an additional mechanism for the cause of renal medullary dysplasia resulting from dysregulated stimulation of cell proliferation through the inactivation of $p57^{KIP2}$ and overexpression of IGF2.

Elaboration of the medullary collecting duct network is thought to require oriented cell divisions that permit elongation of the medullary collecting ducts through proliferation during development. One means by which this occurs is

through the activity of the secreted factor, WNT7b, in up-regulating canonical Wnt signaling in medullary collecting ducts to promote oriented cell division and cell survival [302, 308]. α3β1 integrin and the receptor tyrosine kinase c-Met (receptor for hepatocyte growth factor) appear to coordinately regulate *Wnt7b* expression in the medullary collecting duct [308]. Another member of the Wnt family, *Wnt9b*, also plays a role in elongation and appears to regulate planar cell polarity [309]. Post-transcriptional regulation by miRNAs also drives collecting duct elongation and differentiation [310, 311].

Development of the Ureteral Smooth Muscle

Urinary filtrate removal also requires coordinated ureteric contractions, which in turn is the result of development of a "pacemaker" at the base of the renal papilla and smooth muscle around the ureter. *Bmp4* heterozygous mutant mice develop both hydronephrosis and hydroureter [144], suggesting that *Bmp4* may play additional roles that involve formation of the ureter and renal pelvis. Support for this concept is provided by the finding in kidney explants that recombinant BMP4 induces smooth muscle actin, an early marker for smooth muscle differentiation, in peri-ureteric mesenchymal cells [312]. Both *Bmp4* and *Bmp5* are expressed in mesenchymal cells lining the ureter and the developing renal pelvis [144, 173, 313], and BMP receptors *Bone morphogenetic protein receptor, type 1A* (*Alk3*) and *Bone morphogenetic protein receptor, type 1B* (*Alk6*) are expressed in neighboring collecting ducts [144]. Moreover, mice mutant for *Bmp4* in the ureteric mesenchyme show similar defects in renal pelvis and ureter development with reduced ureter smooth muscle [314]. This study suggested SMAD as a downstream effector of BMP4. Indeed, mice with inactivation of *Smad4* show a similar phenotype with decreased ureteral smooth muscle and abnormal function [315, 316].

In addition to BMP signaling, the Sonic hedgehog (SHH) pathway plays a critical role in regulating ureteral mesenchymal development. SHH is secreted from the medullary collecting duct and ureteric stalk, and signals to the surrounding interstitium through its receptor, Patched1 (Ptch1) [317]. BMP4, together with SHH, induces the expression of the transcription factor, *Teashirt zinc finger homeobox 3* (*Tshz3*), which regulates the development of smooth muscle [318]. Interestingly, loss of the *Gli family zinc finger 3* (*Gli3*) repressor (resulting in inappropriate Hedgehog pathway activation) results in hydronephrosis due to ureteric dyskinesis and reduced numbers of pacemaker cells [319]. Mutations in *GLI3* are associated with Pallister-Hall syndrome in humans [320]. Loss of another zinc factor transcription factor gene, *Gata2*, in the ureteric mesenchyme also leads to abnormal smooth muscle differentiation and dilated ureters [321]. More recently, SHH was shown to use FOXF1-BMP4 signaling to program ureter elongation and differentiation [322].

Mutations in genes encoding components of the renin-angiotensin axis also cause abnormalities in the development of the renal calyces, pelvis and ureter. Mice homozygous for a null mutation in the *Agt* gene demonstrate progressive widening of the calyx and atrophy of the papillae and underlying medulla [323]. Identical defects occur in homozygous mutants for the *Agtr1* gene [324]. The underlying defect in these mutants appears to be decreased cell proliferation of the smooth muscle cell layer lining the renal pelvis, resulting in decreased thickness of this layer in the proximal ureter. Mutational inactivation of *Agtr2* results in a range of anomalies, including vesicoureteral reflux, duplex kidney, renal ectopia, uretero-pelvic or ureterovesical junction stenoses, renal dysplasia or hypoplasia, multicystic dysplastic kidney, or renal agenesis [109]. Null mice demonstrate a decreased rate of apoptosis of the cells around the ureter, suggesting that *Agtr2* also plays a role in morphogenetic remodeling of the ureter.

Other regulators of ureteral smooth muscle differentiation include *Tbx18* [325], *Tbx2* and *Tbx3* (*Tbx2* and *Tbx3*) [326], *Robo2* [327] and retinoic acid signaling [328]. Deletion of *Dicer* also led to impaired ureter morphology and function with ureteral smooth muscle abnormalities [329], suggesting that miRNAs are important in regulating ureter smooth muscle differentiation.

Renal Stroma

Stromal cells are comprised of interstitial cells that secrete extracellular matrix and growth factors that provide a supportive framework and developmental patterning signals around the developing nephrons, collecting system and vasculature. In keeping with this idea, stromal cells are found in close proximity to developing nephrons, ureteric bud branches and blood vessels (Fig. 7.3C). Developmentally, stromal cells are derived from a population of *Foxd1*-expressing stromal progenitors [330]. As the renal cortex and medulla become morphologically distinct regions, stromal cells become defined geographically into two separate populations—cortical stroma, which form interstitium between induced nephrons and express *Foxd1*, *Aldehyde dehydrogenase 1 family*, *member A2* (*Raldh2*), *Retinoic acid receptor α* (*Rarα*) and *Rarβ2*; and medullary stroma, which form interstitium between medullary collecting ducts and express *Fgf7*, *Pod1*, and *Bmp4*. Many of these stromal genes have been shown to be critical for nephrogenesis and ureteric branching morphogenesis in transgenic mouse studies [35, 36, 38, 39, 144, 239]. Further evidence of the importance of the renal stroma for kidney development comes from transgenic animal studies which result in ablation of the renal stroma with diphtheria toxin, causing an abnormally thickened cap mesenchyme and decreased ability of progenitors to differentiate [47, 331]. scRNA-seq studies have recently identified 17 molecularly distinct cell clusters of renal interstitium in the developing kidney [71]. Once nephrogenesis is complete, stromal cells differentiate into a diverse population which includes fibroblasts, lymphocyte-like cells, glomerular mesangial cells, renin-expressing cells and pericytes [330, 332, 333].

One important role for the renal stroma is regulation of *Ret* expression, and hence branching morphogenesis. RARα and RARβ2, members of the retinoic acid receptor (RAR) and retinoid X receptor (RXR) families of transcription factors, are both expressed in stromal cells and *Ret*-expressing ureteric bud branch tips [36]. *Rarα*$^{-/-}$; *Rarβ2*$^{-/-}$ double mutant mice have small kidneys characterized by a decreased number of ureteric bud branches and loss of normal cortical stromal patterning between induced nephrons [36]. In the collecting ducts of *Rarα*$^{-/-}$; *Rarβ2*$^{-/-}$ double mutant mice, *Ret* expression is down-regulated whereas *Gdnf* expression in the metanephric mesenchyme is maintained. The renal defect in these mice can be rescued by overexpressing a *Ret* transgene in the ureteric bud lineage [37]. Recent data suggests that retinoic acid derived from the renal stroma via the enzyme Raldh2 signals to the ureteric bud to up-regulate expression of *Rarα* and *Rarβ2* to induce *Ret* expression in the ureteric bud [193].

The transcription factors *Foxd1* and *Pod1* have roles in regulating the spatially restricted pattern of *Ret* expression during collecting duct development. *Foxd1* is most strongly expressed in the developing kidney in the cortical, or subcapsular, stroma [35, 38] (Fig. 7.3C). In contrast, *Pod1* is most abundant in medullary stromal cells [239, 334]. Homozygous deletion of either *Foxd1* or *Pod1* results in decreased renal branching morphogenesis and misexpression of *Ret* throughout the developing collecting system [35, 239]. These data suggest that stromal cues expressed under the control of *Foxd1* and *Pod1* are involved in inhibiting *Ret* expression in the truncal segments of the developing ureteric bud. It is not clear from the analyses of these mutants whether secreted stromal factors directly block *Ret* expression in collecting ducts. Since *Foxd1* and *Pod1* mutants show additional defects in nephron morphogenesis [35, 239], these stromal genes may indirectly control *Ret* expression through the production of nephron-derived factors that secondarily act on collecting duct cells to inhibit *Ret* expression.

Although the mechanisms by which the renal stroma regulates nephron differentiation are not entirely clear, Wnt and Hippo signaling have been implicated. With regards to Hippo signaling, loss of the protocadherin *Fat atypical cadherin 4* (*Fat4*) in the stroma results in expansion of the cap mesenchyme, and this appears to be due to binding to *Dachsous 1/2* (*Dsch1/2*) on the cap mesenchyme [47, 335]. Interestingly, activation of β-catenin in the renal stroma prevents the differentiation of nephron progenitors and results in histological and molecular features similar to Wilm's tumour [336].

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Disorders of Kidney Formation

8

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Introduction

Congenital anomalies of the kidneys and urinary tract, otherwise known as CAKUT, are classical disorders of development that are the most common cause of renal failure in children [1–3]. These disorders encompass a spectrum of entities including renal agenesis, renal hypodysplasia (RHD), multicystic kidney dysplasia, duplex renal collecting systems, ureteropelvic junction obstruction (UPJO), ureterovesical junction obstruction, megaureter, posterior urethral valves and vesicoureteral reflux (VUR). While congenital disorders like autosomal recessive and autosomal dominant polycystic kidney disease (PKD), nephronophthisis, and heritable nephrotic syndrome could also be considered as disorders of kidney formation, these generally occur later in kidney development as part of terminal cell differentiation events. However, some of the genes that cause nephronophthisis are also associated with CAKUT. In this chapter, we discuss disorders that arise during the early inductive events that lead to the formation of the kidneys and the urinary tracts. Both tissues arise from a common primordial tissue known as the mesonephric duct and thus, congenital kidney and urinary tract malformations commonly co-occur. In this chapter, we will focus on renal disorders encompassed within CAKUT and discuss their etiology, clinical manifestations and management. Other disorders within CAKUT with significant urinary tract pathology like UPJO, ureterovesical junction obstruction, megaureter, posterior urethral valves and VUR are discussed in other chapters.

Classification and Definition of Renal Malformations

Congenital malformations of the kidney can be defined at the macroscopic level by changes in size, shape, location, or number or microscopically by changes within specific lineages like the ureteric bud, the metanephric mesenchyme, or combinations of both [4]. In clinical practice, most congenital renal malformations are defined grossly using imaging methods like ultrasound and nuclear medicine scans. Sometimes renal tissue is obtained from biopsies or from nephrectomies, and in these cases, histological definitions

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of renal hypoplasia, renal dysplasia, and multicystic renal dysplasia can be utilized for classification. One can group congenital malformations of the kidney as follows:

Changes in size
- Renal hypoplasia
- Renal dysplasia

Changes in shape
- Multicystic dysplastic kidney (MCDK)
- Renal fusion

Changes in location
- Renal ectopia
- Renal fusion

Changes in number
- Renal duplication
- Renal agenesis

When induction events do not occur at the right time or location during embryogenesis, the kidneys may fail to form (agenesis, hypoplasia, dysplasia), the kidneys may form, but in the wrong location (ectopia ± hypoplasia/dysplasia), the kidneys may fail to migrate to the correct location (fusion ± hypoplasia/dysplasia) or there may be multiple induction events that arise (duplication). Malformations can be either unilateral or bilateral. Importantly, from animal models and human studies, disorders of renal formation are frequently observed with concurrent lower urinary tract malformations. In these cases, it is not clear if the impairment in induction of the kidney is primary or secondary to urinary tract obstruction. Renal agenesis refers to congenital absence of the kidney and ureter. Typically, renal malformations defined as renal hypoplasia or dysplasia are grossly small in size, defined as less than 2 SD below the mean for kidney length or weight [4–6]. Usually, renal hypoplasia or dysplasia is defined based on the presence of a small hyperechogenic kidney from ultrasound imaging [7]. Simple renal hypoplasia is defined as a small kidney with a reduced number of nephrons and normal architecture. Renal dysplasia is defined by the presence of malformed kidney tissue elements. Characteristic microscopic abnormalities include abnormal differentiation of mesenchymal and epithelial elements, a decreased number of nephrons, loss of corticomedullary differentiation and the presence of dysplastic elements including cartilage and bone (Fig. 8.1). As stated, dysplastic or hypoplastic kidneys are typically small, but can range in size and appear normal or even large due to the presence of multiple cysts or coincident urinary tract obstruction with hydronephrosis. The MCDK is an extreme form of renal dysplasia and is defined grossly as a non-reniform collection of cysts.

In addition to defects in renal formation that affect size, shape, location or number, and tissue patterning, congenital anomalies of the kidneys also encompass defects in the number of nephrons formed. Nephron number in the lower range of that normally observed, but not so low that it would result in renal insufficiency during childhood and/or adolescence, typically manifests in adulthood with hypertension and/or chronic kidney disease (CKD) [8–10].

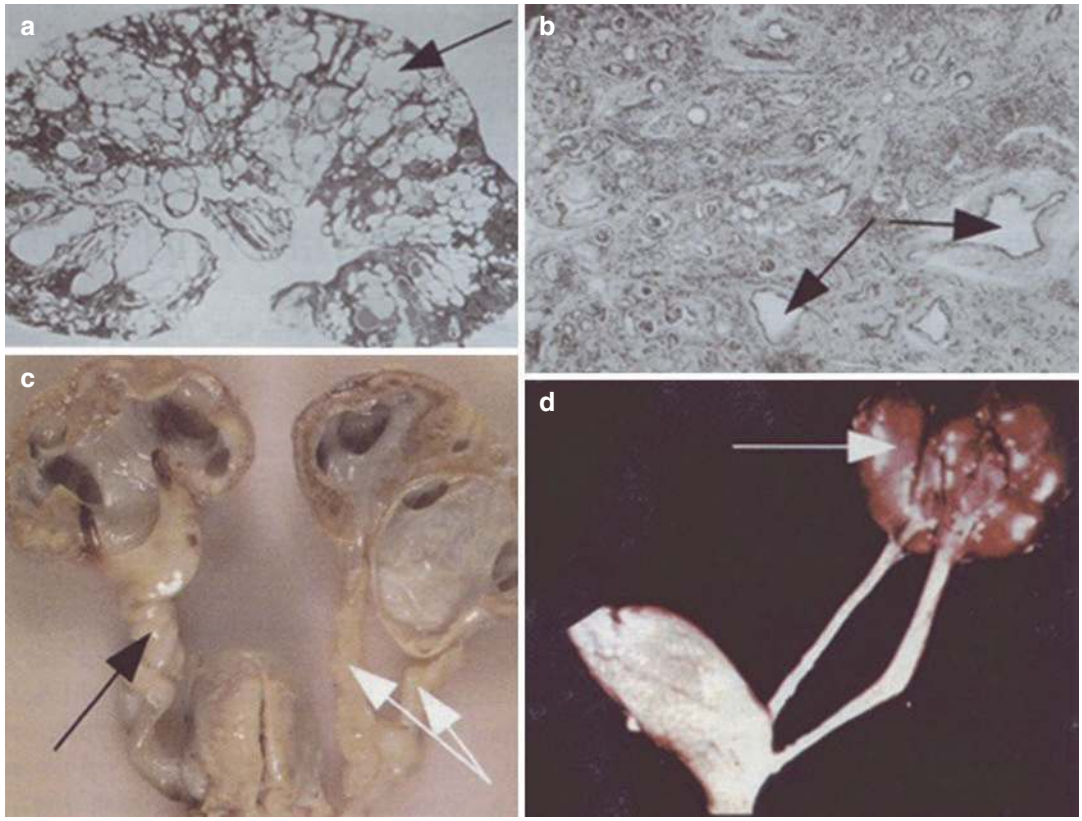

Fig. 8.1 Anatomical features of human renal and lower urinary tract malformations. (**a**) MCDK characterized by numerous cysts (arrow) distorting the renal architecture. (**b**) Dysplastic renal tissue demonstrating lack of recognizable nephron elements, dilated tubules, large amounts of stromal tissue and primitive ducts (arrows) characterized by epithelial tubules with fibromuscular collars. (**c**) Ureteral duplication (right, white arrows) and dilated ureter (left, black arrow) associated with a ureterocele. All ureters are obstructed at the level of the bladder and are associated with hydronephrosis. (**d**) Crossed fused ectopia with fused orthotopic and heterotopic kidneys (arrow)

Epidemiology and Longterm Outcomes of Renal Malformations

The prevalence of renal and urinary tract malformations is 0.3–17 per 1000 liveborn and stillborn infants [11, 12]. Due to their common embryonic origin from the mesonephric duct, lower urinary tract abnormalities are found in about 50% of patients with renal malformations and include VUR (25%), UPJO (11%), and ureterovesical junction obstruction (11%) [13, 14]. Renal malformations are commonly detected in the antenatal period and account for 20–30% of all anomalies detected [15]; upper urinary tract dilatation is the most frequent abnormality that is observed. All major organs are formed between the 4th and 8th week of gestation: the neural tube closes, the aortic arches undergo transformation, the cloacal membrane ruptures, and the kidneys begin to form. Renal malformations are therefore observed in association with non-renal malformations in about 30% of cases [12]. Indeed, there are over 100 multiorgan syndromes associated with renal and urinary tract malformations [16, 17] (Table 8.1).

Bilateral renal agenesis occurs in 1:3000–10,000 births and males are affected more often than females. Unilateral renal agenesis has been reported with a prevalence of 1:1000 autopsies. The incidence of unilateral hypoplasia/dysplasia is 1 in 3000–5000 births (1:3640 for the MCDK) compared to 1 in 10,000 for bilateral dysplasia

Table 8.1 Most frequent syndromes, chromosomal abnormalities and metabolic disorders with renal or urinary tract malformation

Syndromes
Beckwith-Wiedemann
Cerebro-oculo-renal
CHARGE
DiGeorge
Ectrodactyly, ectodermal dysplasia and cleft/lip palates
Ehlers Danlos
Fanconi pancytopenia syndrome
Fraser
Fryns
Meckel
Marfan
MURCS Association
Oculo-auriculo-vertebral (Goldenhar)
Oculo-facial-digital (OFD)
Pallister-Hall
Renal Cyst and Diabetes
Simpson-Golabi-Behmel (SGBS)
Tuberous sclerosis
Townes Brock
VATER
WAGR
Williams Beuren
Zelweger (cerebrohepatorenal)
Chromosomal abnormalities
Trisomy 21
Klinefelter
DiGeorge, 22q11
45, X0 (Turner)
(XXY) Kleinfelter
Tri 9 mosaic, Tri 13, Tri 18, del 4q, del 18q, dup3q, dup 10q
Triploidy
Metabolic disorders
Peroxysomal
Glycosylation defect
Mitochondriopathy
Glutaric aciduria type II
Carnitine palmitoyl transferase II deficiency

[18]. The male to female ratio for bilateral and unilateral renal hypo/dysplasia is 1.32:1 and 1.92:1, respectively [19]. Nine percent of first degree relatives of patients with bilateral renal agenesis or bilateral renal hypoplasia/dysplasia have some type of renal malformation [20]. The incidence of renal ectopia is 1 in 1000 from autopsies, while from clinical studies it is estimated to be less frequent at 1 in 10,000 patients [21]. Males and females are equally affected. Renal ectopia is bilateral in 10% of cases; when unilateral, there is a slight predilection for the left side. The incidence of fusion anomalies is estimated to be about 1 in 600 infants [22].

While congenital renal malformations are relatively frequent birth defects, they become clinically evident at variable times during life and comprise a wide spectrum of outcomes ranging from no symptoms at all to CKD, which causes early mortality. The range in phenotypic severity makes it extremely difficult to counsel patients with certainty. Melo et al. reported a prevalence of CAKUT of 1.77 per 100 live births (524 cases of CAKUT in 29,653 newborns) in a tertiary care unit and a mortality rate of 24% in those affected (126/524) [11]. Amongst the 524 cases, risk factors for early mortality were the co-existence of non-renal and non-urinary tract organ disease, prematurity, low birth weight, oligohydramnios, and renal involvement (renal agenesis, RHD, multicystic renal dysplasia). Quirino et al. reported on the clinical course of 822 children with prenatally detected CAKUT that were followed for a median time of 43 months [23]. Their results demonstrate that most affected children do well: 29% of the children had urinary tract infection, 2.7% had hypertension, 6% had CKD, and 1.5% died during follow-up. Celedon et al. studied 176 children with chronic renal failure secondary to renal dysplasia, reflux nephropathy or urinary tract obstruction with a minimum of 5 years of follow-up [24]. They noted that patients with a urine albumin to creatinine ratio greater than 200 mg/mmol deteriorated faster compared to those with less than 50 mg/mmol (−6.5 mL/min/1.73 m^2 year vs. −1.5 mL/min/1.73 m^2 year change in estimate glomerular filtration rate [eGFR]). They also observed that those children with more than two febrile urinary tract infections deteriorated faster than those with fewer than two infections (median −3.5 mL/min/1.73 m^2 vs. −2 mL/min/1.73 m^2 year change in eGFR). Similar differences were noted for children with hypertension when compared to those without. Finally, they noted that the rate of decline in eGFR was greater during puberty (−4 mL/min/1.73 m^2/year vs. −1.9 mL/min/1.73 m^2/year change in eGFR). They noted

no differences in deterioration of eGFR when comparing children with one or two functioning kidneys. In contrast, Sanna-Cherchi et al. examined the risk of progression to end-stage kidney disease (ESKD) in patients with CAKUT. They found that by 30 years of age, 58 out of 312 patients had initiated dialysis. They also noted that the risk for dialysis was significantly higher for patients with a solitary kidney [25]. The same group reported that patients with bilateral hypodysplasia, solitary kidney, or posterior urethral valves with RHD had a higher risk of dialysis requirement at 30 years when compared to patients with unilateral RHD or horseshoe kidney, and the risk was even higher if there was coexistence of VUR. Wuhl et al. compared patients with CAKUT to age-matched patients with other causes of renal failure who were receiving some form of renal replacement therapy (RRT) and registered within the European Dialysis and Transplant Association Registry [26]. Of 212,930 patients ranging in age from 0 to 75 years who commenced RRT, only 2.2% had renal failure secondary to CAKUT. Importantly, the median age for requirement of RRT was 31 years in the CAKUT cohort versus 61 years in the non-CAKUT cohort, suggesting that most children are likely to require dialysis and/or transplantation as adults. CAKUT was the most frequent cause of need for RRT in all pediatric age groups and peaked in incidence in the 15–19-year-old group.

Low birth weight and prematurity are associated with low nephron number and have therefore been studied as surrogate markers of low nephron number. The Helsinki Study followed approximately 20,000 people born between 1924 and 1944 until death or age 86 years and established that low birth weight was a risk factor for CKD in males, whereas prematurity (birth before 34 weeks of gestation) was a risk factor for CKD in females [9]. Similarly, Crump et al. demonstrated that preterm birth, defined as <37 weeks, and extreme preterm birth, defined as <28 weeks, were strongly associated with an increased risk of CKD in childhood and in adulthood [8]. Keller et al. demonstrated that low nephron number is a risk factor for hypertension in middle-aged adults compared with age-, sex-, and race-matched controls without hypertension [10].

Taken together, many questions remain in understanding the long-term outcome of CAKUT, but clearly most children are surviving into adulthood, and thus there is a need for adult nephrologists to understand these disorders.

Abnormal Molecular Signaling in the Malformed Kidney

Human renal development is complete by 34 weeks gestation [4]. Thus, by definition, renal malformation is a problem of disordered renal embryogenesis. The morphologic, cellular, and genetic events that underlie normal renal development are reviewed in Chap. 7. During human kidney development, two primordial tissues, the ureteric bud and the metanephric mesenchyme, undergo epithelial morphogenesis to form the final metanephric kidney [27]. The kidneys and the ureters arise from two epithelial tubes that extend along the length of the embryo, the mesonephric ducts. An epithelial swelling emerges from the mesonephric duct and is known as the ureteric bud. The ureteric bud invades the adjacent undifferentiated mesenchyme and induces the formation of the metanephric mesenchyme. Reciprocal signaling between the ureteric bud and the metanephric mesenchyme induces the ureteric bud to elongate and bifurcate in a process known as branching morphogenesis that ultimately gives rise to the collecting duct system of the adult kidney. The process of ureteric bud branching morphogenesis is critical for kidney development: each ureteric bud tip induces the adjacent ventrally located metanephric mesenchyme to undergo mesenchymal-to-epithelial transition and this determines the final number of nephrons formed *in utero*. Perturbations in ureteric bud outgrowth, branching morphogenesis and mesenchymal-to-epithelial transition are thought to underlie the majority of the malformations described in humans.

Failure of ureteric bud outgrowth and invasion of the metanephric blastema are events antecedent to renal agenesis or severe renal dysplasia.

Studies in the mouse embryo, a model of human renal development, have identified genes that control ureteric bud outgrowth, ureteric bud branching morphogenesis, and mesenchymal-to-epithelial transition. Some of these genes are mutated in human renal malformations also characterized by agenesis or severe dysplasia (reviewed by [28]). If the ureteric bud fails to emerge, the ureter and the kidney do not develop, while if the ureteric bud emerges from an abnormal location, the ureter that forms will not connect to the bladder properly and potentially result in obstruction and/or VUR with a malformed kidney. Indeed, a pathogenic role for abnormal ureteric bud outgrowth from the mesonephric duct was first hypothesized based on the clinical-pathological observation that abnormal insertion of the ureter into the lower urinary tract is frequently associated with a duplex kidney. Moreover, the renal parenchyma associated with the ureter with ectopic insertion into the bladder is frequently dysplastic [29]. The local environment of transcription factors and signalling pathways is therefore critical to the successful formation of an intact kidney and urinary tract. While a large number of transcription factors and ligand-receptor signalling pathways have been identified that regulate kidney development [28, 30], we will focus on the function of a few selected molecules that have been implicated in human congenital renal malformations: *Gdnf-Ret*, *EYA1*, *Six1*, *Sall1*, *Pax2*, *HNF1b*, *Shh*, and components of the renin-angiotensin-system (RAS).

The central ligand-receptor signalling pathway that leads to the outgrowth of the ureteric bud from the mesonephric duct is the GDNF-GFRα1-RET signalling pathway. Glial cell derived neurotrophic factor (GDNF) is a ligand expressed by the metanephric blastema that interacts with the tyrosine kinase receptor, RET, and its co-receptor GFRα1, both expressed on the surface of the mesonephric duct, to initiate outgrowth of the ureteric bud. Mutational inactivation of *Gdnf*, *Gfra1*, or *Ret* in mice causes bilateral renal agenesis due to failure of ureteric bud outgrowth, demonstrating the importance of this pathway [31–34]. Similarly, when GDNF-soaked beads are positioned adjacent to cultured murine mesonephric ducts, multiple ectopic ureteric buds emerge, demonstrating the potency of this signalling pathway [35]. The expression domain of GDNF is therefore tightly regulated in the nephrogenic mesenchyme and the metanephric mesenchyme.

A network of transcription factors promotes *Gdnf* expression: *Eya1*, *Six1*, *Sall1*, and *Pax2*, while *Foxc1* restricts *Gdnf* expression [36]. Another ligand-receptor complex that limits the domain of *Gdnf* expression is the secreted factor SLIT2 and its receptor ROBO2 [37]. *Slit2* is expressed in the mesonephric duct, while *Robo2* is expressed in the nephrogenic mesenchyme Bone morphogenetic protein 4 also negatively regulates the expression domain of *Gdnf* such that the ureteric bud emerges in the correct location [38].

EYA1 is expressed in metanephric mesenchymal cells in the same spatial and temporal pattern as GDNF. Mice with EYA1 deficiency demonstrate renal agenesis and failure of GDNF expression [39]. EYA1 functions in a molecular complex that includes SIX1 and together they translocate to the nucleus to regulate GDNF expression. Therefore, mutational inactivation of *Six1* in mice also results in renal agenesis or severe dysgenesis [40]. Like GDNF, SIX1 and EYA1, SALL1 is expressed in the metanephric mesenchyme prior to and during ureteric bud invasion. Mutational inactivation of *Sall1* in mice causes renal agenesis or severe dysgenesis and a marked decrease in GDNF expression [41]. Thus, EYA1, SIX1 and SALL1 function upstream of GDNF to positively regulate its expression, thereby controlling ureteric bud outgrowth.

PAX2 is another transcription factor that is expressed in the mesonephric duct, the ureteric bud and in metanephric blastema cells induced by ureteric bud branch tips [42]. Mice with a *Pax2* mutation identical in type to that found in humans with renal coloboma syndrome (RCS) exhibit decreased ureteric bud branching and renal hypoplasia. Investigation of the mechanisms controlling abnormal ureteric bud branching in a murine model of RCS ($Pax2^{1Neu}$) revealed that increased ureteric bud cell apoptosis

decreases the number of ureteric bud branches and glomeruli formed. Remarkably, rescue of ureteric bud cell apoptosis normalizes the mutant phenotype [43]. *Pax2* appears to function upstream of *Gdnf* since in *Pax2* null mice no *Gdnf* expression is detected and the PAX2 protein can activate the *Gdnf* promoter [44].

PAX2 and HNF1β, another transcription factor, are co-expressed in the mesonephric duct and the ureteric bud lineage. Constitutive inactivation of HNF1β is embryonic-lethal in the mouse at gastrulation prior to the formation of the kidneys, but by using tetraploid and diploid embryo complementation, homozygous mutant embryos were able to proceed past gastrulation. The latter study demonstrated that HNF1β is critical for mesonephric duct integrity, ureteric bud branching morphogenesis, and early nephron formation [45]. Another group conditionally inactivated HNF1β in the proximal tubule, loop of Henle and collecting ducts and noted that null mice had cystic kidneys with cysts arising predominantly from collecting duct and loop of Henle segments [46]. The renal phenotype was severe, leading to death from renal failure in the newborn period. Importantly, cystic kidneys from null animals demonstrated downregulation of uromodulin, *Pkd2*, and *Pkhd1*, suggesting that HNF1β may regulate genes associated with cyst formation [46]. Compound heterozygous mice bearing null alleles for *Pax2* and *Hnf1β* show severe CAKUT phenotypes, including hypoplasia of the kidneys, caudal ectopic aborted ureteric buds, duplex kidneys, megaureters and hydronephrosis [47]. These phenotypes were much more severe than *Pax2* heterozygous null or *Hnf1β* heterozygous null mice, strongly suggesting that *Pax2* and *Hnf1β* genetically interact in a common kidney developmental pathway.

Sonic hedgehog (SHH) is a secreted protein that controls a variety of critical processes during embryogenesis. In mammals, SHH acts to control gene transcription via three members of the GLI family of transcription factors, GLI1, GLI2 and GLI3. A pathogenic role for truncated Gli3 was demonstrated in mice engineered such that the normal *GLI3* allele was replaced with the truncated isoform. These mice are characterized by renal agenesis or dysplasia similar to humans with Pallister Hall Syndrome (PHS) [48]. Subsequent analysis of renal embryogenesis in mice deficient in SHH suggests that the truncated form of GLI3 represses genes like *Pax2* and *Sall1* that are required for the initiation of renal development [49]. Loss of Hedgehog signalling has also been implicated in nonobstructive hydronephrosis and urinary pacemaker dysfunction in mice [50]. *Shh* is expressed in the epithelium of the ureter during embryonic development and signals to the developing ureteric mesenchyme. *Shh* deficiency results in lack of smooth muscle cell differentiation in the ureter and nonobstructive hydronephrosis [51]. Hedgehog signaling is also active in the embryonic metanephric mesenchyme; genetic deficiency of Hedgehog signaling in this spatial domain also results in nonobstructive hydronephrosis but by a different mechanism. In mice so affected, there is loss of pacemaker cell activity in the renal pelvis and ureter [50] The observation that constitutive expression of GLI3 repressor also causes nonobstructive hydronephrosis and that genetic deficiency of *Gli3* in mice with decreased Hedgehog rescues hydronephrosis demonstrates the critical role of GLI3 repressor downstream of Hedgehog signaling. Interestingly, some patients with Pallister Hall Syndrome manifest hydronephrosis, although the underlying mechanisms in these patients are unclear.

Analysis of mice with constitutive activation of Hedgehog signaling and human ureter tissues, implicates Hedgehog signaling in the pathogenesis of UPJO. Mice with deficiency of *Patched1*, a cell surface receptor that inhibits Hedgehog signaling, in renal progenitors that give rise to both nephrogenic and stromal cells are characterized by UPJO due to formation of an ectopic cluster of stromal cells that block the UPJ. Analysis of obstructing ureteric tissue in infants and children with congenital UPJO demonstrated upregulation of hedgehog signaling effectors and stromal genes, suggesting that increased hedgehog signaling may contribute to the pathogenesis of human UPJO, as well [52].

In postnatal renal physiology, the RAS plays a critical role in fluid and electrolyte homeostasis

and in the control of blood pressure. Renin cleaves angiotensinogen (AGT) to generate angiotensin (Ang) I which is cleaved by angiotensin-converting enzyme (ACE) to yield Ang II. Ang II is the main effector peptide growth factor of the RAS and acts on two major receptors: AT1R and AT2R. The role of the RAS during kidney development appears to differ somewhat in humans versus rodents, but the metanephric kidney expresses all components of the pathway in both species. Ang is expressed in the ureteric bud lineage and the stromal mesenchyme, while renin is expressed by mesenchymal cells destined to form vascular precursors in the kidney. ACE is expressed slightly later during kidney development in differentiated mesenchymal structures including glomeruli, proximal tubules and collecting ducts. The receptors AT1R and AT2R are expressed in the ureteric bud lineage and in metanephric mesenchymal cells [53]. Mutations of *AGT*, *renin*, *ACE*, or *AT1R* all result in CAKUT phenotypes in the mouse that are characterized by renal malformations with hypoplasia of the medulla and the papillae and hydronephrosis [54]. Mice with mutations in *ATR2* also exhibit CAKUT, but a wider range of phenotypes is observed that includes renal hypo/dysplasia, duplicated collecting systems, VUR, and hydronephrosis [55]. Importantly, genetic inactivation of the RAS pathway in mice does not result in renal tubular dysgenesis (RTD) as observed in humans with similar mutations. It is postulated this may be due to differences between the species: in humans, RAS activity (renin and ANG II levels) peaks during fetal life while nephrogenesis is occurring, while in rodents, RAS activity peaks postnatally from weeks 2–6, when nephrogenesis has ceased. These temporal differences likely explain the lack of concordance between genetic mouse models and affected humans [56].

Ureteric bud branching and modelling of the lower urinary tract with its insertion into the bladder is also controlled by vitamin A and its signaling effectors [57, 58]. Expression of RET, the receptor for GDNF, is controlled by members of the retinoic acid receptor family of transcription factors that function in the vitamin A signaling pathway. These members, including RAR alpha and RAR beta2, are expressed in stromal cells surrounding *Ret*-expressing ureteric bud branch tips [58, 59]. Mice deficient in these receptors exhibit fewer ureteric bud branches and diminished expression of *Ret*. These observations are consistent with the finding that vitamin A deficiency during pregnancy causes renal hypoplasia in the rat fetus [60]. A similar observation has been noted in a human study where maternal vitamin A deficiency was associated with congenital renal malformation [61].

In summary, genetic and nutritional factors like vitamin A and folic acid [61–63] interact to control ureteric bud outgrowth, ureteric bud branching, nephrogenesis, and ureter formation. The number of nephrons is likely determined by a complex combination of factors including genetic variants, environmental events and stochastic factors. This could explain the variable number of nephrons in humans, ranging from approximately 230,000 to 1,800,000 [64]. Loss-of-function mutations in developmental genes can impair nephron formation *in utero* and depending on the magnitude of this effect, renal insufficiency may present at birth, childhood, adolescence or adulthood. Despite evidence in animals that depletion of protein, total calories or micronutrients causes renal hypoplasia, their contribution to human CAKUT remains unclear and an important area of future investigation.

Human Renal Malformations with a Defined Genetic Etiology

In humans, congenital renal malformations are more frequently sporadic than familial in occurrence. This may be due to the fact that infants with severe renal malformations have only recently survived; prior to the late 1970s, chronic dialysis was not offered as a therapy for children, and this continues to be the case in much of the developing world because of a lack of resources. Therefore, it is only in the past 30 years that children with congenital renal malformations have survived and been able to reproduce and potentially transmit deleterious gene mutations.

Therefore, congenital renal malformations appear as sporadic events over time. Genetic haploinsufficiency for many of the aforementioned transcription factors (*EYA1*, *SIX1*, *PAX2*, etc.) can result in a severe renal developmental phenotype, therefore *de novo* heterozygous mutations continue to arise. However, as reported by others, incomplete penetrance with variable expressivity is frequently observed in genetic studies of CAKUT, especially in relation to many of the transcription factors described previously [65]. Congenital renal malformations can occur in isolation, as part of CAKUT, or as part of a syndrome with organ malformations. Importantly, familial cases and extra-renal symptoms are sometimes unrecognized if carefully phenotyping is not performed. A careful evaluation of family history reveals a clustering of isolated or syndromic urinary tract and renal malformations in more than 10% of the cases [66]. Knowledge of the most frequent syndromes, a careful clinical examination and appropriately selected investigations are critical to the clinical approach to these disorders.

Mutations in more than 30 genes have been identified in children with renal development anomalies, generally as part of a multiorgan syndrome (Table 8.2). Some of these syndromes and their associated genes are described here or in some recent reviews [16, 17]. The most frequent syndromes in which renal malformations are encountered are listed in Tables 8.1 and 8.2. For a

Table 8.2 Human gene mutations exhibiting defects in renal morphogenesis

Primary disease	Gene(s)	Kidney phenotype	References
Alagille syndrome	*JAGGED1, NOTCH2*	Cystic dysplasia	[177–179]
Apert syndrome (overlaps with Pfeiffer syndrome and Crouzon syndrome)	*FGFR2, FGFR1*	Hydronephrosis, VUR	[180, 181]
Beckwith-Wiedemann syndrome	*CDKN1C*($p57^{KIP2}$), *H19, LIT1, NSD1*	Medullary dysplasia, nephromegaly, collecting duct abnormalities, cysts, VUR, hydronephrosis, Wilms tumor	[182, 183]
Branchio-Oto-Renal (BOR) syndrome	*EYA1, SIX1, SIX5*	Unilateral or bilateral agenesis/dysplasia, hypoplasia, collecting system anomalies	[79, 80, 184]
Campomelic dysplasia	*SOX9*	Dysplasia, hydronephrosis	[185, 186]
Duane Radial Ray (Okihiro) syndrome	*SALL4*	UNL agenesis, VUR, malrotation, cross-fused ectopia, pelviectasis	[187]
Fraser syndrome	*FRAS1, GRIP1, FREM2, FREM1*	Agenesis, dysplasia, CAKUT	[188, 189–191]
Hypoparathyroidism, sensorineural deafness and renal anomalies (HDR) syndrome	*GATA3*	Dysplasia, VUR, CAKUT, mesangioproliferative glomerulonephritis	[192, 193]
Kallmann syndrome	*KAL1, FGFR1, FGF8, PROK2, PROK2R, CHD7, NELF, HS6ST1*	Agenesis	[133, 194]
Mammary-Ulnar syndrome	*TBX3*	Dysplasia	[195]
Pallister-Hall syndrome	*GLI3*	Dysplasia	[49, 196]
Renal-Coloboma syndrome	*PAX2*	Hypoplasia, vesicoureteral reflux	[109]
Renal tubular dysgenesis	RAS components, *REN, AGT, AGTR1, ACE*	Tubular dysplasia	[139]
Renal cysts and diabetes syndrome	*HNF1β*	Dysplasia, hypoplasia	[197]
Simpson-Golabi Behmel syndrome	*GPC3*	Medullary dysplasia	[198]
Smith Lemli Opitz syndrome	*DHCR7*	Agenesis, dysplasia	[199]
Townes-Brock syndrome	*SALL1*	Hypoplasia, dysplasia, VUR	[91]
Zellweger syndrome	*PEX1*	VUR, cystic dysplasia	[200]

complete list of syndromes featuring renal malformations, the reader is referred to McKusick's Online Mendelian Inheritance in Man.[1]

For most children with renal malformations, neither a syndrome nor a Mendelian pattern of inheritance is obvious. However, genetic studies incorporating chromosomal microarrays, targeted gene panels or whole exome sequencing (WES) have identified a genetic cause for the renal malformation in anywhere from 5% to 30% of cases [16]. One of the first such studies of 100 patients with RHD and renal insufficiency demonstrated that 15% had mutations in two transcription factors [65]: *TCF2* (HNF1β) (especially in the subset with kidney cysts) and *PAX2*. *EYA1* and *SALL1* mutations were found in single cases. Some of the mutations that were identified in these genes were *de novo* mutations explaining the sporadic appearance of RHD. Careful analysis of patients with *TCF2* and *PAX2* mutations revealed the presence of extrarenal symptoms in only half, supporting previous reports that *TCF2* and *PAX2* mutations can be responsible for isolated renal tract anomalies or at least CAKUT malformations with minimal extrarenal features [67, 68]. This study demonstrates that subtle extrarenal symptoms in syndromal RHD can easily be missed. Genetic testing in children with RHD should be preceded by a thorough clinical evaluation for extrarenal symptoms, including eye, ear, and metabolic anomalies. The presence of nonrenal anomalies increases the likelihood of detecting a specific genetic abnormality (Table 8.5). In addition, mutations in genes that are usually associated with syndromes can occur in patients with isolated RHD.

The GDNF/RET Signaling Pathway

The proto-oncogene *RET*, a tyrosine kinase receptor, and its ligand, GDNF, play a pivotal role during early nephrogenesis and enteric nervous system development. Activating *RET* mutations cause multiple endocrine neoplasia, whereas inactivating mutations lead to Hirschsprung disease. A number of human studies have demonstrated that patients with CAKUT have mutations in the RET/GDNF signaling pathway [69–72]. A study of 122 patients with CAKUT identified heterozygous deleterious sequence variants in *GDNF* or *RET* in 6/122 patients, 5%, while another group screened 749 families from all over the world and identified 3 families with heterozygous mutations in RET [69, 73]. Similar findings have been reported in studies of fetuses with bilateral or unilateral renal agenesis [70, 71].

Branchio-Oto-Renal Syndrome

The association of branchial (B), otic (O) and renal (R) anomalies was first described by Fraser and Melnick [74, 75]. Major diagnostic criteria consist of hearing loss (95%), branchial defects (49–69%), ear pits (83%) and renal anomalies (38–67%) [76, 77]. The association of these three major features defines the classical BOR syndrome (OMIM # 113650). Yet, many patients have only one or two of these major features in association with other minor features such as external ear anomalies, preauricular tags or other facial abnormalities (Table 8.3). Hearing loss can be conductive, sensorineural, or mixed.

The frequency of BOR syndrome has been estimated to be 1 in 40,000 births [78]. The transmission is autosomal dominant with incomplete penetrance and variable expressivity. Renal malformations include unilateral or bilateral renal agenesis, hypodysplasia as well as malformation of the lower urinary tract including VUR, pyeloureteral obstruction, and ureteral duplication. Different renal malformations can be observed in

Table 8.3 Major and minor criteria for the diagnosis of BOR syndrome

Major features	Minor features
Deafness	External ear anomalies
Branchial anomalies	Preauricular tags
Preauricular pits	Other facial anomalies
Renal malformations	Cataracts Lacrimal duct stenosis

[1] http://www.ncbi.nlm.nih.gov/.

the same family; moreover, some individuals have normal kidneys (BO syndrome, OMIM 120502). Other infrequent abnormalities have been described in patients with the BOR syndrome. These include aplasia of the lacrimal ducts, congenital cataracts and anterior segment anomalies [74, 75]. Characteristic temporal bone findings include cochlear hypoplasia (4/5 of normal size with only 2 turns), dilation of the vestibular aqueduct, bulbous internal auditory canals, deep posterior fossae, and acutely angled promontories [77].

Approximately 40% of patients with BOR syndrome have a mutation in *EYA1* [76]. Mutations in in *SIX1*, *SIX5*, and *SALL1* have also been identified in patients with BOR syndrome, but at lower frequencies [79–81]. Both EYA1 and SIX1 are co-expressed in the developing otic, branchial and renal tissue, where they function in a transcriptional complex that regulates cell proliferation and cell survival [82, 83]. EYA1 and SIX1 control the expression of PAX2 and GDNF in the metanephric mesenchyme [84]. The EYA1 protein contains a highly conserved region called the *eyes absent* homologous region encoded within exons 9–16, which is the site of most mutations identified to date.

A reasonable approach is to perform genetic analysis in families in which at least one member fulfils the criteria for classical BOR syndrome (Table 8.3). Investigations should include a family history, and examination of relatives to look for preauricular pits, lacrimal duct stenosis, and branchial fistulae and/or cysts. Hearing studies and renal ultrasound should be done in all first-degree relatives.

Molecular testing can confirm the diagnosis and provide genetic recurrence risk information to families. However, variability of the phenotype even with the same mutation does not permit accurate prediction of the disease severity. Within the same family, a given mutation may be associated with renal malformation in some individuals, but not in others. This discrepancy might be explained by stochastic factors that impact the formation of the kidneys or by other unlinked genetic events that may act in synergy with the EYA1 protein during nephrogenesis.

Townes-Brocks Syndrome and VATER/VACTERL Associations

Townes-Brocks syndrome (TBS) is an autosomal dominant malformation syndrome usually defined by a triad of anomalies including imperforate anus, dysplastic ears, and thumb malformations [85]. A wide spectrum of additional features includes renal malformations, congenital heart defects, hand and foot malformations, hearing loss, and eye anomalies [86, 87]. Intelligence is usually normal. REAR Syndrome (renal-ear-anal-radial) has also been used to describe this condition [88]. Its incidence is reported to be 1:250,000 live births [89]. The presentation of TBS is highly variable within and between affected families. Importantly, *SALL1*, is the only gene implicated in TBS and it encodes a C_2H_2 zinc finger transcription factor that is required for the normal development of the limbs, nervous system, ears, anus, heart and kidneys [90, 91].

The detection rate of *SALL1* mutations in patients with TBS appears to be higher when malformations of the hands, the ears, and the anus are present [92]. However genetic testing is further complicated by the fact that the phenotypic features of TBS can resemble other disorders like VACTERL association, Goldenhar syndrome, Oculo-Auriculo-Vertebral spectrum, Pallister-Hall syndrome and even BOR syndrome. TBS features overlap those seen in the VACTERL association (anal, radial and renal malformations). In contrast to VACTERL association, TBS is associated with ear anomalies and deafness and it is not characterized by tracheo-oesophageal fistula or vertebral anomalies.

VACTERL association is defined by the presence of at least three of the following congenital malformations: vertebral anomalies, anal atresia, cardiac defects, tracheo-esophageal defects, renal malformations, and limb anomalies [93]. It is reported to occur in 1:10,000–40,000 of all live births. Renal anomalies are reported in 50–80% of patients and include unilateral or bilateral renal agenesis, horseshoe kidney, cystic kidneys, and dysplastic kidneys; they can be accompanied by urinary tract and genital defects [93, 94]. Ninety percent of VACTERL cases appear to be

sporadic with little evidence of heritability [93]. In a subset of patients there is evidence of heritability [93, 95, 96], and genes that interact with the Sonic Hedgehog pathway have been implicated [97, 98]. The presence of a single umbilical artery on ultrasound has been associated with a variety of congenital birth defects, including VACTERL syndrome [99]. It has been hypothesized that the single umbilical artery is a risk factor for a placental defect that may affect nutrient supply for multiple organs simultaneously during development [100].

An important diagnosis to consider in patients suspected to have VACTERL syndrome is Fanconi's anemia. Patients with Fanconi's anemia can phenocopy VACTERL syndrome, but also exhibit bone marrow failure manifest as pancytopenia. They can also develop malignancies like acute myelogenous leukemia secondary to their propensity for chromosomal instability manifest as spontaneous cytogenetic aberrations. Patients with Fanconi's anemia also frequently demonstrate skin pigmentation (café au lait spots), microcephaly, growth retardation and microphthalmia. There are at least nine different gene mutations implicated in Fanconi's anemia and they are inherited as X-linked or recessive disorders. It has been reported that approximately 5% of patients with confirmed Fanconi's anemia have features consistent with VACTERL syndrome [101]. Therefore, the diagnosis of Fanconi's anemia needs to be carefully considered in all patients with VACTERL syndrome and confirmed if needed by performing chromosomal breakage studies [101].

Renal-Coloboma Syndrome

Renal Coloboma Syndrome (RCS) (also named papillo-renal syndrome) is an autosomal dominant disorder characterized by the association of renal hypoplasia, VUR and optic nerve coloboma from a mutation in *PAX2* [102]. The prevalence of the syndrome is unknown, but approximately 100 families have been reported [103]. A wide range of renal malformations are observed in RCS. Oligomeganephronic hypoplasia, renal dysplasia and VUR are the most frequent malformations, but multicystic dysplasia [104] and UPJO have also been described [104]. Similarly, the ocular phenotype is extremely variable. The most common finding is an optic disc pit associated with vascular abnormalities and cilio-retinal arteries, with mild visual impairment limited to blind spot enlargement, the "morning glory" anomaly [105]. In other cases, the only ocular anomaly is optic nerve dysplasia with an abnormal vessel pattern and no functional consequence (Fig. 8.2). In contrast, a large coloboma of the

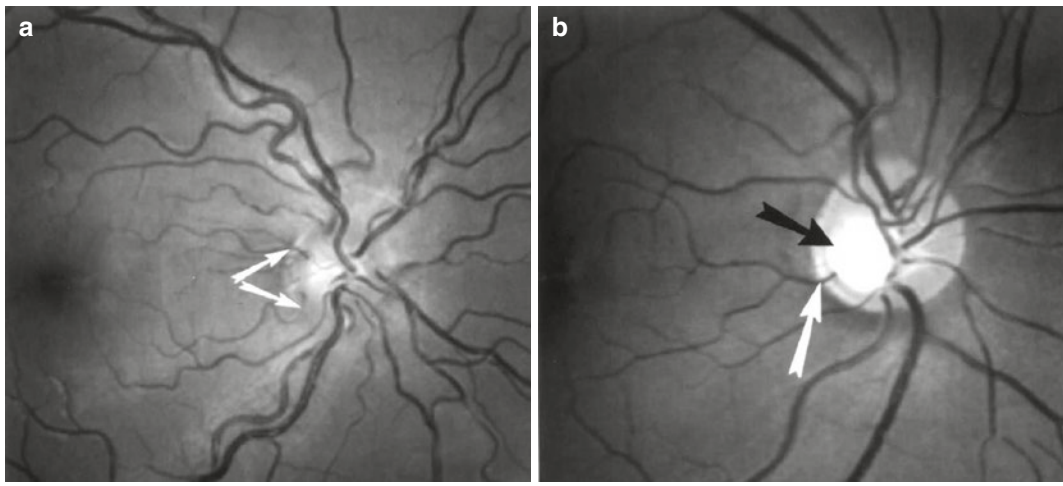

Fig. 8.2 Optic disc appearance in two patients with Renal Coloboma Syndrome and PAX2 mutations: (**a**) Characteristic features of optic disk coloboma with a deep temporal excavation (arrowheads). (**b**) The optic disk is dysplastic with thickening (arrow) and emergence of abnormal vessels ("morning glory anomaly")

optic nerve or of the choroid and retina and the morning glory anomaly can be responsible for a severe visual impairment [106]. Coloboma and the related anomalies are probably the consequence of an incomplete closure of the embryonic fissure of the optic cup. Other extrarenal manifestations can include sensorineural hearing loss, joint laxity, Arnold-Chiari malformation and seizures of unknown cause [107, 108]. In addition to its expression in the developing kidney and in the optic fissure, *PAX2* is also expressed in the hindbrain during its development. However, neurological symptoms are not usually present in RCS.

PAX2 is a transcription factor of the paired-box family of homeotic genes that is expressed in the mesonephros and in the metanephros during renal development. In 1995, Sanyanusin et al reported heterozygous mutations in two RCS families [109]. Since then, more than 30 mutations have been reported, most of them lying in exons 2–4 that encode the paired domain that binds to DNA or in exons 7–9 that encode the transactivation domain [103]. Other gene(s) are probably also responsible for this syndrome since *PAX2* mutations are not found in approximately 50% of RCS patients. Importantly the RCS phenotype is highly variable, even in patients harboring the same *PAX2* mutation, suggesting that modifier genes might be implicated.

Optic nerve coloboma occurs frequently as an isolated anomaly or as a feature of many other multiorgan syndromes such as the CHARGE association, the COACH syndrome and the acro-renal-ocular syndrome. As optic nerve coloboma and the related disorders can be easily misdiagnosed, it is likely that the prevalence of RCS is underestimated. It is wise to examine the fundus in every patient with RHD, and conversely to perform renal ultrasound and serum creatinine in every patient with optic nerve coloboma.

Even in the absence of optic nerve colobomas, mutations in *PAX2* are one of the more common genetic causes of RHD [65] and they also appear to be associated with low nephron number. Barua et al. described families diagnosed with FSGS anywhere from 7 to 68 years of age due to dominantly inherited mutations in *PAX2*. One patient had a kidney biopsy sample that exhibited glomerulomegaly, which could be secondary to low nephron endowment at birth [110]. Some of the affected individuals had imaging studies that revealed other CAKUT phenotypes including small kidneys and hydronephrosis. Vivante et al. identified heterozygous mutations in *PAX2* in three families and one child with steroid-resistant nephrotic syndrome [111]. The patients developed their steroid-resistant nephrotic syndrome or FSGS either during infancy or in adolescence. Here again, the FSGS lesion could be secondary to low nephron endowment at birth.

Renal Cyst and Diabetes Syndrome

Mutations in the *TCF2* gene encoding the transcription factor HNF1β were initially found in patients with maturity onset diabetes of the young, type 5 (MODY5), an autosomal dominant disorder [112, 113]. Diabetes mellitus is present in approximately 60% of all the cases reported, usually occurs before 25 years of age, and is often associated with pancreatic atrophy [114–116]. In some patients, a subclinical deficiency of pancreatic exocrine functions has been demonstrated. Additional features have been described, including a wide spectrum of renal phenotypes (Table 8.4). The presence of cysts is the most consistent feature of the renal phenotype, leading

Table 8.4 Renal cyst and diabetes syndrome

Main features[a]
Fetal large hyperechoic kidneys
Renal hypodysplasia with cortical microcysts
Diabetes mellitus (MODY type 5)
Occasional features
Genital malformations
Female: vaginal aplasia, rudimentary or bicornuate uterus
Male: epididymal cysts, atresia of the vas deferens, asthenospermia, hypospadias
Hyperuricemia, rarely gout (reduced uric acid fraction excretion)
Hypomagnesemia
Moderate elevation of liver enzymes
Subclinical defect of exocrine pancreatic functions

[a] Age at onset and severity of these symptoms are highly variable

to the name, "Renal Cysts and Diabetes (RCAD) Syndrome". The cysts are usually cortical, bilateral, and small [68]. Mutations in the *TCF2* gene have also been found in association with a variety of isolated renal development disorders such as RHD, MCDKs, renal agenesis, horseshoe kidneys, UPJO as well as clubbing and tiny diverticula of the calyces [117–119]. The most specific finding when histology is available is the presence of cortical glomerular cysts with dilatation of the Bowman spaces (glomerulocystic dysplasia) [120]. Other nonspecific lesions such as cystic renal dysplasia, interstitial fibrosis or oligomeganephronia have also been reported. Antenatal presentations with enlarged hyperechoic kidneys or macroscopic cysts can occur [118, 121].

Various genital tract malformations have been reported mostly in females. These include vaginal aplasia, rudimentary uterus, bicornuate uterus, uterus didelphys and double vagina. In males, hypospadias, epididymal cysts, and agenesis of the vas deferens have been reported [114]. These genital anomalies have been described in approximately 10–15% of patients with *TCF2* mutations, but these malformations might be underestimated especially in paediatric reports. Reduced fractional excretion of uric acid (<15%) and moderate hyperuricemia is observed in some cases and is usually asymptomatic. The hyperuricemia is thought to reflect altered urate transport by the kidney and impaired glomerular filtration [114]. Serum hypomagnesemia has also been reported and this may be due to the fact that HNF1b regulates FXYD2 that is needed for distal tubule reabsorption of magnesium [122]. A similar mechanism may explain the altered urate transport observed in these patients since HNF1b can activate the promoter of URAT1 that regulates urate transport in the proximal tubule [123]. Moderate elevation of liver enzymes is a common finding, but severe hepatopathy has not been reported.

HNF1β is a homeobox-containing basic helix-turn-helix transcription factor, which is involved in the development of the pancreas, the kidneys, the liver and intestine. More than 50 mutations have been reported, most of which are located in the first four exons that encode the DNA-binding domain. In more than one-third of the cases, the gene is entirely deleted [68, 115, 123]. Such alterations are not detected by conventional amplification and screening methods. Importantly, deletions are infrequently transmitted by the parents but appear *de novo* in the proband. Analysis of *TCF2* can thus be recommended not only in patients with a family history of RCAD syndrome but also in cases with renal cysts when polycystic disease or nephronophthisis are unlikely. The presence of cortical bilateral cysts is probably the most typical finding. Reduced uric acid fractional excretion, elevation of liver enzymes, hypomagnesemia, glucose intolerance and abnormalities of the genital tract should be systemically sought and *TCF2* analyzed if one of these symptoms is present. As observed in other syndromes, phenotypic variability can be observed between families and also in family members with the same mutation, suggesting a role for environmental and genetic factors.

Longitudinal follow-up of genetically proven HNF1β nephropathy has been reported in a group of 62 children and adolescents, of whom 87% were diagnosed with bilateral renal dysplasia. Among these patients, 74% and 16% had visible bilateral or unilateral cysts, respectively, at the end of an average of 4 years of follow-up [124]. During this period, 28% of patients had an increase in cyst number, which was associated with a greater decline in GFR compared to patients without an increase in cyst number. Eight percent of patients developed ESKD at a median age of 15 months. Hypomagnesemia was present in 19 of 52 patients evaluated by a median age of 1 year, while recurrent hyperglycemia was observed in 4 of 50 evaluated patients. Increased uric acid was detected in 37% of patients. HNF1β mutations were varied in type, were familial or de novo, and were not correlated with phenotype. The issue of which patients should be screened for a mutation in *HNF1β* has been investigated in a cohort of 433 pediatric patients with known *HNF1β* status and CAKUT using a 17-item weighted score inclusive of abnormalities in renal, pancreatic, genital, electrolyte, and liver function as well as family history. A score of ≥8

was reported to have a sensitivity of 98.2%, specificity of 41.1%, positive predictive value of 19.8% and a negative predictive value 99.4% [125]. The possible utility of this scoring system, particularly in predicting absence of a HNF1β mutation, needs to be validated in other cohorts with HNF1β-associated disease.

Kallmann Syndrome

Kallmann syndrome (KS) is defined by the presence of hypogonadotropic hypogonadism and deficiency of the sense of smell (anosmia or hyposmia) [126]. Some affected individuals exhibit unilateral renal agenesis, cleft lip and/or palate, selective tooth agenesis, bimanual synkinesis and hearing impairment [127]. Other CAKUT phenotypes including duplex systems, hydronephrosis, and VUR have been rarely reported. Anosmia/hyposmia is related to the absence or hypoplasia of the olfactory bulbs and tracts. Hypogonadism is due to a deficiency in gonadotropin-releasing hormone (GnRH). The GnRH-synthesizing neurons migrate during development from the olfactory epithelium to the forebrain along the olfactory nerve pathway [128]. KS is genetically heterogeneous with at least 8 genes reported including *KAL1*, an X-chromosome encoded gene that gives rise to the extracellular matrix protein anosmin-1 [129], *FGF8* (Fibroblast growth factor 8) [130], *FGFR1* (Fibroblast Growth Factor Receptor 1) [131], *PROK2* (prokinectin-2) and *PROKR2* (prokinectin-2 receptor) [132], *CHD7*, *NELF*, and *HS6ST1* [133]. Chromodomain helicase DNA-binding protein 7 (CHD7) is a transcriptional regulator that binds to enhancer elements in the nucleus. It is implicated in CHARGE syndrome, that is characterized by choanal atresia, malformations of the heart, the inner ear, and the retina, and in Kallmann syndrome. In the largest study to date of 219 patients with Kallmann syndrome, mutations were most commonly observed in the FGF pathway (either FGF8 or FGFR1), in KAL1, in the PROK2/PROKR2 pathway and in CHD7 [133]. Importantly in this study, unilateral renal agenesis was only observed in patients with KAL1 mutations (reported in 18%, 3/17), or in patients with no mutation in the above-mentioned 8 genes, where the frequency was similar at 17% (4/23). Patients with *KAL1* mutations are typically male since the disorder is X-linked and they demonstrate a much more severe reproductive phenotype compared to patients with other mutations with small testes, absent puberty, and micropenis. Females with KAL1 mutations typically present with partial pubertal development manifesting as spontaneous breast development in the absence of hormonal treatment. KAL1 is expressed in the developing human metanephric kidney at 11 weeks of gestation [134].

Renal Tubular Dysgenesis and Mutations of RAS System Elements

The differential diagnosis of oligohydramnios with neonatal renal failure includes a spectrum of diagnoses including bilateral renal dysplasia, posterior urethral valves, and PKD. All of these diagnoses are detectable and distinguishable on antenatal ultrasound imaging of the kidneys and the urinary tracts. The presence of normal kidneys on antenatal ultrasound in combination with oligo- or anhydramnios should strongly suggest the diagnosis of RTD [135]. RTD is a severe perinatal disorder characterized by absence or paucity of differentiated proximal tubules, early severe oligohydramnios, and perinatal death. The latter is usually due to pulmonary hypoplasia and skull ossification defects [136]. This condition has also been described in clinical conditions associated with renal ischemia, including the twin–twin transfusion syndrome, major cardiac malformations, severe liver diseases, fetal or infantile renal artery stenosis [137] and in fetuses that are exposed *in utero* to ACE inhibitors, Ang II receptor antagonists [138] or non-steroidal anti-inflammatory medications [135]. All of these environmental insults are postulated to lead to chronic hypoperfusion of the fetal kidneys with upregulation of the RAS. The absence or paucity of proximal tubules is believed to be secondary to chronic renal hypoperfusion [56]. Mutations in

the genes which encode components of the RAS have been identified in some families [139]. Mutations in the *ACE* gene are seen in 65.5 % of cases, while mutations in the *renin* (*REN*) are observed in 20 % of cases. Mutations in *AGT* and in the *AGT type I receptor* (*ATR1*) occur much less frequently [56]. It has been suggested that if there is no expression of the renin protein on immunohistochemistry of the kidneys, then the *renin* gene should first be assessed. Similarly, the plasma renin activity should be measured in the newborn with suspected genetic RTD and if elevated, this should prompt an analysis of genes downstream of the *REN* gene [140].

CHD1L, CHD7 and CHARGE Syndrome

Chromodomain helicase DNA binding protein 1-like protein, CHD1L, belongs to the Snf2 family of helicase-related ATP-hydrolyzing proteins and contains a helicase-like region that is similar to other family members, such as CHD7 which is the major gene that causes CHARGE syndrome. CHARGE syndrome is characterized by Colobomas, Heart defects, choanal Atresia, Retarded growth and development, Genital hypoplasia, and Ear anomalies with deafness. CHARGE syndrome is associated with CAKUT phenotypes including horseshoe kidneys, renal agenesis, VUR and renal cysts [141]. Chromatin-remodelling and -modifying enzymes like CHD1L and CHD7 are predicted to play key roles in differentiation, development and tumour pathogenesis via effects on chromatin structure and accessibility. Brockeschmidt et al. screened 85 patients with CAKUT and identified 3 patients with heterozygous missense variants in CHD1L [142]. The same paper reported that CHD1L was expressed in early ureteric bud and comma- and S-shaped structures during human kidney development. In the postnatal human kidney, CHD1L was expressed in the cytoplasm of tubular cells in all nephron segments. Similarly, Hwang et al. reported that 5 out of 650 families had heterozygous mutations in CHD1L: the affected individuals had a spectrum of CAKUT phenotypes including renal dysplasia, posterior urethral valves, UVJ obstruction and horseshoe kidneys [73]. It is not yet known if these patients will also be at greater risk for malignancies given that CHD1L is known to be an oncogene in hepatocellular carcinoma [143].

DSTYK and CAKUT

DSTYK is a dual serine-threonine and tyrosine protein kinase that is co-expressed with fibroblast growth factor receptors in the developing mouse and human kidney in both metanephric mesenchyme and ureteric bud cells. Sanna-Cherchi et al. discovered that 7/311 patients with CAKUT had heterozygous mutations in this gene [144]. The CAKUT phenotypes observed in these patients included UPJO, VUR, and RHD.

Copy Number Variants, CAKUT and Neuropsychiatric Disorders

Copy number variants are stretches of DNA that are larger than 1 kb in length with the potential to contribute to functional variation and disease. Rare CNVs have been implicated in neuropsychiatric and craniofacial syndromes, and in syndromes with CAKUT [145, 146]. Sanna-Cherchi et al. examined the burden of rare CNVs in individuals with congenital renal malformations and identified disease-causing CNVs and potentially pathogenic CNVs in 10% and 6%, respectively, of the 522 affected individuals analyzed. This burden of CNVs in CAKUT was compared to 0.2% in population controls [146]. A subsequent analysis of 2824 individuals with CAKUT highlighted an increased prevalence of large, rare, exonic CNVs compared to population-based controls [147]. In this study, genomic abnormalities were identified in 4% of patients with the majority within six pathogenic loci including chromosome 17q12 (RCAD Syndrome), 22q11.2 (DiGeorge Syndrome), 16p11.2, 1q21.1, 4p– (Wolf-Hirschhorn Syndrome), and 16p13.11. In addition, 90% of the CNVs associated with congenital renal malformations were previously reported to predispose to developmental delay or

neuropsychiatric disease, suggesting that there are shared pathways implicated in renal and central nervous system development. Similarly, Handrigan et al. demonstrated that copy number variants at chromosome 16q24.2 are associated with autism spectrum disorder, intellectual disability, and congenital renal malformations [145].

Environmental Factors and Renal Malformations

As mentioned earlier in this chapter, genetic causes of CAKUT can be identified in at most 30% of all cases, which suggests that environmental factors or epigenetic factors explain the remaining cases (Table 8.5). Epigenetics refers to changes in gene expression rather than changes in the gene sequence itself and usually arises from DNA methylation and histone modifications that can silence or enhance gene expression. Maternal obesity and diabetes are major risk factors for CAKUT. From questionnaire data of 562 parents of children with CAKUT, maternal obesity was more highly associated with duplex kidneys and VUR, while maternal diabetes was particularly associated with posterior urethral valves [148]. Dart et al. demonstrated that pregestational diabetes was significantly associated with CAKUT (odds ratio, 1.67; 95% confidence interval, 1.14–2.46), which implies a 67% increased chance of CAKUT in the children of mothers with pregestational diabetes compared to the general population (8.3 vs. 5.0 per 1000 births, respectively) [149]. These findings in humans are strongly supported by animal models in which the pregnant dam has diabetes. Animal models have also shown that maternal undernutrition and uteroplacental insufficiency are risk factors for impaired nephrogenesis and CAKUT. Maternal use of medications, alcohol and illicit drugs like cocaine are also risk factors for impaired nephrogenesis and CAKUT. Maternal use of RAS inhibitors such as ACE inhibitors, Ang II receptor blockers and direct renin inhibitors during pregnancy have been linked to an increased risk of fetopathy in humans. The majority of children manifest hyperechogenic and enlarged kidneys with proximal tubular dysplasia, thickening of arterial walls and multiple small cysts [150, 151]. Maternal supplementation with folate may also decrease the risk of CAKUT. CAKUT phenotypes have been observed in infants exposed to folic acid antagonists *in utero*, such as carbamazepine, phenytoin, primidone, phenobarbital or valproic acid, suggesting that folate is important for kidney and urinary tract development [63, 152]. Fertility treatment with in vitro fertilization or intrauterine semination are also risk factors for CAKUT, possibly through epigenetic effects on the developing zygote. Identifying the environmental risk factors that predispose to CAKUT needs to be addressed in future research.

Table 8.5 Clinical indications to search for a renal anomaly

Exposure to teratogens
ACE inhibitors and Angiotensin receptor blockers
Alcohol
Alkylating agents
Cocaine
Folic acid antagonists
Vitamin A congeners
Maternal diabetes
Findings on physical examination
High imperforate or anteriorly positioned anus
Abnormal external genitalia
Supernumerary nipples
Preauricular pits and ear tags, cervical cysts or fistula
Hearing loss
Aniridia
Coloboma or optic disc dysplasia
Hemihypertrophy
Single umbilical artery
Other
Hyperglycemia

Clinical Approach to Renal Malformation

The majority of renal malformations are now diagnosed antenatally, largely because of the widespread use and sensitivity of fetal ultrasound. The sensitivity of prenatal ultrasound screening for renal malformations is about 82% and the mean time at which these malformations

are detected is 23 weeks gestation [12]. In general, urinary tract malformations detected antenatally are isolated and present as mild hydronephrosis with no therapeutic consequences. Parents should be reassured. In contrast, bilateral forms of renal agenesis, severe dysgenesis, bilateral ureteric obstruction, or obstruction of the bladder outlet or the urethra can cause severe oligohydramnios as early as 18 weeks. Because amniotic fluid is critical to lung development, oligohydramnios as early as the second trimester can result in lung hypoplasia, a potentially fatal disorder. The oligohydramnios sequence, termed Potter's syndrome, in its most severe form consists of a typical facial appearance characterized by epicanthal folds, recessed chin, posteriorly rotated, flattened ears and flattened nose, as well as decreased fetal movement, musculoskeletal features including clubfoot and clubhand, hip dislocation, joint contractures and pulmonary hypoplasia. The renal prognosis can be evaluated antenatally. Poor outcome can be predicted when there is severe oligohydramnios, and small and hyperechogenic kidneys. Normative data on kidney dimensions including kidney length from antenatal ultrasound imaging is available from the 15th week of gestation and can be used to determine if a kidney is small, suggesting some type of renal dysplasia, or increased in size, as observed in autosomal recessive PKD (ARPKD) or autosomal dominant PKD (ADPKD) [153]. Indeed, fetal renal hyperechogenicity with renal cysts suggests the fetus may have ARPKD, ADPKD or a mutation in *HNF1B*. If there is concurrent severe oligohydramnios, then ARPKD is the most likely diagnosis. Amniotic fluid analysis may be of help in some cases if the fetus is suspected to have a trisomy. Trisomy 21, 18, and 13 are all associated with CAKUT [154–156]. Antenatal diagnosis and assessment of the renal prognosis are important for consideration of early termination in cases of fatal (or eventually severe renal disease) and to prepare parents and medical staff for the likelihood of neonatal renal insufficiency. Other organ malformations should be sought carefully and, if detected, a karyotype should be done. Some authors have suggested that fetal urine analysis may be helpful to determine fetal renal prognosis and to decide on *in utero* therapy if congenital lower urinary tract obstruction is noted. Morris et al. performed a systematic review of the literature on fetal urine analysis and concluded that none of the analytes examined had sufficient accuracy to predict poor postnatal renal function [157].

Oligo/anhydramnios from CAKUT is associated with a high incidence of fetal death *in utero*, severe pulmonary hypoplasia, umbilical cord compression and perinatal asphyxia. The earlier that renal oligohydramnios (ROH) is identified in the pregnancy, the more severe the pulmonary hypoplasia [158, 159]. Pulmonary hypoplasia is defined as deficiency in the number of lung cells, airways and alveoli, leading to a reduced surface area for gas exchange. Postnatally, pulmonary hypoplasia is suspected in cases of ROH when there is respiratory failure with the need for high ventilatory support and the chest x-ray reveals a bell-shaped chest, an elevated diaphragm, and/or pneumomediastinum or pneumothorax. Ultrasound and MRI have been used to assess lung volumes antenatally, but there is no consensus on whether they are reliable predictors of pulmonary hypoplasia postnatally. Therefore, it remains difficult to predict pulmonary prognosis postnatally.

When isolated CAKUT or CAKUT with other organ defects is diagnosed in the fetus, genetic counseling and referral to a multidisciplinary team should be offered. An individualized approach that is in accordance with the parental wishes for more information is advised [160]. A screen for gross chromosomal abnormalities can be performed using a chromosomal microarray from the amniotic fluid (if less than 25 weeks) or from fetal blood (greater than 25 weeks or severe oligohydramnios). If the chromosomal microarray is normal, then a targeted gene panel or WES on the parents and the fetus could be considered. Given the incomplete penetrance and variable expressivity of many mutations implicated in CAKUT, it remains to be demonstrated whether targeted gene panels or WES are beneficial for antenatal decision-making.

The clinical presentation of renal malformation in the postnatal period is dependent on the

amount of functioning renal mass, the presence of bilateral urinary tract obstruction and the occurrence of urinary tract infection. Bilateral renal agenesis or severe dysplasia is likely to present soon after birth with decreased renal function. This may be accompanied by oliguria. Alternatively, patients may present with a flank mass or an asymptomatic abnormality detected by renal imaging.

A detailed history and careful physical examination should be carried out on all infants with an antenatally detected renal malformation. An early (within 24 h of life) renal ultrasound is recommended for newborns with a history of oligohydramnios, progressive antenatal hydronephrosis, distended bladder on antenatal sonograms, and bilateral severe hydroureteronephrosis. In male infants, a distended bladder and bilateral hydroureteronephrosis may be secondary to posterior urethral valves, a condition which requires immediate renal imaging and clinical intervention. In general, unilateral anomalies do not require urgent investigation after birth. Renal ultrasound for unilateral hydronephrosis is not recommended within the first 72 h of life because urine output gradually increases over the first 24–48 h of life as renal plasma flow and glomerular filtration rate increase [161]. Thus, the degree of urinary tract dilatation can be underestimated during this period of transition.

A careful examination of the genitalia and the position of the anus are part of the initial assessment since CAKUT can occur in the context of cloacal malformations and with genital tract defects in females and males. The mesonephric duct gives rise to the developing kidneys, urinary tracts and the male genital tracts; therefore, a careful examination of the testes, the epididymis, and the ductus deferens is important. Congenital epididymal cysts are the most frequent anomaly noted in association with mesonephric duct anomalies and are usually asymptomatic. Other male genital duct anomalies that may occur in the context of CAKUT include an absent, ectopic or duplicated ductus deferens. Seminal vesicle cysts may also arise and typically present after puberty as pelvic pain or with urinary symptoms like dysuria, polyuria, or urinary retention [162]. Adjacent to the mesonephric ducts are the paired Müllerian or paramesonephric ducts that give rise to the fallopian tubes, the uterus, the cervix, and the upper two thirds of the vagina. Because Müllerian duct development is tightly linked to the growth and elongation of the mesonephric ducts, CAKUT is also observed with concurrent female Müllerian duct anomalies. Indeed, the Mayer-Rokitansky-Kuster-Hauser syndrome describes women with normal female external genitalia, but Müllerian duct anomalies that include aplasia of the uterus, the cervix, and the upper vagina. In a large cohort of 284 women with this syndrome, roughly 30% of them had associated CAKUT anomalies including renal agenesis, horseshoe kidney, ectopic kidney, and urinary tract defects including duplications [163]. Females with Müllerian ducts anomalies are typically discovered because of primary amenorrhea, dyspareunia, infertility, and/or obstetric complications [164]. In females with CAKUT and a suspected Müllerian duct anomaly, MRI imaging may be indicated to define the anatomical defect with better precision.

Clinical Approach to Specific Malformations

Unilateral Renal Agenesis

A diagnosis of unilateral renal agenesis depends on the certainty that a second kidney does not exist in the pelvis or some other ectopic location. Since absence of one kidney induces compensatory hypertrophy in the existing kidney, the presence of a large kidney on one side suggests the possibility of unilateral renal agenesis. Interestingly, compensatory hypertrophy has been observed to begin as early as 20 weeks of gestation: van Vuuren et al. examined 67 fetuses with a diagnosis of MCDK or unilateral renal agenesis and noted that 87% of the cases of MCDK and 100% of the cases of unilateral renal agenesis exhibited compensatory hypertrophy of the contralateral kidney with kidney length greater than the 95th percentile for gestational age [165]. Since unilateral agenesis is associated with contralateral urinary tract abnormalities

including UPJO and VUR in 20–40% of the cases [166, 167], imaging of the contralateral side is suggested. Management of affected patients involves determining the functional status of the contralateral kidney. If the contralateral kidney is normal, the long-term renal functional outcome is usually excellent, although a recent study suggests that some patients may in fact have a poor long-term outcome and require dialysis [25]. It is therefore reasonable to propose that individuals with a single functioning kidney should have their blood pressure measured, urine tested for protein, and renal function measured periodically throughout life. While some have suggested that children with single kidneys should avoid contact/collision sports, at least one study suggests that kidney injuries occur much less frequently than other organ injuries, and thus sports restriction may not be indicated solely on the basis of having a single kidney [168].

Renal Hypoplasia

Unless associated with other malformations, renal hypoplasia can be asymptomatic. Unilateral hypoplasia is often discovered as an incidental finding during an abdominal sonogram or other imaging study. In contrast, patients with bilateral renal hypoplasia are at risk for decreased renal function and CKD.

Renal Dysplasia

The dysplastic kidney is generally smaller than normal. However, cystic elements can contribute to large kidney size, the most extreme example being the MCDK (see below). During the antenatal period, unilateral disease is likely to be discovered as an incidental finding. This may also be the case for bilateral renal dysplasia unless it is associated with oligohydramnios. After birth, bilateral renal dysplasia may limit GFR, causing renal failure that is usually progressive. Postnatal ultrasonography of the dysplastic kidney is characterized by small size, increased echogenicity, loss of corticomedullary differentiation and cortical cysts. Renal dysplasia is strongly associated with dilatation of the upper and lower urinary tract from VUR, posterior urethral valves, and/or other urinary tract obstruction [169]. Accordingly, imaging of the lower urinary tract should be performed to determine whether these abnormalities are present.

Multicystic Dysplastic Kidney

The MCDK presents by ultrasonography as a large cystic non-reniform mass in the renal fossa and by palpation as a flank mass. The MCDK is nonfunctional, a condition that can be demonstrated by imaging with MAG3 or DTPA radionuclide scanning. The MCDK is usually unilateral. If bilateral, it is fatal. Complications of MCDK include hypertension (0.01–0.1%). Wilms tumour and renal cell carcinoma have also been described in MCDK, but the incidence of malignant complications is not significantly different from the general population [170]. In 25% of cases, the contralateral urinary tract is abnormal. Contralateral abnormalities can include rotational or positional anomalies, renal hypoplasia, VUR and UPJO [18]. Contralateral UPJO occurs in 5–10% of cases.

Gradual reduction in renal size and eventual resolution of the mass of the MCDK is common. At two years, an involution in size by ultrasound has been noted in up to 60% of the affected kidneys. Complete disappearance of the MCDK can occur in a minority of patients (3–4%) by the time of birth, and in 20–25% by two years. Increase in the size of MCDK can be seen in some cases. Several reports suggest that if the kidney length of the MCDK is less than 6.2 cm on the initial postnatal US, then complete resolution is likely to occur [171, 172]. The contralateral kidney usually shows compensatory hypertrophy by ultrasound evaluation. If the contralateral kidney does not show hypertrophy, it could be hypoplastic.

Management of patients with MCDK has shifted from routine nephrectomy in the past, to observation and medical therapy. Because of the risk of associated anomalies in the contralateral kidney, the possibility of VUR should be evaluated and blood pressure should be measured. For children with isolated MCDK and a contralateral kidney that is structurally normal with compensatory hypertrophy, the prognosis is excellent. While there exist no evidence-based guidelines

for long-term follow-up of these children, a review of published evidence and expert opinion supports serial investigation by ultrasound and urinalysis within the first two years of life to monitor MCDK involution and contralateral renal growth, and then very intermittent examination of renal growth, blood pressure and urine protein excretion through the end of puberty [173]. For the small number of patients with unilateral MCDK who develop hypertension, estimated to be 5.4 out of 1000 children [170], medical therapy is usually effective. Nephrectomy may be curative in resistant cases.

Renal Ectopia

Normally, the kidneys lie on either side of the spine in the lumbar region and are located in the retroperitoneal renal fossae. Rapid caudal growth during embryogenesis results in migration of the developing kidney from the pelvis to the retroperitoneal renal fossa. With ascension, comes a 90° rotation from a horizontal to a vertical position with the renal hilum finally directed medially. Migration and rotation are complete by 8 weeks of gestation.

Simple congenital ectopy refers to a low-lying kidney that failed to ascend normally. It most commonly lies over the pelvic brim or in the pelvis and is termed a pelvic kidney. Less commonly, the kidney may lie on the contralateral side of the body, a state that is termed crossed ectopy without fusion. Clinical presentation can be asymptomatic or symptomatic. Asymptomatic presentation is when the ectopic kidney has been diagnosed coincidentally such as might occur during routine antenatal sonography. Symptomatic presentation occurs with urinary tract infections. Symptoms such as abdominal pain or fever may occur. On examination, an abdominal mass may be palpable. Other presenting features include hematuria, incontinence, renal insufficiency and hypertension [21]. A high incidence of urological abnormalities has been associated with renal ectopia. VUR is the most common, occurring in 20% of crossed renal ectopia and 30% of simple renal ectopia. In bilateral simple renal ectopia, there is a higher incidence of VUR, occurring in 70% of cases. Other associated urological abnormalities include contralateral renal dysplasia (4%), cryptorchidism (5%) and hypospadias (5%) [21]. Reduced renal function is commonly observed by radionuclide scan in the ectopic kidney. Female genital anomalies such as agenesis of the uterus and vagina [174] or unicornuate uterus [175] have also been associated with ectopic kidneys. Other anomalies described include adrenal, cardiac and skeletal anomalies. Clinical assessment should therefore include a careful physical examination for other anomalies. Renal ultrasonography will help with diagnosis and defining the underlying anatomy. A VCUG should be undertaken, particularly if there is hydronephrosis, given the risk of VUR and obstruction. A DMSA scan is also recommended to assess for differential renal function.

Renal Fusion

Renal fusion is defined as the fusion of two kidneys. The most common fusion anomaly is the horseshoe kidney, in which fusion occurs at one pole of each kidney, usually the lower pole. The fused kidney may lie in the midline (symmetric horseshoe kidney) or the fused part may lie lateral to the midline (asymmetric horseshoe kidney). In a crossed fused ectopic kidney, the kidney from one side has crossed the midline to fuse with the kidney on the other side. Fusion is thought to occur before the kidneys ascend from the pelvis to their normal dorsolumbar position. This is usually between the fourth to ninth week of gestation. As a result, fusion anomalies seldom assume the high position of normal kidneys. The blood supply may therefore come from vessels such as the iliac arteries. Abnormal rotation is also associated with early fusion of the developing kidneys. The pelvis of each kidney lies anteriorly and the ureter, therefore, traverses over the isthmus of a horseshoe kidney or the anterior surface of the fused kidney. Ureteric compression may occur due to external compression by a traversing aberrant artery. The majority of patients are asymptomatic. Some, however, develop obstruction which presents with loin pain, hematuria and may be associated with urinary tract infections due to urinary stasis or VUR. Renal calculi may occur in up to 20% of cases [176].

Other associated urological anomalies include ureteral duplication, ectopic ureter and retrocaval ureter. Genital anomalies such as bicornuate and/or septate uterus, hypospadias, and undescended testis have also been described. Associated non-renal anomalies involve the gastrointestinal tract (anorectal malformations such as imperforate anus, malrotation, and Meckel diverticulum) the central nervous system (neural tube defects), and the skeleton (rib defects, clubfoot, or congenital hip dislocation). Investigations should include static imaging (renal ultrasound) and functional imaging (DMSA scan) and a VCUG.

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Part III

Ciliopathies

Ciliopathies: Their Role in Pediatric Kidney Disease

9

Miriam Schmidts and Philip L. Beales

Cilia in the Historic Context

Cilia are evolutionarily well conserved, hair-like structures projecting from the surface of most cells in vertebrates and are broadly divided into motile and non-motile cilia. While non-motile cilia can be found as single organelles on most cells in mammals, the occurrence of motile cilia in bundles of multiple (hundreds) is restricted to certain tissues in vertebrates such as the respiratory tract, the reproductive system (epididymidis and oviduct), the ependyma lining the brain ventricles and the embryonic node where they are involved in fluid movement and mucociliary clearance. Also, the flagellum of mammalian sperm has a very similar structure to motile cilia. In vertebrate photoreceptor cells within the retina, a modified ciliary structure called "connecting cilium" links inner and outer segment of those cells. See Figs. 9.1a–c and 9.2a–k for examples of cilia visualisation.

Although the existence of cilia has been described as early as the 1800s by Purkinje and Valentin [3, 4], their significance for mammalian development, organ maintenance and clinical disease has only been fully appreciated in the last two decades. Cilia were regarded as functionless cellular extensions for many years as no link between this organelle and human disease was made despite the fact that dextrocardia had already been visualised by Leonardo da Vinci in the fifteenth century and in 1793, the Scottish pathologist Matthew Baillie mentioned situs inversus in his book *The Morbid Anatomy of Some of the Most Important Parts of the Human Body*. As published by Afzelius in 1979 [5], we realise today that situs abnormalities result from (mainly motile) ciliary dysfunction in the embryonic node. The function of non-motile, so called primary cilia remained elusive for even longer; however, when "rediscovered," their role in (cystic) kidney disease was one of the initial ciliary functions acknowledged [6, 7], Since then, a large number of (inherited) human diseases have been identified to result from ciliary malfunction [8–10]. See Table 9.1 for a summary of ciliary diseases with renal involvement and their underlying genetic cause.

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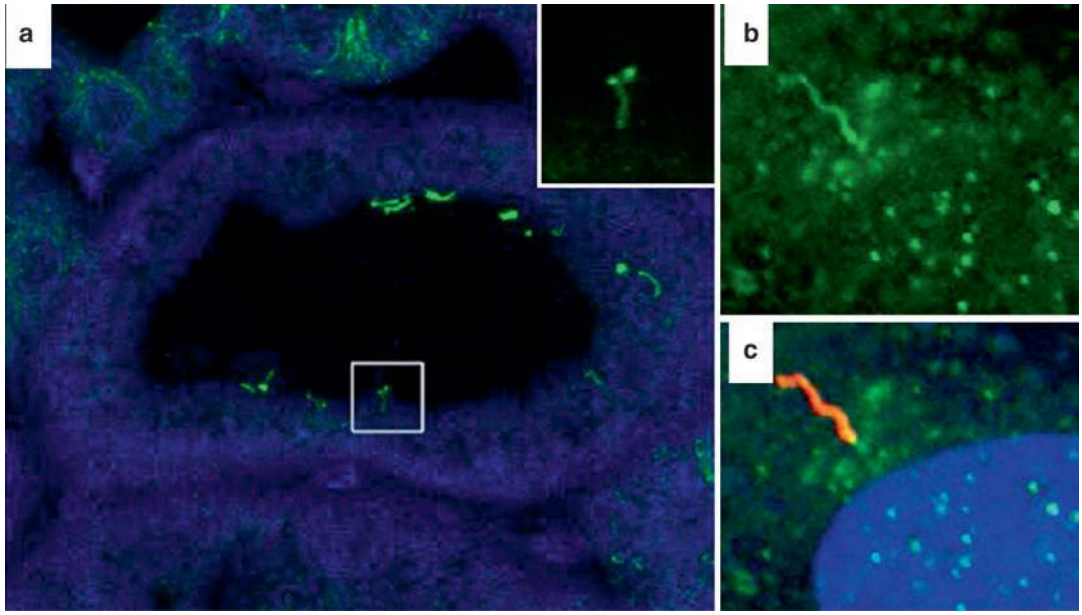

Fig. 9.1 (**a**–**c**) Mammalian cilia visualised by immunofluorescence. (**a**, **b**) Antibody staining of WT IFT172 in human control fibroblasts showing axonemal and pericentriolar localization in comparison to acetylated tubulin (anti-acetylated alpha tubulin, mouse monoclonal antibody) marking the ciliary axoneme shown in (**c**). (Images reprinted with permission from "Defects in the IFT-B component IFT172 cause Jeune and Mainzer-Saldino syndromes in humans", Halbritter et al. [1])

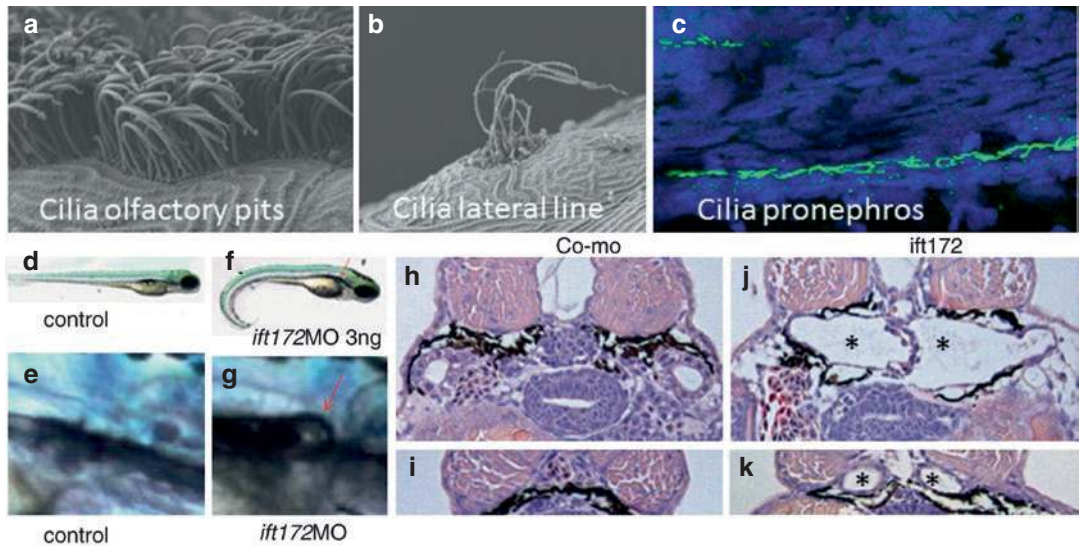

Fig. 9.2 (**a**–**k**) Zebrafish cilia and *ift172* knockdown mimicking the human renal phenotype: (**a**, **b**) Motile Cilia in the olfactory pits and Cilia in the lateral line organ (scanning electron microscopy). (**c**) *Green*: Cilia in the zebrafish pronephric duct visualised by immunofluorescence (mouse anti-acetylated tubulin antibody followed by anti-mouse monoclonal antibody). Nuclei are shown in *blue* and visualised using DAPI. (**d**) Wildtype zebrafish embryo 4 days post fertilisation, shown from the side compared to a 4 days old embryo after *ift172* knockdown using antisense morpholino (**f**). *Ift172* knockdown results in formation of a glomerular cyst (**g**) not present in the wildtype embryo (**e**). (**j**, **k**) HE staining of zebrafish embryo sections showing glomerular cysts after *ift172* knockdown not present in the wildtype embryo (**h**, **i**). ((**d**–**g**) Used with permission from Halbritter et al. [1]; (**h**–**k**) Used with permission from Westhoff et al. [2])

Table 9.1 Summary of ciliary diseases with renal involvement and their underlying genetic causes

Disease	Renal phenotype	Retinopathy	Skeletal phenotype	Obesity	Developmental delay	Situs inversus	Other	Gene
Autosomal-dominant polycystic kidney disease (ADPKD)	Always; polycystic	–	–	–	–	–	Increased risk for cerebral blood vessel aneurysma, liver involvement possible	PKD1 PKD2
Autosomal-recessive polycystic kidney disease (ARPKD)	Always; polycystic	–	–	–	–	–	Frequent liver disease, possible pancreatic cysts	Fibrocystin (PKHD1)
Medullary cystic kidney disease (MCKD1/2)	Always; cysts at the cortico-medullary junction		–	–	–	–	Extensive salt wasting	MUC1, uromodulin
Nephronophthisis (NPHP)	Always; NPHP	–	No	–	–	Rarely, especially *NPHP2*/*INVS* mutation carriers		NPHP1, NPHP2 (Inversin), NPHP3, NPHP4, NPHP5 (IQCB-1) NPHP6 (CEP290), NPHP7 (GLIS2), NPHP8 (RPGRIP1L), NPHP9 (NEK8), NPHP10 (SDCCAG8), NPHP11 (TMEM67, Meckelin), NPHP12 (TTC21B), NPHP13 (WDR19), NPHP14 (ZNF423), NPHP15 (CEP164), NPHP16 (ANKS6)
Joubert syndrome (JS) and JS related disorders, including senior-Loken syndrome, Cogan syndrome	Often; mainly NPHP-like, rarely cystic	Frequent in JSRD	Sometimes polydactyly	–	Very often	Rarely	Pathognomic "molar tooth sign" on brain MRI images; ataxia, ocular motor apraxia, hypoventilation	TMEM216, AHI1, NPHP1, CEP290, TMEM67, RPGRIP1L, ARL13B CC2D2A, CXORF5, TTC21B, KIF7 TCTN1, TMEM237, CEP41, TMEM138, C5ORF42, TCTN3, ZNF423, TMEM231, CSPP1, PDE6D

(continued)

Table 9.1 (continued)

Disease	Renal phenotype	Retinopathy	Skeletal phenotype	Obesity	Developmental delay	Situs inversus	Other	Gene
BBS	~30%, mainly NPHP-like, rarely cystic	+	Often polydactyly	Always	Very often	Rarely	Hypogonoadism	BBS1, BBS2, BBS3 (ARL6), BBS4, BBS5, BBS6 (MKKS), BBS7, BBS8 (TTC8), BBS9, BBS10, BBS11 (TRIM32), BBS13 (MKS1), BBS14 (CEP290), BBS15 (C2ORF86), BBS17 (LZTFL1), BBS18 (BBIP1), BBS19 (IFT27)
Alstrom syndrome	Often	+	–	Always	–	–	Frequent cardiomyopathy, sensorineural hearing loss, hepatic disease	Alms1
Meckel-Gruber syndrome	Often; NPHP like or cystic	na	Often orofacial clefting		na (early lethality)	Sometimes	Occipital encephalocele	MKS1, MKS2 (TMEM216), MKS3 (TMEM67), MKS4 (CEP290), MKS5 (RPGRIP1L), MKS6 (CC2D2A), MKS7 (NPHP3), MKS8 (TCTN2), MKS9 (B9D1), MKS10 (B9D2), MKS11 (TMEM231)
Orofacial digital syndrome (OFD)	Renal malformations	Usually not	Often polysyndactyly, orofacial clefting	–		Rarely	Lobulated tongue, heart defects, agenesis of the corpus callosum, conductive hearing loss, cerebellar atrophy described	OFD1 (CXORF5), TCTN3, C5orf42, DDX59

Short rib-polydactyly syndrome (SRPS)	Often; NPHP-like or cystic	Usually not evident	Often polydactyly, short ribs, shortened long bones, brachydactyly, abnormal pelvis configuration, sometimes orofacial clefting	–	na (early lethality)	Rarely	Always lethal perinatally due to cardiorespiratory insufficiency	DYNC2H1, NEK1, WDR60, WDR34, WDR35
Jeune asphyxiating thoracic dystrophy (JATD)	<30%, mainly NPHP-like, rarely cystic	Rarely	Short ribs, short long bones, rarely polydactyly, abnormal pelvis configuration, scoliosis, cone shaped epiphyses	Single cases	–	–	Sometimes retinal degeneration, mainly in cases with renal disease;	DYNC2H1, WDR34, WDR60, IFT80, IFT172, IFT140, WDR19 (IFT144), TTC21B (IFT139), CSPP1
Mainzer-Saldino-syndrome (MSS)	Always; mainly NPHP-like, rarely cystic	Always	(Mildly) shortened ribs, cone shaped epiphyses	–	Single cases	Not described	Always retinal degeneration	IFT140, IFT172
Sensenbrenner syndrome (CED)	Very often; mainly NPHP-like	Often	(Mildly) shortened ribs, brachydactyly, craniosynostosis	–	Sometimes	Usually not	Thin and sparse growing hair, nail dysplasia (ectodermal defects), heart defects	IFT122, WDR19 (IFT144) IFT43, WDR35

Ciliary Ultrastructure

The ciliary research field originates from the protozoan flagellar research undertaken since the 1950s and was therefore initially more focussed on motile cilia, possibly also because those were easier to detect due to their moving features. Electron microscopy is an essential imaging technique for visualisation these cilia also lack the central pair (9 + 0 structure). In motile cilia, so-called inner and outer dynein arms extend from the outer microtubule pairs and those outer pairs are connected to the central pair via radial spokes. This complex construction enables sliding of the microtubules generating ciliary movement. Dynein arms and radial spokes are absent from non-motile primary ciliary.

This ciliary *axoneme* is anchored to and extends from the *basal body* which itself lies within the cytosol and is derived from the mother centriole after cell division. Along its vertical axis, the primary cilium can be structurally divided into different sub-compartments: *the ciliary tip*, *the ciliary body*, *the ciliary necklace* [11], *the transition zone* [12], *the so-called inversin compartment* [13] *and the basal body* [14, 15]. The specialise cellular plasma membrane at the ciliary insertion site is referred to as the *ciliary pocket* (Fig. 9.3 for a schematic of ciliary structures). As cilia lack organelles such as endoplasmatic reticulum and Golgi and therefore no protein synthesis occurs within the cilium, all ciliary proteins are produced within the cellular cytosol, and transported to the cilium. Within the

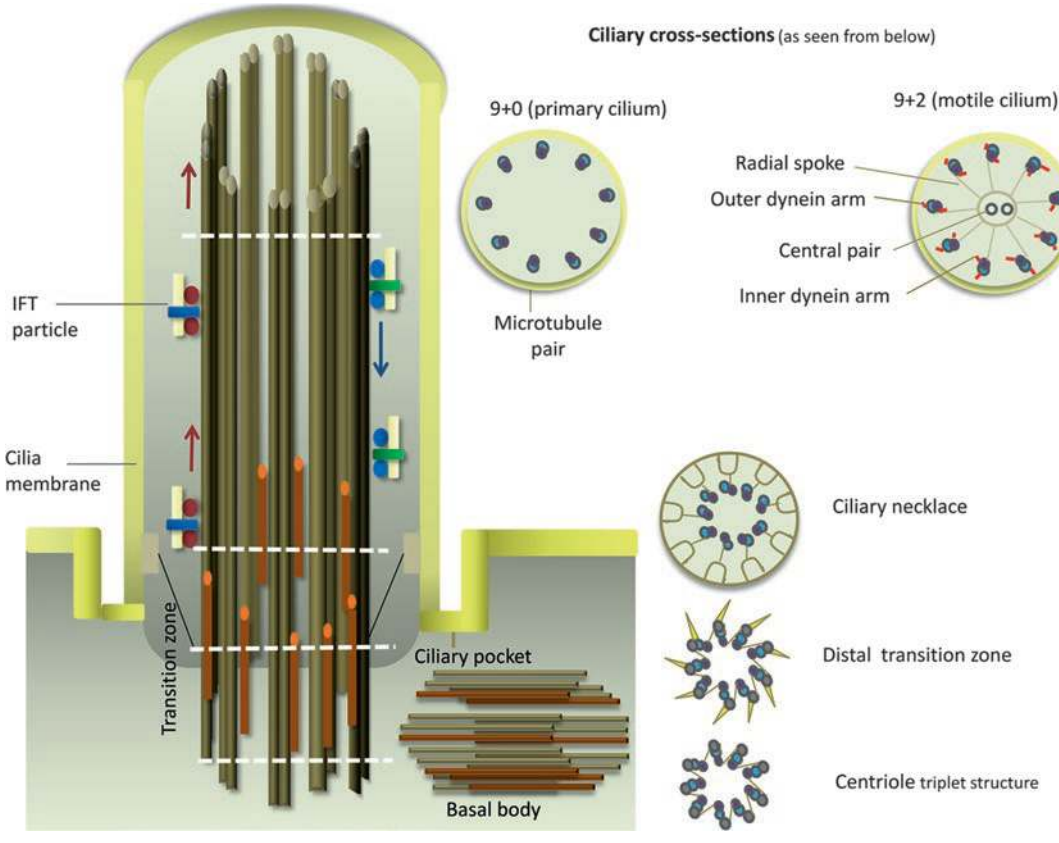

Fig. 9.3 Simplified schematic of the ciliary ultrastructure

cilium, so called **I**ntra **f**lagellar **T**ransport (**IFT**) enables protein trafficking from the ciliary base to the tip and vice versa (Fig. 9.3 for a schematic of ciliary ultrastructure and Fig. 9.4 for a schematic of IFT). Figure 9.5a–c shows a schematic of ciliary protein complex localisation and IFT defects are visualised in Fig. 9.6a, b.

Although cilia extend from the cell body and are surrounded by specialised plasma membrane forming the " *ciliary membrane*," cilia represent a distinct cellular compartment with the so called " *transition zone*" which acting as a barrier between the cilium and the rest of the cell [22, 23]. At the transition zone, the microtubule trip-

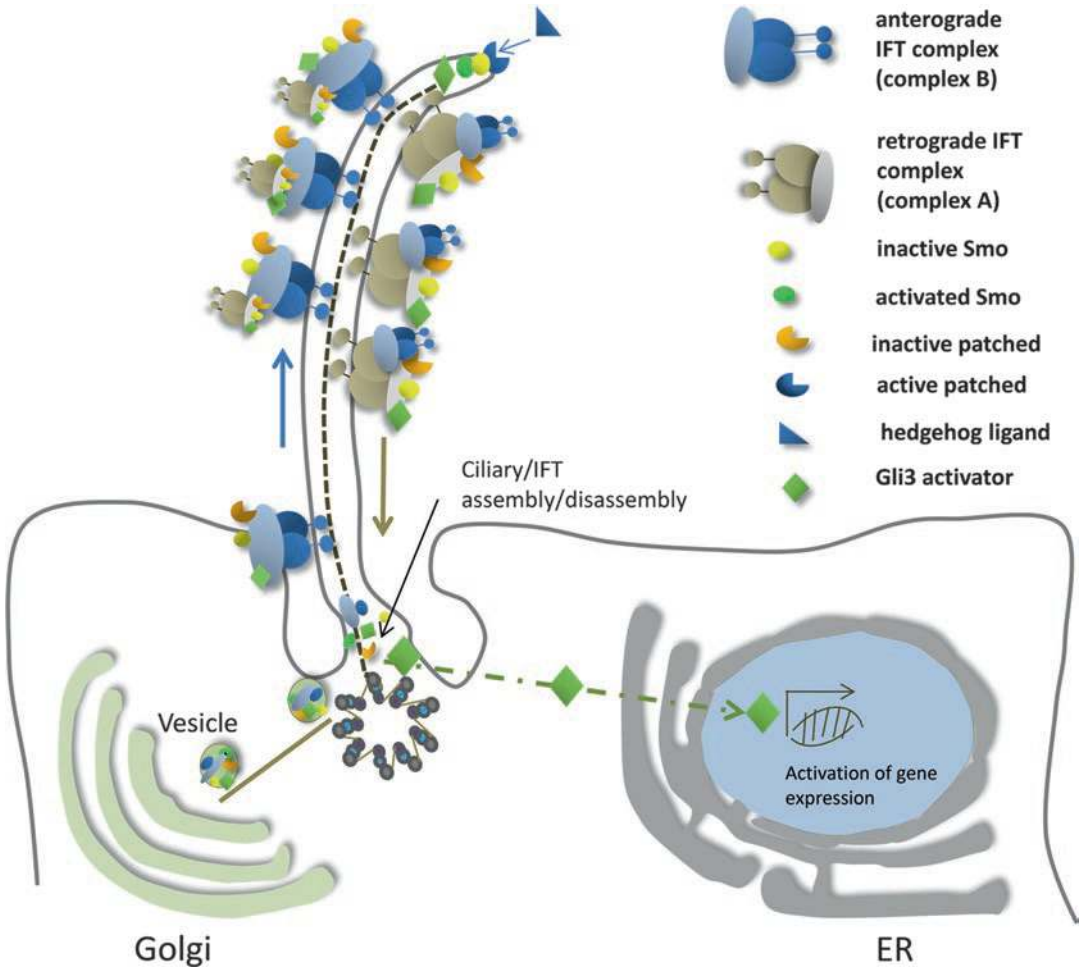

Fig. 9.4 Simplified schematic of Intraflagellar Transport (IFT) and it's relation to hedgehog signalling. Hedgehog signalling pathway components such as smoothened (smo) and patched localise to the cilium and require anterograde IFT to localise to the ciliary tip where binding of the hedgehog ligand (*blue triangle*) activates the smoothened inhibitor patched (*orange*) which in turn releases smoothened (*yellow*). Activated smoothened (*green ball*) releases GLI3 activator from its inhibitor SUFU (not shown). Gli3 activator (*green rectangle*) requires retrograde IFT to translocate to the nucleus where it activates genes involved in chondrogenic and osteogenic differentiation [16–19]

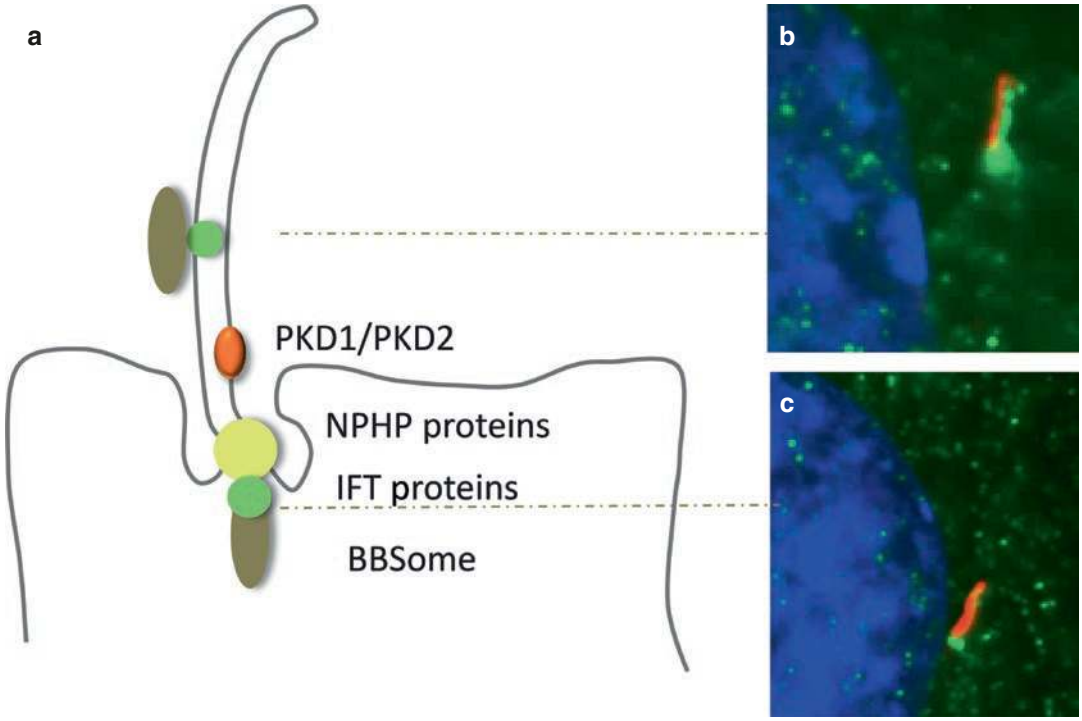

Fig. 9.5 (**a**–**c**) Localisation of major ciliary protein complexes. (**a**) Schematic of protein-complex localisation: While Bardet-Biedl-Syndrome (BBS) proteins and intraflagellar transport (IFT) proteins are detected both at the ciliary base and along the ciliary axoneme, many proteins encoded by genes mutated in nephronophthisis localise to the ciliary transition zone. (**b**) Immunofluorescence image of axonemal IFT140 localisation (*green*). (**c**) Immunofluorescence image of IFT140 localisation at the base of the cilium (*green*). The ciliary axoneme is marked in *red* using anti-acetylated tubulin antibody. ((**b**, **c**) Used with permission of John Wiley and Sons from Schmidts et al. [20])

lets of the basal body transform into the axonemal microtubule doublets. As the protein and lipid composition of the ciliary axoneme and ciliary membrane is distinct from the cell body, selective recruitment of components and transport of those components into the cilium is required. How this is precisely undertaken is still unclear, but some progress in understanding has been made in recent years. Interestingly, the connecting cilium of retinal photoreceptors is very similar in its structure to the ciliary transition zone and many proteins encoded by genes found to carry mutations leading to nephronophthisis (often with retinal degeneration) localise to the ciliary transition zone as well as the photoreceptor connecting cilium. This implicates a function of these proteins in "gate keeping" (between cytosol and ciliary axoneme as well as between inner and outer photoreceptor segment) [12, 23]. Two highly specialised areas occur within the ciliary membrane: the *ciliary necklace*, initially described by Gilula and Satir [11] where the microtubules of the basal body are connected to the plasma membrane and the base of a plasma membrane invagination around the proximal ciliary axoneme, the ciliary pocket [24]. The "*inversin compartment*" is found in the proximal ciliary region the ciliary necklace, the transitional zone and the basal body [13]. Mutations in *INVS* encoding Inversin cause nephronophthisis type 2. Please also refer to the nephronophthisis chapter of this book (Chap. 13) for details.

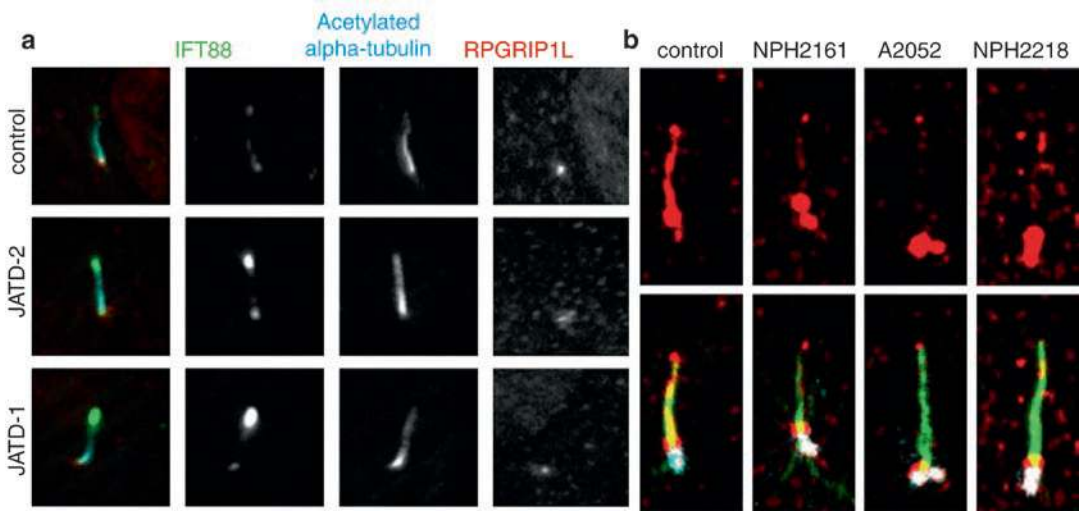

Fig. 9.6 (**a**, **b**) Visualisation of disturbed intraflagellar transport by immunofluorescence. (**a**) Compared with controls, IFT88 accumulates in distal ends of cilia in fibroblasts from Jeune Syndrome patients (JATD-1 and -2) carrying mutations in the dynein-2 complex protein dync2h1 leading to impaired retrograde intraflagellar transport (IFT). Cells were stained with anti-IFT88 (*green*); anti-acetylated α tubulin (marker for the ciliary axoneme, *cyan*); and anti-RPGRIP1L (marker for the ciliary base, *red*) (reprinted with permission from [21]). (**b**) Fibroblasts of patients with biallelic mutations in the anterograde IFT component IFT172 show decreased axonemal and increased basal body staining of IFT140 (*red*) compared to controls. The ciliary axoneme is marked with anti-acetylated-tubulin antibody (*green*), basal bodies are marked in *blue* using anti-g-tubulin antibody. (Reprinted with permission from Exome sequencing identifies DYNC2H1 mutations as a common cause of asphyxiating thoracic dystrophy (Jeune syndrome) without major polydactyly, renal or retinal involvement. (**a**) Used with permission of BMJ Publishing Group from Schmidts et al. [21]; (**b**) Halbritter et al. [1])

Ciliary Assembly

As described above, proteins necessary to build a cilium cannot be synthesized within the cilium but have to be transported to the building site. How exactly ciliary components get to the cilium and the precise process of the transformation of the mother centriole (the older of the two centrioles) into a basal body from where the cilium is assembled has not been understood in all its depth to date. Ciliogenesis is tightly linked to the cell cycle: dividing cells do not exhibit a cilium, cilia are only observed during the G1 cell cycle phase or when cells are quiescent. At least some ciliary proteins seem to reach the ciliary building site by vesicular transport: ciliary vesicles (post-Golgi vesicles) traffic close to the ciliary building site and merge there with the plasma membrane [25]. In this process of sorting membrane proteins to cilia, Bardet-Biedl syndrome proteins have been shown to be essential in assembling a coat that traffics membrane proteins to the cilium [26, 27]. Ciliogenesis is thought to start with the basal body docking to ciliary vesicles which then fuse with the plasma membrane, probably at the site of the ciliary pocket [28–32]. One of the proteins promoting this process has been identified as CEP164 and mutations in the *CEP164* gene cause nephronophthisis in humans [33, 34]. The fact that primary cilia and flagella only ever extend from the mother centriole could be due to the lack of subdistal appendages at the daughter centriole which seem to play a crucial role in anchoring the basal body to the plasma membrane [35]. Cilia stability seems to also depend on posttranslational modification of tubulin, including acetylation [36, 37], glutamylation [38, 39] and farnesylation. The latter seems of particular importance as Thomas et al. identified a homozygous *PDE6D* mutation Joubert syndrome which impaired the targeting of another known joubert protein, INPP5E to the cilium [40].

Cilia and Cell Cycle

As indicated above, ciliogenesis is interlinked with the cell cycle: dividing cells do not normally exhibit cilia, those only become visible after the cell's exit from cell cycle. When cells divide, one centrosome stays within the mother cell while the other can be found in the daughter cell. After cell division, the centrioles duplicate and the original centrioles from which duplication occurs are referred to as "mother centrioles." Once a cell has left cell cycle again, the cilium is built from this mother centriole [41]. When cells re-enter the cell cycle, cilia need to be disassembled which seems to be initiated by Percentrin-1 (PCM1) mediated recruitment of Polo-like kinase 1 (Plk1) to the pericentriolar material [42, 43]. While it has been accepted for many decades that ciliogenesis is linked to mitotic exit of the cell, the possibility that cilia themselves might influence cell cycle progression has only been recently taken into account. For example, the IFT protein Ift88 seems required for spindle orientation during mitosis [44]. Lack of Ift88 in mice leads to a polycystic kidney phenotype [7] and as for cystic renal phenotypes in humans, it remains controversial to which extent and at what time point cellular hyperproliferation contributes to the initiation and/or progression of disease [45]. The INPP5E protein (genetic mutations can cause a Joubert phenotype with renal disease) and the tumor suppressor protein VHL (mutations in the VHL gene cause Von-Hippel-Lindau syndrome which is associated with cystic renal disease) are both involved in stabilising cilia by inhibiting AURKA triggered ciliary disassembly for cell-cycle re-entry [46–48]. The question how cilia influence cell cycle, how they are influenced by cell cycle and how this contributes to ciliopathy, especially polycystic phenotypes, is difficult to resolve as proteins encoded by genes mutated in ciliopathy subjects often have multiple extra-ciliary functions which might influence cell-cycle progression independently of the cilium. For example, several genes mutated in subjects with nephronophthisis such as *ZNF423*, *CEP164* and *NEK8* have been linked to DNA damage repair and therefore directly to cell cycle progression [34, 49]. It is, however, of note that, except for VHL, no increased rate of malignancies has been demonstrated for humans affected by ciliopathies to date.

Intraflagellar Transport (IFT)

Transport of ciliary proteins along the axoneme is undertaken via IFT, an energy dependent process. Kosminski et al. were the first to notice this process in 1993 [50]. IFT is a highly conserved transport mechanism along cilia and flagellae from the green algae *Chlamydomonas* to vertebrates and mammals including humans. Building a cilium from the base to the tip is largely dependent on anterograde IFT [51]. Counter-intuitively, the anterograde IFT complex transporting proteins from the ciliary base to the tip is named "complex B" while the complex enabling retrograde transport from the ciliary tip back to the base is called "complex A." Motor for the anterograde complex is kinesin-2 while the cytoplasmic dynein-2 complex enables transport from the ciliary tip back to the base. Although named "cytoplasmic dynein," the latter complex localises to the ciliary axoneme and should not be confused with the cytoplasmic dynein-1 complex which enables transport along microtubules within the cell body and along neuronal axons. The precise composition of the dynein-2 complex has still not been completely elucidated; however, it is assumed that it resembles dynein-1 complex, a homodimer consisting of two heavy chains, two light-intermediate chains, two intermediate chains and two light chains. IFT-complex A and B are multiprotein assemblies functioning as an adaptor system between the motor complexes and cargo. As IFT-A complex proteins and the dynein-2 complex have to be brought up to the ciliary tip before they can fulfill their function in retrograde transport back from the tip to the base and vice versa, kinesin-2 and IFT complex B must be transported back to the base after they have reached the ciliary tip, it is evident that both complexes must be transported as cargo by each other [25, 52, 53]. Knockout mice for the kinesin-2 component *Kif3a*, the dynein-2 motor heavy

chain *Dync2h1* or IFT components often exhibit fewer or shorter cilia and are lethal around midgestation, indicating the fundamental role of these highly conserved proteins during development [43]. Kidney specific gene disruption or hypomorphic mutations often cause renal cysts in mice [7, 54, 55]. Human mutations in genes encoding dynein-2 complex components and IFT particles mainly cause ciliary chondrodysplasias with variable extraskeletal involvement [1, 20, 21, 56–69] and mutations in the IFT-A component *TTC21B/IFT139* and in the IFT-B component *IFT27* were recently identified in Bardet-Biedl-Syndrome [68, 70]. For more details also see the human disease section below and Fig. 9.4 for a schematic of IFT and its relation to hedgehog signalling.

Ciliary Signalling Pathways

Single non-motile (primary) cilia are considered sensory organelles involved in multiple signalling pathways transducing both signals from the cellular surroundings to the cells as well as modifying cell signalling pathways within the cell [71, 72]. As mentioned above, cilia and ciliary proteins have been highly conserved throughout evolution and mutations in genes encoding ciliary components often result in complex developmental defects in vertebrates. This can be attributed to disturbances of fundamental signalling pathways essential for embryogenesis, organogenesis and proper tissue maintenance.

Hedgehog Signalling

The best explored cilia-regulated cellular pathway is hedgehog signalling [16, 17, 73]. Hedgehog signalling crucially influences chondrogenic (and subsequently osteogenic) proliferation and differentiation [74]. Mouse models of ciliary chondrodysplasias, e.g., knockout mice for *Evc* (mutations in the EVC1 and EVC2 gene cause Ellis-van Creveld-Syndrome in humans), *Dync2h1* and *Ift80* (associated with Jeune Syndrome and Short-Rib-Polydactyly Syndrome type III in humans) indicate that IFT defects lead to imbalances in the hedgehog signalling pathway [18, 75, 76]. Lack of hedgehog signal transduction leads to decreased chondrogenic proliferation and imbalanced chondrogenic differentiation at the growth plates which impairs bone growth. As a result' mice, as well as human subjects with mutations in these genes as well as in *IFT144/WDR19*, exhibit shortened ribs and long bones. Lack of Ift80 or Ift144 also induces polydactyly in mice, a hallmark of dysregulated hedgehog signalling [18, 75–77].

The hedgehog signaling pathway operates via ciliary trafficking: the pathway components smoothened (smo) and patched move via anterograde IFT to the ciliary tip, where smo becomes activated and releases GLI3 activator. The latter is subsequently transported back to the base of the cilium via retrograde IFT and enters the cell body and nucleus where it activates genes regulating chondrogenic and osteogenic differentiation and proliferation (simplified schematic in Fig. 9.4).

Apart from altered bone growth, impaired hedgehog signalling leads to complex developmental defects in mammals including polydactyly, heart defects, midline defects such as clefting and holoprosencephaly. Renal abnormalities, especially ectopic kidneys but also cystic-dysplastic changes, can also be observed in mice [78] and human subjects affected by Smith-Lemli-Opitz syndrome, a condition thought to result from altered hedgehog signalling due to a cholesterol biosynthesis defect [79]. However, neither human subjects with *IFT80*- nor *EVC1/EVC2* mutation nor the corresponding mouse models exhibit a renal phenotype. On the other hand, human subjects with mutations in other IFT genes such as *WDR19/IFT144*, *TTC21B/IFT139*, *IFT140*, *IFT43*, *WDR35* or *IFT172* genes are affected by childhood-onset cystic or nehronopthisis-like renal disease [1, 20, 64, 65, 67–69], and in knockout mouse models for *IFT140* or *IFT172*, early onset cystic (dysplastic) kidney disease is observed [55, 80]. While it seems likely that the skeletal phenotype observed in those subjects is due to imbalances in the hedgehog pathway secondary to IFT defects,

this does not necessarily apply to the renal phenotype as no changes in the hedgehog pathway were noted in kidneys from *Ift140* knockout mice prior to the onset of cystogenesis [55].

Wnt Signalling and Planar Cell Polarity (PCP)

The role of cilia and ciliary proteins in regulating wnt signalling is subject of an ongoing discussion recently reviewed by Wallingford and Mitchell [81]. Wnt signalling can be roughly divided into a so-called "canonical" pathway branch involving wnt/beta-catenin and a so called "non-canonical" or planar cell polarity (PCP) branch. Canonical Wnt/beta-catenin dependent signalling occurs after extracellular wnt ligand binds to transmembrane Frizzled receptors which stabilize beta-catenin. Beta-catenin subsequently localises to the nucleus to activate further target genes [82]. The non-canonical or PCP pathway is mediated via Frizzled and the large transmembrane proteins Vangl2 and Celsr and involves cytoplasmic regulatory proteins such as Dishevelled (Dvl). PCP describes the process of orientation of cells and their structures along an axis in an epithelial plane in a coordinated manner [83]. Classical PCP readouts exist for all species from *drosophila* flies over *xenopus* frogs to mammals, including orientation of hair on the fly wing, convergent extension (axis elongation by cell intercalation) of the anterior-posterior body axis of frog embryos and orientation of hair cells in the inner ear of mice.

The first indication that proteins encoded by genes defective in human ciliopathies play a role in PCP came from Bardet-Biedl-Syndrome mouse models displaying typical PCP-related defects in the cochlea [84]. Further initial experiments suggested that the ciliary protein encoded by *NPHP2*, *Inversin* (*Invs*), inhibits Dvl-mediated transduction of the canonical wnt pathway while promoting a shift towards PCP signalling [85], proposing that Inversin might act as a switch between canonical and non-canonical/PCP Wnt signalling [85]. This would imply that in an inversin-deficient state such as in nephronophthisis patients with Inversin mutations, non-canonical wnt signalling might be expanded and PCP signalling reduced. In line with the assumption that cilia might act as a negative regulator of canonical wnt signalling, increased canonical wnt signalling was found in cells deficient for Bardet-Biedl-Syndrome proteins BBS1, BBS4 and MKKS and the anterograde IFT motor protein KIF3A [86], in mice mutant for *Kif3a*, *Ift88* and *Ofd1* [87] as well as in kidney-specific *Ift20* knockout mice exhibiting a cystic renal phenotype [88]. However, no abnormalities in wnt signalling were found by Ocbina et al. in the absence of cilia in mice mutant for *Kif3a*, *Ift88*, *Ift72* [89] and in *ift88* zebrafish mutants [90]. The relationship between cilia and wnt signalling therefore remains unclear.

Loss of ciliary proteins such as Ift88 and *Kif3a* in mice results in developmental defects resembling those expected for loss of PCP including mis-orientated inner ear kinocilia [91]. Also, renal tubules elongate during development using a process strongly resembling convergent extension, depending on the non-canoncial wnt ligand *Wnt9b*, where loss of Wnt9b leads to renal cyst formation [92]. Furthermore, *Ift20* disruption in mice causes cystic renal disease and in those kidneys, mitotic spindle mis-orientation has been described [88]. As mis-orientated mitotic spindles could affect **o**rientated **c**ell **d**ivision (OCD), and OCD is the basis for planar cell polarity and necessary for postnatal tubule elongation, loss of OCD might contribute to cystic phenotypes [93]. This is supported by observations in Kif3a, Pkd1, Tsc1/2 and Hnf1b-deficient mice [94–96]. Last, the wnt-regulator Dvl might also control apical docking and planar polarisation of basal bodies from which cilia extend in epithelial cells [97]. Ciliary polarity, which in turn could potentially define cellular polarity, is influenced by the anaphase promoting complex APC/C [98]. These findings, together with the suggestion that the nephronophthisis protein Inversin might act as a switch from canonical wnt towards PCP signalling [85], has led to the hypothesis that cystic and NPHP-phenotypes observed in ciliopathies might result from disturbed PCP. However, loss of OCD and/or PCP might not be the cyst-initiating event

as mice mutant for the murine homologue of PKHD1, the Fibrocystin gene causing ARPKD in humans, do not develop cysts despite disrupted OCD [99]. Moreover, cysts can be present despite normal OCD as shown in mice lacking IFT140 in the kidneys [55]. Recent work in the Xenopus model suggests nevertheless that tubular morphogenesis requires planar cell polarity (PCP) and non-canonical Wnt signalling [100].

Flow Hypothesis, Ca-Signalling/ Mechanosensation and mTOR

In 2001, Praetorius and Spring observed increased intracellular calcium levels after flow induced bending of primary cilia on canine kidney cells [101] and subsequently demonstrated that loss of cilia abolishes the flow-induced calcium influx [102]. This was subsequently confirmed in mice with ciliary defects due to *Ift88* deficiency [103]. The *Polycystin-1* and *Polycystin-2* (*PKD1* and *PKD2*) genes, mutated in subjects with ADPKD, are thought to play a role in this mechanosensation and calcium influx process [104, 105]. However, loss of mechanosensation and/or flow induced calcium influx alone is probably not sufficient to cause cystic kidney disease and Polycystin-1, although required for maintenance of tubular morphology in the long run, does not appear essential in the short or intermediate term. Possibly a second exogenous harmful event such as kidney injury is required for cyst formation in the long term [93]. How lack of flow and subsequently reduced calcium influx might lead to cystogenesis has not been clearly established. Delayed or reduced clearance of intracellular cAMP may be involved as accumulation of cAMP was noticed in cystic renal tissue [106]. This in turn might lead to increased MAP kinase signalling stimulating both cell proliferation and fluid secretion into the cyst lumen. Interestingly, fluid flow also induces phosphorylation of a key regulator of cardiac hypertrophy, histone deacetylase 5 (HDAC5) via polycystin-mediated mechanosensation. This leads to myocyte enhancer factor 2C (MEF2C)-dependent transcriptional events in the nucleus and kidney-specific knockout of *Mef2c* results in extensive renal tubule dilatation and cysts whereas *Hdac5* heterozygosity or treatment with an HDAC inhibitor reduces cyst formation in *Pkd2−/−* mouse embryos, indicating a potential treatment target [107].

Based on the observations that human subjects with tuberous sclerosis due to *TSC2* mutations develop cystic renal disease, TSC2 (Tuberin) is considered a negative regulator of mTor signalling [108], Polycystin-1 interacts with Tuberin and mTor activity is elevated in cystic tissues [109, 110], a hypothesis evolved that mTor signalling might be involved in the initiation and/or progression of cystic renal disease and that inhibition of such signalling could delay disease progression. However, while in a rodent model the mTORC1 inhibitor Rapamycin significantly delayed cyst progression [111], a clinical trial using everolimus in human PKD subjects ended with disappointing results: despite slowing down cyst expansion, renal function was not better preserved in everolimus treated subjects compared to controls. Possibly the administered dose was insufficient or more likely, the timepoint of treatment initiation was chosen too late with regards to the disease course to achieve stabilisation of renal function [112]. Nevertheless, mTor signalling seems to play a role in progression of cystic kidney disease and is potentially connected to flow-induced cilia bending: when cells are grown in a flow chamber, flow leads to Lkb1 induced mTor inhibition resulting in smaller cell size [113]. Flow might further regulate cilia length, potentially also through mTor signalling as cilia length is reduced under flow and increased within cysts where flow is absent [95]. Likewise, mTor inhibitors seem to decrease cilia length reflecting the effects of flow [93, 114].

Yap-Hippo Signalling

The hippo-signalling pathway has emerged in recent years as an important pathway controlling cell and organ growth, stem cell function, regeneration functions and tumor suppression. Dysregulation of hippo signalling was initially

noticed to lead to initiation and maintenance of cancerous growth [115]. More recently evidence for this pathway has emerged indicating its fundamental role for developmental processes, including kidney and eye development in mammals. Knockout mice for one of the main pathway components, *Yes-associated protein-1* (*Yap1*), die during early gestation with major developmental defects including yolk sack and axis elongation defects [116] while heterozygous *YAP1* loss of function mutations lead to optic fissure closure defects and possibly also cleft-lip palate, hearing loss, learning difficulties and hematuria with incomplete penetrance in humans [117]. More importantly, inactivation of another major pathway molecule, TAZ, in mice leads to polycystic kidneys (glomerulocystic disease), severe urinary concentrating defects and pulmonary emphysema [118, 119]. The renal phenotype partially resembles that of nephronophthisis patients. Further, NPHP3, NPHP4 and NPHP9/NEK8, products of genes mutated in subjects with nephronophthisis and Meckel-Gruber Syndrome, seem to be involved in Hippo-pathway regulation [120–122]. It is of note, however, that YAP1 is not only directly implicated in the hippo signalling pathway but also modulates WNT signalling via β-catenin-interaction [123], BMP signalling via interaction with Smad7 [124] as well as NOTCH signalling through up-regulation of *JAG1* [125] so that the observed developmental defects in patients with *YAP1* mutations are probably not solely effects of impaired hippo signalling. Moreover, *YAP1* itself is up-regulated by hedgehog signalling [126] raising the possibility that disturbed hedgehog signalling in ciliopathy/nephronophthisis subjects might contribute to the observed abnormalities in hippo signalling.

The substantial cross-talk between major developmental signalling pathways including wnt, hedgehog, hippo, notch and mTor [127] leaves the actual molecular basis of a developmental defect such as nephronophthisis uncertain. Nonetheless, in view of the clear renal phenotype observed in TAZ-deficient mice and well established protein-protein interactions between NPHP proteins and TAZ, dysregulated hippo signalling is one of the best candidates so far regarding the primary molecular pathomechanism leading to nephronophthisis like disorders.

Other Cilia Associated Cell Signalling Pathways

Excellent overviews of how the cilium orchestrates cellular signalling pathways during development and tissue repair have been provided by Christensen et al. [128] and Satir et al. [129]. Due to space constraints, not all pathways can be discussed here in detail so we will only scratch the surface of the relationship between cilia and TGF-beta signalling. Clathrin-dependent endocytosis governs TGF-beta signalling and interestingly, TGF-β receptors localise to endocytotic vesicles at the ciliary base and to the ciliary tip in vitro. Activation of SMAD2/3 at the ciliary base has been observed. TGF-β signalling seems to be reduced in the absence of cilia in fibroblasts lacking Ift88 suggesting that cilia might regulate TGF-β signalling and that the cilium could represent a compartment for clathrin-dependent endocytotic regulation of signal transduction [130]. Increased TGF-beta signalling has been associated with increased interstitial fibrosis in progressive renal dysfunction in ADPKD and other renal conditions. Most interestingly, the PPAR-γ agonist rosiglitazone, reversing a downstream effect of TGFß, was nephro-protective and prolonged survival in an ADPKD rodent model via inhibition of TGF-ß induced renal fibrosis [131]. If this effect is also applicable to humans remains to be established.

Cilia in Renal Disease

The clinical aspects of human ciliopathies with renal involvement are discussed in more detail in the polycystic kidney disease and nephronophthisis chapters of this book (Chaps. 12 and 13, respectively). We will here give a short introduction and overview, mainly focusing on conditions resulting from mutations in IFT and BBS genes. Please also see Table 9.1 for a summary of ciliopathies with renal involvement.

Polycystic Kidney Disease (ADPKD and ARPKD)

The classic ciliary polycystic renal diseases are represented by autosomal dominant polycystic kidney disease (ADPKD) and autosomal recessive polycystic kidney disease (ARPKD). The hallmark of both diseases is extensive cystic enlargement of both kidneys. ADPKD is one of the most common monogenetic disorders with an incidence of 1 in 600–800 live births in the western world and clinical signs of ADPKD usually become manifest in adulthood [132]. In affected subjects, heterozygous mutations in either PKD1 or PKD2, encoding for Polycystin 1 and Polycystin 2, have been identified and it is common belief that a second acquired somatic mutation is necessary for the initiation of cystogenesis ("second hit hypothesis"). Gene dosage at least of PKD1 probably plays a role for the severity of the phenotype, which rarely can mimic ARPKD [133].

With a frequency of 1:20,000 live births, ARPKD is a lot less common than ADPKD and is inherited in an autosomal-recessive manner. Two mutated germline alleles are present from the very beginning causing a very early disease onset due to loss of Fibrocystin function, the protein encoded by the *PKHD1* gene. Increased renal echogeneity and cysts are usually present prenatally and biliary dysgenesis resulting in intrahepatic bile duct dilatation and congenital hepatic fibrosis (Caroli disease) occurs frequently. Fibrocystin co-localises with PKD2 at the ciliary axoneme and the ciliary base; however, its exact function has remained elusive [134].

As outlined above, flow mediated ciliary bending might activate calcium influx and it has been suggested that PKD1 and PKD2 proteins together function as a Ca^{2+}-permeable receptor channel complex [104, 135, 136]. Further, Polycystins interact with the tuberous sclerosis protein Tuberin which is known to influence mTor signalling and the mTor pathway was found to be overactivated in cystic epithelia from Polycystin-deficient kidneys. However, despite major efforts over the past two decades, the exact pathomechanism for cystogenesis in ADPKD and ARPKD remains to be defined and to date, efficient pharmacological treatment remains to be developed. For more details on ciliary signalling pathways please refer to the above sections and the polycystic kidney disease chapter of this book, Chap. 12.

Syndromal Nephronophthisis (NPHP)

For a detailed description of Nephronophthisis, including isolated Nephronophthisis, Joubert Syndrome, Senior-Loken Syndrome and Cogan Syndrome, please refer Chap. 13. Nephronophthisis can be translated as "vanishing nephrons" or "vanishing kidney" and in contrast to ADPKD and ARPKD, where increasing numbers of cysts and increasing cyst volumes lead to larger kidneys, in subjects affected by NPHP or NPHP-like renal disease kidneys appear normal or small in size, often with increased echogenicity in ultrasound images. Many genes have been identified to date causing either isolated NPHP or NPHP combined with extrarenal symptoms which could be described as "syndromal" NPHP. These extrarenal symptoms include retinal degeneration, cerebellar malformations, skeletal dysplasia including polydactyly, situs inversus, obesity and learning difficulties. There is excessive genetic and phenotypic heterogeneity, meaning that not only the clinical symptoms overlap between different syndromes with NPHP- or NPHP-like renal phenotypes but also mutations in one and the same gene can cause different phenotypes. We will focus in this chapter on selected phenotypes of "syndromal NPHP" including Bardet-Biedl-Syndrome resulting from mutations in BBS genes and ciliary skeletal dysplasias such as Short-rib polydactyly syndrome (SRPS), Jeune Syndrome (JATD), Mainzer-Saldino Syndrome and Sensenbrenner Syndrome (CED), consequences of impaired intraflagellar transport (IFT).

Bardet-Biedl-Syndrome (LMBBS, BBS)

Among the first human ciliopathy diseases recognised as such was Bardet-Biedl-Syndrome (BBS,

Laurence-Moon-Bardet-Biedl-Syndrome, LMBBS) and therefore this condition is often referred as an example of a classical complex developmental phenotype resulting from hereditary cilia malfunction. The main features of BBS are polydactyly, developmental delay, obesity, retinal degeneration, cystic or, more commonly, NPHP-like renal disease and hypogenitalism [137]. BBS is very rare with an estimated frequency varying between 1:160,000 in northern European populations to 1:13,500 and 1:17,500 in isolated consanguineous communities such as in Kuwait and Newfoundland. Like other ciliopathies, BBS is a genetically very heterogeneous disease with mutations in 19 genes identified to date and there is considerable genetic and phenotypic overlap (especially regarding eyes and kidneys) with other ciliopathies such as Meckel-Gruber-Syndrome and Senior-Loken-syndrome. Perinatally, BBS can be difficult to distinguish from McKusick-Kaufman syndrome if polydactyly and hydrometrocolpos are present [138]. Although BBS represents an autosomal-recessive condition, in individual cases more than two mutated alleles at two different loci have been found to be necessary to cause the phenotype ("triallelic inheritance") [139, 140]. However, the vast majority of cases are inherited in the classical recessive manner [141]. Nevertheless, several "modifier" genes have been described which may influence the phenotype. While genotype-phenotype correlations have proven difficult to establish, mutations in certain genes seem to predispose for more severe kidney involvement. Mutations in *BBS6*, *BBS10* and *BBS12* are associated with renal disease at an overall frequency of 30–86%; however, this includes minor anomalies and the majority of patients do not progress to renal failure [142].

While some of the proteins encoded by genes mutated in BBS localise to the ciliary transition zone (e.g., CEP290), others localise further proximal to the basal body area and axonemal localisation has been observed as well. BBS proteins seem to form two different major protein complexes: the so-called BBSome and the BBS chaperone complex where the formation of the BBSome requires the function of the BBS chaperone complex. Data from mouse and zebrafish models indicate a role for BBS genes in intracellular and intraflagellar trafficking of ciliary components. Therefore, although the BBSome does not seem to be required for ciliary assembly itself, due to its trafficking function specific signalling receptors and transmembrane proteins no longer reach the cilium in subjects affected by BBS, leading to organ-specific signalling abnormalities and subsequently the characteristic developmental defects [26, 143, 144]. While the loss of function of BBS and IFT proteins conceivably leads to retinal degeneration due to impaired transport of molecules such as rhodopsin along the connecting cilium between the inner and outer segments of photoreceptor cells, the mechanism for (NPHP-like) renal disease in BBS and IFT-related diseases as largely remains elusive. As discussed above, imbalances in the hippo signalling pathway seem to play a role in classical nephronophthisis. To which extent this applies to BBS has not yet been investigated. While polydactyly in BBS as well as indirectly IFT-dependent disorders point towards a hedgehog-based mechanism at least for the skeletal phenotype, a contribution of misregulated hedgehog signalling to the renal phenotype has yet to be proven. Clinical hallmarks of BBS are shown in Fig. 9.7a–h.

Alström Syndrome

Alström syndrome is a very rare autosomal-recessive ciliopathy occurring with a frequency of 1:500,000–1:1,000,000 but can be more common amongst consanguineous populations. Over 100 mutations in a single large gene, *Alms1*, have been published to date. Clinical characteristics include obesity, retinal dysfunction, cardiomyopathy, hearing loss, hepatic involvement, renal disease and hypogonadism, resembling the BBS phenotype; however, with higher lethality due to cardiac complications [146]. In contrast to BBS, polydactyly and developmental delay are not common features. The Alms1 protein localises to the base of the cilium [147] and knockdown of *Alms1* in kidney epithelial cells in vitro causes

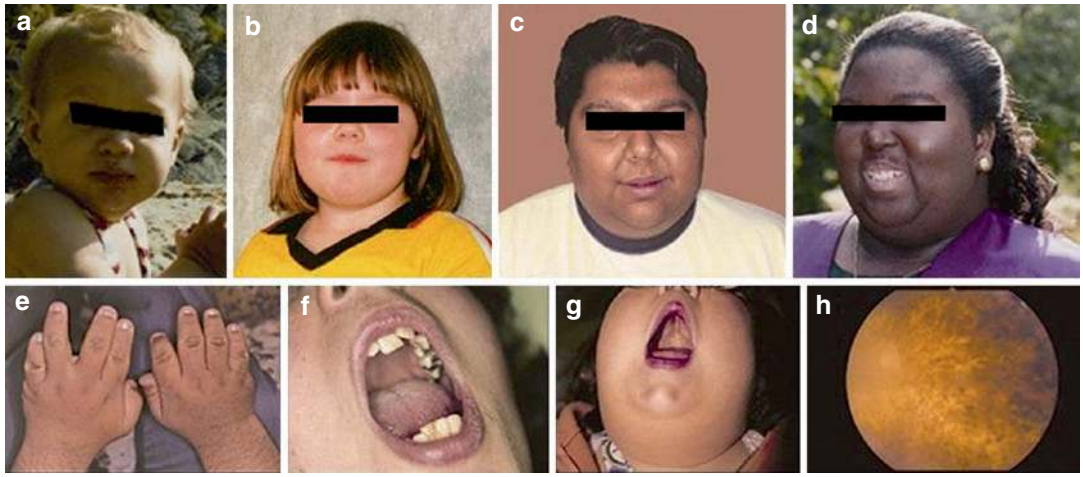

Fig. 9.7 (**a**–**h**) Clinical hallmarks of Bardet-Biedl-Syndrome (BBS). (**a**–**d**) Dysmorphic facial features including a flat nasal bridge, retrognathia, small mouth, malar hypoplasia, deep-set eyes, downward slanting palpebral fissures, hypertelorism (**e**) Brachydactyly and scars from removal of accessory digits. (**f**, **g**) High arched palate and dental crowding. (**h**) Rod-cone dystrophy in fundoscopy. (Used with permission of Nature Publishing Group from Forsythe and Beales [145])

shortened cilia and seems to abrogate calcium influx in response to mechanical stimuli. In a mouse model of Alström syndrome, loss of cilia from the kidney proximal tubules was observed [148]. *Alms1*-disrupted mice also recapitulate the neurosensory deficits observed in humans with Alström Syndrome and their cochleae display signs of disturbed planar cell polarity abnormal orientation of hair cell stereociliary bundles which seemed to be prematurely lost when the mice grow older [149]. Alms1 has further been implicated in cell cycle control, and intracellular transport as well as the recycling endosome pathway [150]. To which extent disturbance any of these processes contributes to the renal phenotype still requires further investigation.

Joubert-Syndrome Related Disorders (JSRD)

Joubert syndrome (JS) is a very rare neurodevelopmental, mostly autosomal recessive but rarely X-linked disorder characterised by the so-called molar tooth sign (MTS). MTS represents a complex midbrain-hindbrain malformation visible on brain imaging. Anatomical correlate is hypodysplasia of the cerebellar vermis, abnormally deep interpeduncular fossa at the level of the isthmus and upper pons, and horizontalized, thickened and elongated superior cerebellar peduncles [151]. The estimated incidence is 1/80,000–1/100,000 live births. Clinically, neurological features such hypotonia at birth, ataxia, developmental delay, abnormal eye movements and neonatal breathing dysregulation are predominant but multiple extra-neurological symptoms occur. JS and Joubert Syndrome related disorders (JSRD) can be classified in six phenotypic subgroups: isolated JS; JS with ocular defect; JS with renal defect; JS with oculo-renal defects; JS with hepatic defect and JS with orofaciodigital defects.

JS/JSRD are genetically heterogeneous and there is marked phenotypical variability even within families. JS with renal defect (JS-R) is characterized by additional juvenile nephronophthisis but no retinal disease and mainly caused by mutations in *NPHP* and *RPGRIP1L*. JS with oculo-renal defects (JS-OR) occurs mainly due to mutations in *CEP290*, a transition zone protein encoding gene also found defective in BBS. Mutations in *TMEM67*, a gene also found to cause Meckel-Gruber Syndrome, have

been identified in JS with hepatic defects, chorioretinal or optic nerve colobomas and NPH, indicating that Meckel-Gruber Syndrome (MKS) can be clinically regarded as the severe end of the JS spectrum. Genetically, MKS is allelic to JSRD in 7 loci. JS with oro-facio-digital defects (JS-OFD) displays a slightly different phenotype from all of the above with bifid or lobulated tongue (sometimes referred to as hamartomas in the literature), multiple oral frenulae and mesaxial polydactyly with Y-shaped metacarpals and occurs as a consequence of mutations in *TMEM216*. Mutations in this gene can also cause isolated JS as well as MKS and mutations in *C5orf42* have recently been identified in subjects with an OFDVI phenotype in addition to subjects with isolated JS [152–155].

Many of the genes involved in JS/JSRD have been found to localise to the transition zone in cilia where they might exhibit a special gatekeeping function for ciliary protein entry [156]. For more details on the renal phenotype please refer to Chap. 13.

Ciliary Chondrodysplasias

An excellent overview of skeletal chondrodysplasias is given in a recent review article by Huber and Cormier-Daire [157]. Ciliary chondrodysplasias represent a genetically and phenotypically heterogeneous group of very rare (1:20,000 to <1:1,000,000), mainly autosomal-recessively inherited conditions with the major hallmark of skeletal involvement, mainly short/hypoplastic ribs and polydactyly, but also a variable degree of extraskeletal involvement. While *Short-Rib Polydactyly Syndrome* (*SRPS*) is inevitably lethal perinatally, 20–60% lethality has been reported for *Jeune Asphyxiating Thoracic Dystrophy Syndrome* (*JATD*). Polydactyly, a ubiquitous feature in SRPS, is rarely observed in JATD. Radiologically, trident acetabulum with spurs is a pathognomic sign for JATD but can also be observed in some SRPS cases as well as Ellis-van Creveld syndrome (EVC). In SRPS, extraskeletal manifestations such as cardiac defects, orofacial clefting, cerebral, renal, liver or pancreatic abnormalities as well has situs inversus have been observed. In JATD, up to 30% of the patients develop kidney disease, often accompanied by retinal degeneration. Elevated liver enzymes are common but only a handful cases of liver failure have been reported in the literature. Clinical hallmarks of JATD are shown in Fig. 9.8a–f.

JATD also shares clinical features with *Mainzer-Saldino Syndrome*, which can be described as JATD with obligate renal and retinal disease; both conditions are summarised under "cono-renal syndromes" as cone shaped epiphyses of the phalanges are frequently observed on X-rays [157]. Clinical hallmarks of Mainzer-Saldino-Syndrome are shown in Fig. 9.9a–h.

Mutations in genes encoding several components of the retrograde IFT motor complex dynein-2 such as *DYNC2H1*, *WDR34* and *WDR60* as well as the IFT-B component *IFT80* have been identified in patients with *JATD and SRPS*, indicating that these conditions are allelic and JATD represents the milder end of the SRPS phenotypic spectrum [56, 58, 60–62, 158, 159], Mutations in genes encoding retrograde IFT (IFT-A) components such as *IFT43* [67], *IFT121/WDR35* [65], *IFT122* [63], *IFT139/TTC21B* [68], *IFT144/WDR19* [64] and *IFT140* [20, 69] have likewise been found to cause ciliary chondrodsyplasias with renal involvement such as Sensenbrenner Syndrome/Cranioectodermal Dysplasia (CED), JATD/Mainzer-Saldino Syndrome and SRPS, and isolated Nephronophthisis (*WDR19*). Interestingly, in contrast to most JATD patients with mutations in genes encoding dynein-2 components who present with a severe thorax phenotype but usually preserved kidney function if they survive, the majority of patients described with mutations in IFT-A encoding genes as well as the IFT-B component IFT172 present with a milder thoracic phenotype but experience severe renal involvement with end-stage renal disease [1, 20, 21, 56–69]. In striking contrast to other ciliopathies such as BBS where up to two thirds of affected human subjects carry two null alleles (stop- or frameshift mutations), patients with ciliary chondrodysplasias usually harbour at least 1 missense

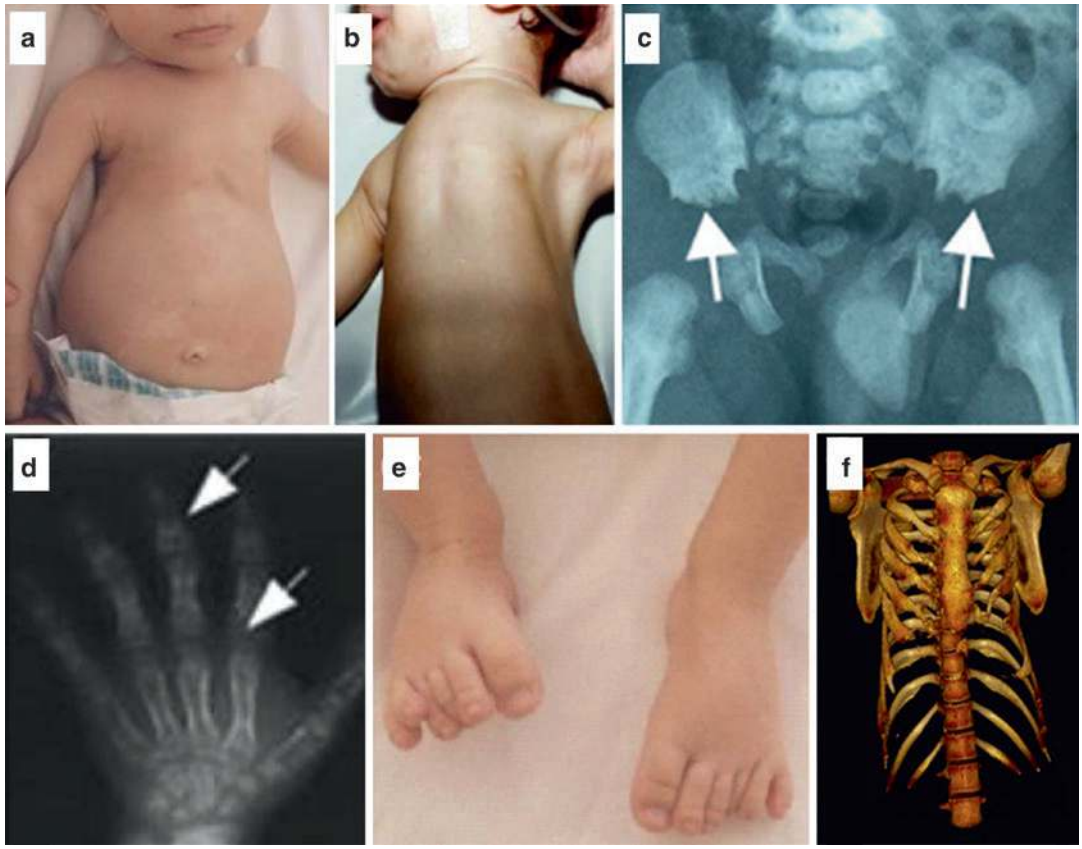

Fig. 9.8 (**a**–**f**) Clinical hallmarks of Jeune Syndrome. (**a**, **b**) Narrow thorax with short ribs. (**c**) Trident acetabulum with spurs. (**d**) Cone shaped epiphyses. (**e**) Polydactyly. (**f**) 3D-reconstruction from thoracic CT scan demonstrating long narrow thorax with short ribs. ((**a**, **e**) Used with permission from Halbritter et al. [1]; (**b**, **c**, **f**) Used with permission of BMJ Publishing Group from Schmidts et al. [21]; (**d**) Used with permission of John Wiley and Sons from Schmidts et al. [20])

allele. This is in line with findings from IFT-mutant mouse models where complete loss of IFT-protein function results in early embryonic death before midgestation (see also IFT section of this chapter) and one can assume that this also applies to humans, suggesting a more important role for IFT proteins compared to BBS proteins for ciliary function and embryonic development.

Likewise, human mutations have only been identified in two anterograde IFT (IFT-B) complex components to date: *IFT80* and *IFT172*. While *IFT80* mutations seem to cause a mild thoracic phenotype without extraskeletal involvement [57], *IFT172* mutations lead to a more complex phenotype with renal, liver and retinal involvement [1]. This might be due a more central role of IFT172 within complex-B or could also result from additional roles of IFT172 outside of IFT. The fact that in humans, mutations in more genes encoding IFT-A than IFT-B components have been identified to date again could point to a more essential role of IFT-B compared to IFT-A so that IFT-B mutations might be incompatible with embryonic development beyond very early stages. See also Fig. 9.6a, b for a visualisation of IFT defects observed in fibroblasts from ciliary chondrodysplasia patients.

SRPS can also be caused by mutations in the serine-threonine kinase *NEK1* [160] which acts within the DNA damage response pathway [161].

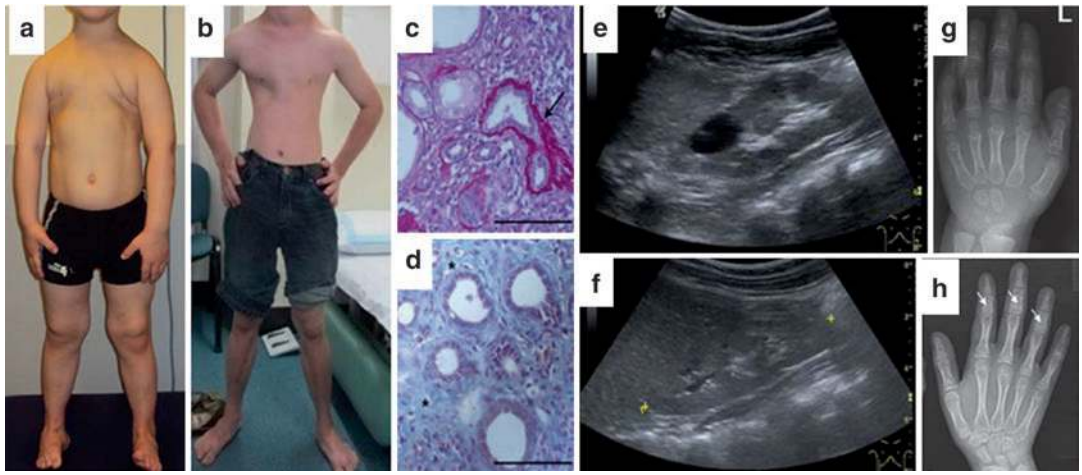

Fig. 9.9 (**a**–**h**) Clinical hallmarks of Mainzer-Saldino-Syndrome. (**a**, **b**) Mildly narrowed thorax. (**c**, **d**) Histological pictures of a renal biopsy. (**e**, **f**) Ultrasound images showing increased echogeneity and small renal cysts. (**g**, **h**) Cone shaped epiphyses. ((**b**–**h**) Used with permission of Elsevier from Perrault et al. [69]; (**a**, **e**–**g**) Used with permission of John Wiley and Sons from Schmidts et al. [20])

In humans affected by SRPS, kidney function cannot be followed due to neonatal death from respiratory failure, but mice carrying mutations in *Nek1* exhibit cystic renal disease [162]. Nek1 binds to the kinesin-2 component Kif3a [163], and kidney specific loss of Kif3a causes cystic disease [54]. Moreover, NEK1 and TAZ proteins interact physically to maintain normal levels of Polycystin 2 [164], so it seems possible that surviving patients would present with cystic or nephronophthisis-like renal involvement. Surprisingly, while impaired function of IFT proteins and Kif3a results in cystic kidney disease in mice, the combined knockout of *Kif3a* and *Pkd1* resulted in a milder rather than more severe phenotype, suggesting the existence of a cilia-dependent, as yet unidentified cyst growth promoting pathway [165]. No human mutations have been identified in *KIF3A* or other components of the kinesin-2 complex to date; presumably such mutations would lead to an early embryonic lethal phenotype.

Although mutations in some genes seem to be able to cause both JATD and *Sensenbrenner-Syndrome/CED* as mentioned above, the clinical phenotype is slightly different in CED where additional ectodermal defects such as dysplastic finger- and toe-nail, sparse and slow growing hair and teeth abnormalities are frequently observed. The thoracic phenotype is usually milder than in JATD and polydactyly not usually observed; however, craniosynostosis has been described and human subjects frequently present with renal involvement [166]. Clinical hallmarks of Sensenbrenner syndrome are shown in Fig. 9.10a–c.

While the skeletal features observed in skeletal chondrodysplasias, especially polydactyly, point towards imbalances in the hedgehog pathway as a molecular origin of disease, the molecular pathogenesis of renal disease in subjects with IFT and dynein-2 mutations has remained elusive as neither hedgehog- nor wnt signalling defects could be established as causative for the kidney phenotype in mouse models (see the IFT and Hedgehog Signalling sections of this chapter for more details). However, given the NPHP-like phenotype observed, hippo signalling might be a good candidate pathway leading to the renal phenotype in this group of ciliopathies.

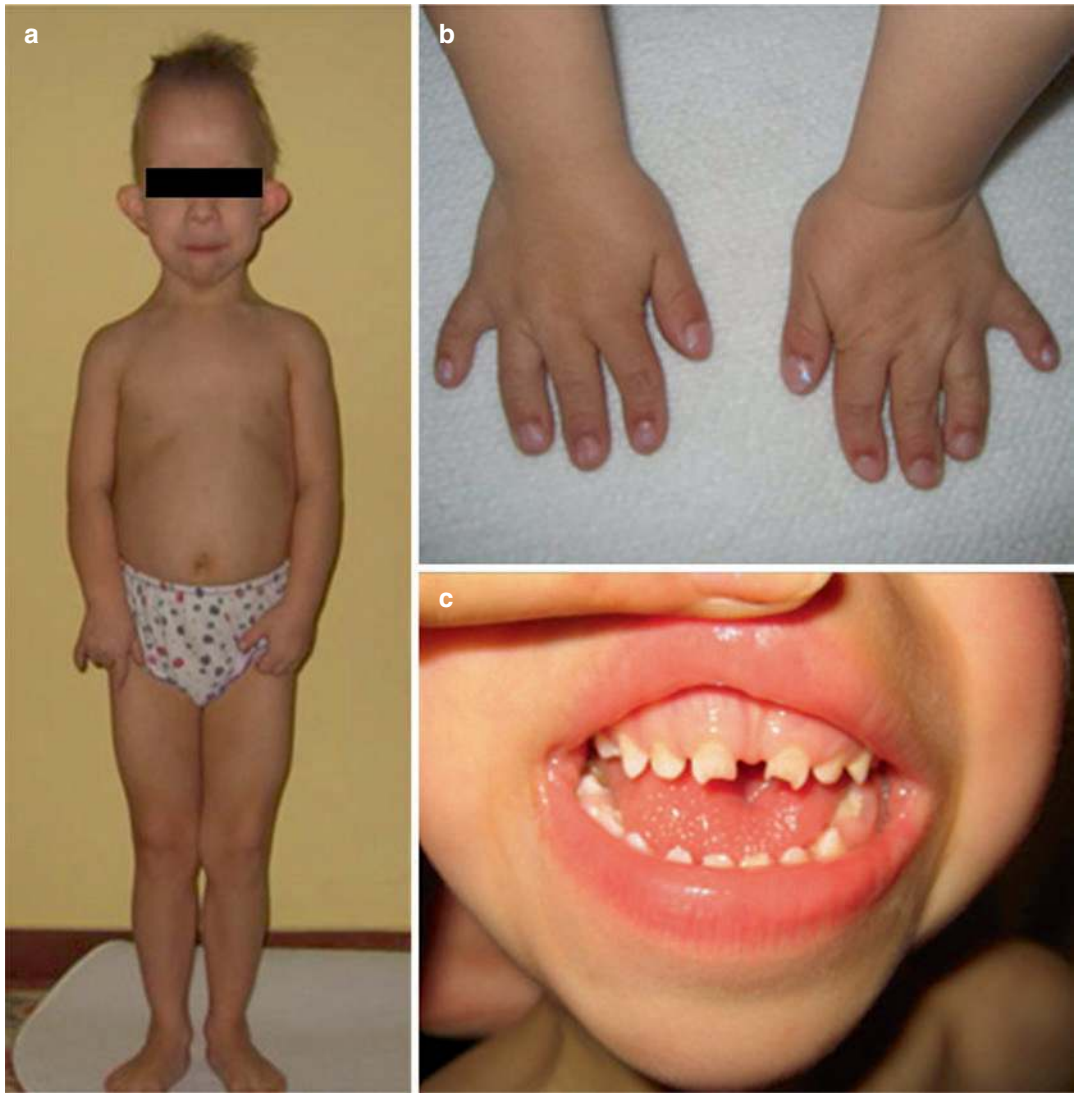

Fig. 9.10 (**a**–**c**) Clinical hallmarks of Sensenbrenner Syndrome (CED). Four-year-old girl displaying short extremities, mildly shortened and narrowed thorax, thin sparse hair, dolichocephaly, prominent forehead, full, hypertelorism, small flat nose, prominent auricles (**a**). (**b**) Brachydactyly. (**c**) Small abnormally shaped teeth. (Used with permission of Elsevier from Walczak-Sztulpa et al. [63])

Summary and Conclusion

Cilia are antenna-like structures projecting from most cells and hundreds of years after their first notion, we begin to acknowledge some of their essential function in human development, including the kidney. Extensive phenotypic and genetic heterogeneity has created a slightly chaotic picture of ciliopathies in the past and many aspects of these complex inherited conditions have remained unclear. Despite linking ciliopathies to imbalances in multiple fundamental cell signalling pathways, the molecular basis of disease, especially kidney mal-development, is still largely elusive to date.

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Polycystic Kidney Disease: ADPKD and ARPKD

10

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Introduction

Polycystic kidney diseases (PKD) are among the most common causes of chronic kidney disease (CKD) and end stage kidney disease (ESKD) both in children and adults [1]. The two main forms of genetic cystic kidney disorders are Autosomal Dominant and Autosomal Recessive Polycystic Kidney Disease (ADPKD and ARPKD). ADPKD is a disorder mainly manifesting at adult ages and is oligosymptomatic in childhood, although it is nowadays accepted that cysts may be present even prenatally and early pediatric involvement raises increased attention [1, 2]. In contrast, ARPKD is usually presenting in early childhood. However, mild phenotypes with disease onset at adult age have been reported anecdotally [1, 3, 4]. Recent cell biological and clinical research approaches have considerably expanded our knowledge on both PKDs. Still, many important questions remain to be solved. This chapter aims to give an overview of the current knowledge of ADPKD and ARPKD with a special focus on pediatric clinical aspects. For genetics we focus on the main genes, but would like to emphasize that there is a growing list of genes that when mutated may mimic ADPKD or ARPKD.

Classification and Differential Diagnosis of Cystic Kidney Diseases

In their seminal studies, Osathanondh and Potter systematically classified cystic kidney diseases into four distinct types [5]. While this historical classification has had a great impact for concise pathoanatomical description, it is hard to reconcile with our current understanding of clinical and genetic entities. Accurate diagnosis is essen-

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tial both in the management of patients with PKD and in counselling their families. Notably, cystic kidneys are an important feature of numerous genetic syndromes, including both dominant disorders like tuberous sclerosis complex (TSC) and recessively inherited diseases such as Joubert syndrome.

Clinical Aspects Guiding Diagnosis in Patients with Kidney Cysts

The differential diagnosis of cysts in the kidney is broad and in addition to ARPKD and ADPKD includes, e.g. single simple cysts, multicystic dysplastic kidneys and cystic dysplasia, nephronophthisis and other ciliopathies, as well as *HNF1B*-nephropathy or TSC. Six straightforward questions that can be answered by the patient and sonographic examination may be helpful to narrow down a potential clinical diagnosis [6].

In a first general consideration, it is important to differentiate a **kidney with a single or a few cysts from a cystic kidney**. Simple kidney cysts are fairly common in elderly persons [7] and usually do not impose problems or require treatment. However, simple cysts are not common in young children and even a single cyst should raise suspicion in pediatric patients**. Family history** is important. Whereas only 10–20% of ADPKD patients lack a family history [8], ARPKD and nephronophthisis (NPH) are usually inherited recessively with healthy parents and are found more frequently in offspring of consanguineous couples. Kidney ultrasound in the parents or grandparents is a useful investigation in the evaluation of a child with early-onset cystic kidney disease of unknown origin. A third important question addresses the **age of the patient** at the time of clinical presentation. ARPKD fetuses and a minor subset of ADPKD patients may be identified with oligo- or anhydramnios already during pregnancy. Juvenile nephronophthisis (NPH) classically presents with polyuria and polydipsia in school children. Most ADPKD patients are adults. While cysts in the kidney may appear during childhood or even prenatally in ADPKD and can be found by ultrasound, most children do not yet show clinical symptoms. Fourthly, the **localization of cysts** may give a hint. Cysts in NPH are found at the cortico-medullary border, whereas in ADPKD cysts localize to all parts of the kidney. **Kidney and cyst size** can also point to the correct diagnosis. Kidneys in NPH tend to be normal-sized or small, whereas ARPKD and ADPKD kidneys are large. Cysts are typically tiny in early stages of ARPKD, but may resemble large ADPKD macrocysts during the course of the disease. Finally, cystic kidney disorders may be accompanied by **extra-renal symptoms**, such as retinitis pigmentosa presenting with initial night blindness later progressing to almost complete loss of vision in case of syndromes accompanied by NPH, liver cysts in ADPKD and congenital hepatic fibrosis in ARPKD.

With these six pieces of information at hand, a potential diagnosis can be made clinically in many cases. Nonetheless, clinical diagnosis can be challenging due to overlapping syndromes and symptoms, and establishing a confirmatory genetic diagnosis can be helpful.

General Considerations on Genetic Diagnostics for Polycystic Kidney Disease

Marquardt first postulated genetic heterogeneity of polycystic kidney diseases when stating in 1935: “In surviving individuals, cystic kidneys are inherited dominantly. In non-viable individuals, cystic kidneys are recessive.” [9]. It took more than 35 years from that point of view before Blyth and Ockenden demonstrated in a systematic analysis that age at presentation alone is not a reliable criterion for defining genetic heterogeneity [10].

Indications for genetic testing and recommendations for preferred testing strategies have changed significantly over recent years. In all patients with PKD manifesting prenatally or in early childhood, genetic testing is highly recommended as a first-line diagnostic procedure as part of the initial patient evaluation [11]. Testing is not required in children with a single cyst,

absent extra-renal anomalies and a negative family history of ADPKD, but may be indicated in children with progressive disease indicated by kidney cysts increasing in size or number.

Genetic testing may be recommended due to the following aspects: The high gene detection rate in cystic kidney diseases allows to rapidly establish a definite diagnosis and avoid a "diagnostic odyssey" with unnecessary diagnostic measures for the majority of patients. To establish a definite diagnosis often is of **psychological benefit** for patients and families. Knowledge of the genotype may point to renal and **extra-renal comorbidities**, which would otherwise have taken considerably longer to diagnose and may benefit from early detection and disease monitoring (e.g. diabetes mellitus in *HNF1B* disease). Valid information on the recurrence **risk for future children** or other family members is only possible with knowledge of the genotype. The genotype can also be relevant for the inclusion of patients in clinical trials and the future choice of treatment options (see below).

Professional genetic counseling is highly recommended due to variable expressivity and the variety of extra-renal features seen in patients with cystic kidneys. It can also address the complex aspects of prenatal testing and preimplantation diagnostics in line with regional practices and regulations. Given the large clinical and genetic heterogeneity and vast pleiotropy, a **comprehensive gene testing** approach is recommended for cystic kidney diseases. A stepwise approach might only be indicated in a minority of patients in which there is clear phenotypic evidence for a specific disease, such as von Hippel-Lindau syndrome for which only one single (small) gene is known and variant detection rate is high.

Whatever primary strategy is chosen, an expanded gene panel or exome sequencing, the testing approach should be able to detect copy-number variations (CNVs) (e.g., deletions account for 50% of anomalies in *HNF1B*) and to cover complex genomic regions such as in *PKD1* [12]. Analysis of the *PKD1* gene is most complicated and needs expert knowledge due to genomic duplication of the first 33 exons at six other sites on chromosome 16p. Many of these pseudogenes are expressed as mRNA transcripts, but probably do not encode proteins. Both *PKD1* and *PKD2* variants are scattered throughout the genes' coding regions exhibiting marked allelic heterogeneity, with most variants being unique to single families ('private' variants) [1, 8, 13].

Ethical concerns exist on the question whether or not an ADPKD diagnosis should be actively sought in asymptomatic children. This includes genetic screening in addition to ultrasound examination. There is consensus on the need for early monitoring of modifiable risk factors (hypertension, proteinuria) and of early treatment of potential complications in children at risk for ADPKD [14]. As there is currently no established disease-controlling targeted treatment that would need to be started during childhood, it has, however, been argued that one should not take the right of self-determination from these children before these individuals are old enough to decide on their own what they consider best for them. Next to the last KDIGO recommendations, a recent consensus has highlighted the need of counselling these families and involving them in a process of shared decision-making between the child, parents and physicians [14, 15]. An algorithm has been proposed to facilitate the counseling of these ADPKD families in accordance to their own decision [16].

Autosomal Dominant Polycystic Kidney Disease (ADPKD)

Epidemiology and Morphology

ADPKD is the most common inherited kidney disease and one of the commonest Mendelian human disorders overall with a frequency of 1/500–1000 [1, 15, 17]. This approximates to about 12.5 million affected individuals worldwide. ADPKD is among the most common causes of ESKD; about 5–10% of all patients requiring kidney replacement therapy (KRT) as kidney transplant or dialysis are affected by ADPKD. Overall, the disease is a major health care issue of socio-economic interest.

Histopathologically, kidney cysts are fluid-filled epithelia-lined cavities. ADPKD is characterized by the formation and progressive enlargement of kidney cysts from all segments of the nephron and additional kidney fibrosis. In contrast to ARPKD in which the cysts usually remain connected with the tubular lumen, cysts in ADPKD become disconnected from the tubular space. Kidney cysts in ADPKD vary considerably in size and appearance, from a few millimeters to many centimeters (Fig. 10.1).

ADPKD is a systemic disorder with profound extra-renal cystic and non-cystic complications. Their prevalences in adults compared to children are summarized in Table 10.1.

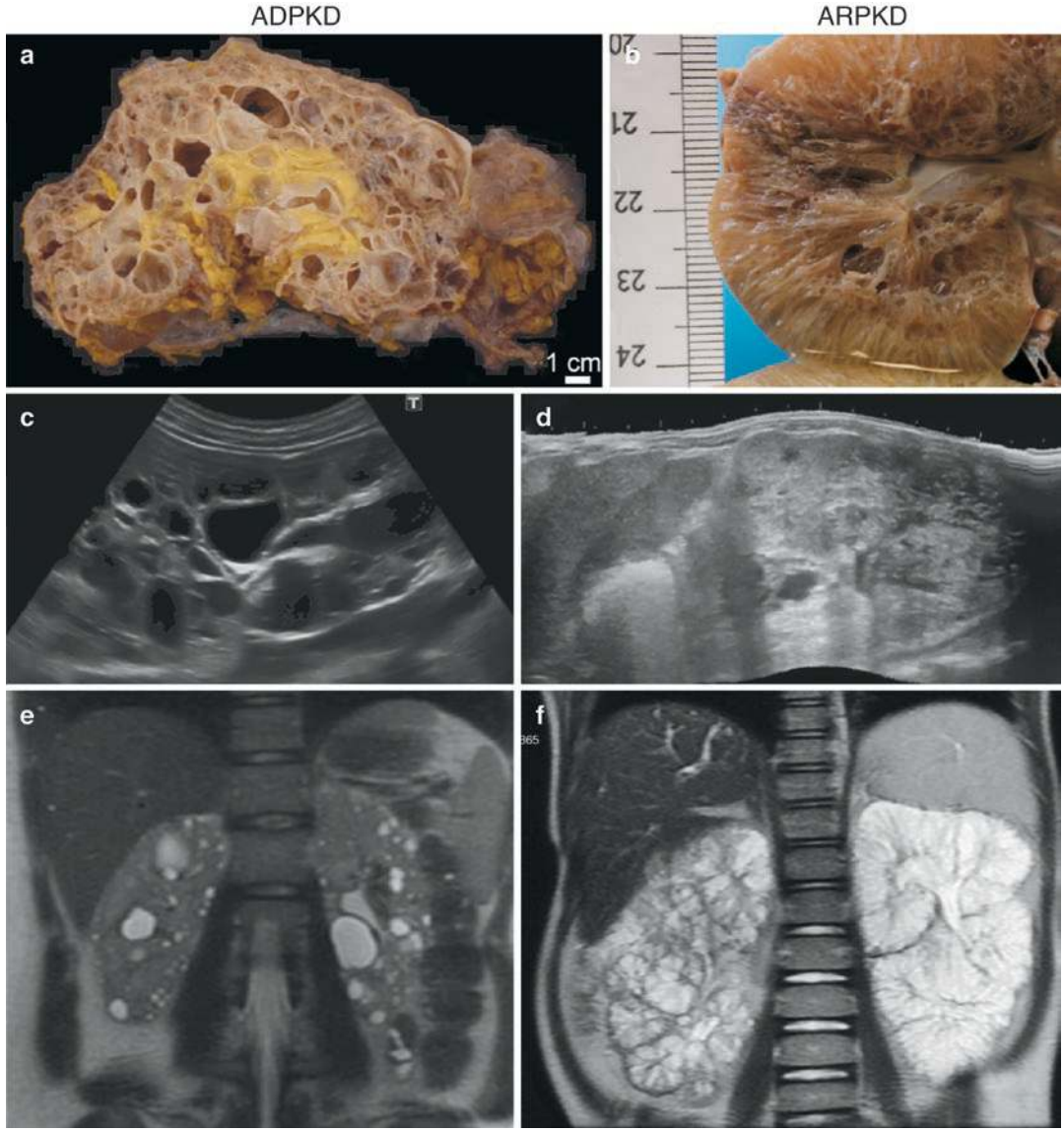

Fig. 10.1 (**a**) Macroscopic appearance of advanced-stage ADPKD (**a**) and ARPKD kidneys (**b**). On cut section, multiple cysts in the cortex and medulla can be seen that vary considerably in size and appearance, from a few millimetres to diameters of many centimeters in the ADPKD kidney, while ubiquitous small dilatations can be seen in the ARPKD kidney. These findings can also be recapitulated by ultrasound (**c**, **d**) and T2-weighted magnetic resonance imaging (MRI; **e**, **f**). (From Liebau and Serra [6] with permission)

Table 10.1 Extra-renal manifestations of ADPKD (according to [2, 198])

Manifestation	Prevalence in adults	Prevalence in children
Hepatic cysts	>90%	<5%
Arterial hypertension	Up to 70%	20–35%
Left ventricular hypertrophy	Up to 70%	
Valvular abnormalities	Up to 20%	12%
Intracranial aneurysm	6–16%	Rare
Abdominal aorta aneurysm	5–10%	
Diverticula	Up to 40%	
Hernias	Up to 45%	16%
Bronchiectasis	Up to 37%	
Genitourinary cysts	39–60%	
Depression	Up to 60%	
Pain	Up to 60%	10–20%

Clinical Course

Kidney Function

The kidneys are usually in the center of disease burden in ADPKD. A common classification differentiates a PKD1 subtype of patients with variants in the *PKD1* gene from a PKD2 subtype of patients with variants in *PKD2* [1]. ESKD presents in about 50% of ADPKD patients by the age of 60 years. On average, PKD2 is regarded to be significantly milder than PKD1 with a 20 years later median age of onset of ESKD (58.1 vs. 79.9 years) and a lower prevalence of arterial hypertension and urinary tract infections [1, 18].

Diagnosis by Imaging and Emerging Imaging Markers Predicting Clinical Courses

ADPKD diagnosis is usually established by ultrasound. Whereas 95% of patients exhibit characteristic ultrasonographic features at 20 years and almost all patients at 30 years of age, the proportion of affected children that can be identified by ultrasound is less clear. Even a single kidney cyst in a pediatric patient should raise suspicion and result in a diagnostic work-up encompassing a careful record of family and medical history, physical examination and, where required, further abdominal imaging [7, 14]. As novel treatment approaches are emerging it may become more important to identify ADPKD patients at an early stage of disease [16]. Various early markers of kidney disease are under investigation [19]. Approximately 60% of children younger than 5 years with a *PKD1* variant and 75–80% of those aged 5–18 years have kidney cysts detectable by ultrasound [20]. In the early 1990s, Bear et al. proposed a false negative diagnosis rate by ultrasound of about 35% below the age of 10 years [21]. The false positive yield of ultrasound is considered extremely low since simple cysts are extremely rare in childhood [22]. Ravine et al. found nil prevalence of cysts in healthy individuals aged 15–29 years [23].

Especially in children with a family history, ADPKD will be the most likely underlying condition for a child with kidney cysts. In adults aged ≤39 years with a family history of ADPKD, the diagnosis of ADPKD can be established by the presence of three uni- or bilateral kidney cysts [24]. Two cysts on each side for patients aged 40–59 years and four cysts on each side for patients aged >60 years make ADPKD the very likely diagnosis according to the modified Ravine criteria. The presence of less than two cysts in persons at risk aged >40 years practically excludes ADPKD. In 420 children with a family history of ADPKD, ultrasound screening detected kidney cysts in 46% of individuals at the age of 15 years [25].

Children affected by ADPKD also exhibit enlarged kidneys with an increased rate of kidney growth. ADPKD children who are hypertensive have larger kidneys than normotensive patients [26–28].

Magnetic resonance imaging (MRI) and computed tomography (CT) have higher detection rates, especially of small cysts. Given this, MRI may be a helpful tool to discover small cysts, e.g. to detect affected persons prior to living kidney donation, but unmodified application of the Ravine criteria to MRI and CT data would frequently yield false-positive results [29, 30]. Novel high-resolution ultrasound equipment may also be more sensitive in detecting small cysts. New reference datasets have therefore been

established for MRI-based cyst detection, revealing at least one cyst in about 60% of healthy adults [30]. Men appear to have more cysts than women and cysts grow with age by size and number.

A study by the CRISP (Consortium for Radiologic Imaging Studies of Polycystic Kidney Disease) consortium on ADPKD patients suggested that total kidney volume (TKV) as measured by MRI predicts kidney function decline in ADPKD. Total TKV values should be standardized to body height, especially in children. Height-adjusted TKV is considered to be a reasonably likely surrogate parameter for disease severity in ADPKD, even before kidney function declines [6].

The CRISP consortium used annual MRI as well as GFR measurements to monitor kidney and cyst volume in 232 young ADPKD patients with a GFR of >60 mL/min/1.73 m^2 over a period of 3 and 8 years [31]. The progression of cyst growth and kidney volume occurred prior to functional impairment. Rapid kidney growth was associated with faster decline in kidney function and lower kidney blood flow [31, 32]. Kidney blood flow and age as well as gender, hypertension, and kidney volume predicted the loss of kidney function [33]. In addition to height-adjusted TKV, novel radiomics approaches like image texture analysis may become an important tools to monitor the disease course and the response to interventions and to predict kidney function decline in ADPKD [34].

The applicability of MRI and CT for disease monitoring in children is limited. MRI requires sedation, and CT is associated with high radiation exposure. For daily clinical work, ultrasound remains the method of choice [6, 7], even more so as the resolution of modern ultrasound equipment is excellent. Recently, Breysem et al. validated 3D ultrasound for measuring TKV in children compared to the 2D ultrasound ellipsoid method and MRI-based volumetry in a pediatric ADPKD cohort [35]. 3D ultrasound manual contouring volumetry outperformed the 2D ellipsoid method and was comparable with MRI volumetry in children, especially for smaller kidneys. While these findings require further validation, 3D-ultrasound holds promise as a potential alternative to MRI in pediatric imaging assessment.

Early-Onset ADPKD and Clinical Spectrum of Kidney Disease in Pediatric ADPKD

Clinical symptoms of ADPKD usually do not arise before the fourth to fifth decade of life. However, there is striking phenotypic variability not only between but even within families, indicating that modifying genes, environmental factors and/or other mechanisms considerably influence the clinical course in ADPKD [36, 37]. In line with this, a small proportion of ADPKD patients presents with an early-manifesting clinical course [1]. Early manifestation in ADPKD is usually defined as clinical symptoms (e.g., arterial hypertension, proteinuria, impaired urinary concentration, impaired kidney function) occurring before the age of 15 years. Among these are cases with significant peri-/neonatal morbidity and mortality, sometimes indistinguishable from those with severe ARPKD [2]. Conflicting data exist on the precise incidence of early-manifesting ADPKD cases. While most authors propose a figure of about 2%, Sweeney and Avner suggested a prevalence of up to 5% [38, 39]. Given the prevalence rates for ADPKD (1/400–1000) and ARPKD (1/20,000), it is plausible that the total number of patients with early-onset ADPKD seen in pediatric nephrology clinics may be comparable to those of the children with ARPKD. A subset of these ADPKD patients may resemble ARPKD. Importantly, variants in *TCF2*/*HNF1B*, which initially were mainly found in children with bilateral cystic dysplasia [40], can also result in PKD-mimicking phenotypes [11, 41].

Longitudinal studies in children with ADPKD demonstrated that severe kidney enlargement at young age and hypertension are risk factors for accelerated kidney growth [14, 26, 27, 42]. Many clinical symptoms such as pain, hematuria, proteinuria, stones and hypertension are associated with large kidney size. Furthermore, a large cyst number in early childhood is a predictor for faster progression of structural anomalies.

Intriguingly, in children with ADPKD, kidney involvement is commonly asymmetric (including

asymmetric kidney enlargement) and even limited to one kidney in a small minority of cases at early stages of the disease [43]. As in ARPKD, the kidneys can present as large and hyperechoic bilateral masses with decreased corticomedullary differentiation [44, 45]. Unlike ARPKD, ADPKD kidneys are frequently characterized by greatly variable macrocysts (up to several centimeters in diameter) even in small children [6, 7].

Gross hematuria is a risk factor for the progression of kidney disease [46, 47]. Hematuria is not common in children and occurs in only about 10% of ADPKD children at a mean age of 9 years [26].

Arterial Hypertension

Arterial hypertension is common in pediatric ADPKD patients even in the presence of normal kidney function and particularly in children with very early onset ADPKD [47]. Hypertension should be diagnosed as early as possible and be treated aggressively [14, 15].

Cardiovascular disease represents the main cause of mortality in ADPKD. The onset of hypertension before age 35 years constitutes an important prognostic factor of rapid disease progression [46]. In children with ADPKD the prevalence of hypertension is between 20% and 40% [14, 48, 49]. 24-Hour ambulatory blood pressure monitoring (ABPM) represents the gold standard in evaluating hypertension in pediatric populations. ABPM in children with ADPKD revealed isolated nocturnal hypertension with normal daytime blood pressure in 16–18% of patients [11, 48].

The precise pathogenesis of hypertension in PKD still remains to be elucidated. It has been hypothesized that cyst expansion results in intrarenal activation of the renin-angiotensin-aldosterone system (RAAS) with stimulation of cyst growth, fibrosis, and hypertension [50].

Extra-Renal Manifestations

Intracerebral aneurysms (ICAs) are an important, specific cardiovascular comorbidity in ADPKD and are associated with a five-fold higher prevalence than in the general population. The risk to develop ICAs is higher in patients with a family history of ADPKD–associated ICA or subarachnoid hemorrhage (22% versus 6%) [51]. However, PKD associated intracranial aneurysms are extremely unusual in childhood and routine screening is not recommended [52].

Simple, mostly solitary hepatic cysts are common with a prevalence of 2.5–10% in the general population [53]. Women may be more often and more severely affected, especially those who used estrogens, had multiple pregnancies or both [54, 55]. Hepatic cysts can be predominant with multiple cysts throughout the liver in the presence of very few kidney cysts. Atypical or alternate disease including *GANAB*, *ALG9* variants in the case of liver cysts or *HNF1B* with significant pancreatic involvement are also to be considered [56].

Other anomalies may occur including mitral valve prolapse (usually without clinical significance), left ventricular hypertrophy, aneurysms of the abdominal aorta, diverticular disease, hernia, chronic back pain, cystic lesions in the genitourinary tract, the pancreas and the lung, hematuria, urinary tract infection, kidney stones, deregulated phosphate homeostasis, and arachnoidal cysts as well as pain and depression [18, 54] (Table 10.1). Extra-renal manifestations overall are rare in children with ADPKD.

ADPKD Genetics

As the name implies, ADPKD is transmitted in an autosomal dominant fashion, i.e., virtually all individuals who inherit a mutated *PKD1* or *PKD2* germline allele will develop kidney cysts by age 30–40. The majority of ADPKD patients are adults who carry a germline variant in the *PKD1* gene on chromosome 16p13.3 (~80%). About 10–15% harbor a variant in the *PKD2* gene on chromosome 4q21 [1, 18]. The majority of patients with ADPKD are explained by heterozygous variants in either *PKD1* or *PKD2*. However, there is a growing list of genes that when mutated either mimic ADPKD or give rise to more atypical ADPKD phenotypes (*GANAB*, *DNAJB11*, *HNF1ß*, *PKHD1*, *DZIP1L*, *TSC1/2*, *VHL*, *ALG9*, *IFT140*, *OFD1* in women, etc.).

Remarkably, ADPKD variants can occur *de novo* without a family history. This frequently results in some kind of mosaicism and the clini-

cal course may be atypical. In patients with early and severe disease manifestations (discussed in greater detail above), pathogenic *PKD1* variants can affect both disease alleles in a recessive mode of inheritance.

PKD1 and *PKD2* Genes and Their Encoded Polycystin-1 and -2 Proteins

PKD1 is a large gene with a longest open reading frame transcript of 46 exons predicted to encode a 4302 aa multidomain integral membrane glycoprotein (polycystin-1). *PKD2* has 15 exons encoding a 5.3 kb transcript that is translated into a 968 aa protein (polycystin-2). In keeping with the systemic nature of ADPKD, the two polycystins are widely expressed in tissues. The expression of polycystin-1 and -2 is developmentally regulated with highest levels during late fetal and early neonatal life. Intra-renal expression is highest in distal tubule and collecting duct epithelial cells. Notably, the majority of cysts in ADPKD originate from collecting ducts.

Although the two-hit model of tumorigenesis is too simplistic, it may still provide a reasonable basis of our understanding of ADPKD. According to this data, second-hit variant and resulting loss of heterozygosity has been proposed as the mechanism underlying cyst formation in ADPKD. The considerable intrafamilial phenotypic variation, focal cyst formation with evidence of epithelial cell clonality within individual cysts, as well as the detection of somatic variants in cells lining kidney and liver cysts are all in keeping with this theory [57–61]. However, numerous findings rule out the two-hit model of a germline variant on one allele and a somatic variant on the other as the sole cause of cystogenesis. Patients and mice have been described that carry germline variants in both *PKD1* and *PKD2* (double-heterozygotes) and, thus, are to be regarded as "homozygously" affected in every cell of the organism [62, 63]. In contradiction to a simple two-hit theory, not every renal tubular cell or nephron in these individuals may give rise to a cyst. Therefore, the second-hit variant to the other *PKD* gene may act as a modifying factor that boosts the risk of cyst development and/or drives cyst progression, rather than initiating cyst events [64]. As regards mechanisms underlying cystogenesis, it is worth noting that increased as well as decreased polycystin-1 expression may result in cyst formation [1, 65, 66]. Haploinsufficiency of *Pkd1* itself has been demonstrated to suffice to elicit a cystic phenotype [67]. Finally, the timepoint of *Pkd1* inactivation crucially determines the severity of the cystic phenotype in mice [68]. Ischemia [69–71], nephrotoxic injury [72] and immunological events involving, e.g. macrophages [73, 74] may affect cystogenesis and it has been suggested that these events might represent a required third or even further additional hit [75].

Polycystin-1 and polycystin-2 are glycosylated integral membrane proteins (Fig. 10.2). Polycystin-2 has six transmembrane passes with cytoplasmic N- and C-termini. It is believed to function as a divalent cation channel, particularly involved in cellular Ca^{2+} signalling, belonging to the transient receptor potential (TRP) protein superfamily [76].

Polycystin-1 is a huge integral membrane glycoprotein with an extensive amino-terminal extracellular region, 11 transmembrane passes, and a short 200-amino acid cytoplasmic carboxy-terminus. The intracellular C-terminus is supposed to mediate protein interactions, by, e.g., a heterotrimeric G-protein activation site and a coiled-coil domain that interacts with the C-terminus of polycystin-2. The large extracellular portion of polycystin-1 contains numerous structural motifs, which are putatively involved in protein-protein or protein-carbohydrate interactions. It has been proposed that polycystin-1 and polycystin-2 form a chemo- and mechanosensing protein complex which senses fluid flow in the renal tubule and controls cell growth and differentiation [1]. Bending of the primary cilia leads to an increase in intracellular calcium potentially via a polycystin-1-dependent mechanism [77, 78] and the ratio of polycystin-1 to polycystin-2 regulates pressure sensing [79]. However, the exact mechanisms remain unknown [80, 81]. Polycystin-1 is involved in the regulation of multiple intracellular signalling pathways [1, 82–85]. Hogan et al. found urinary exosomes with abundant expression of polycystin-1, poly-

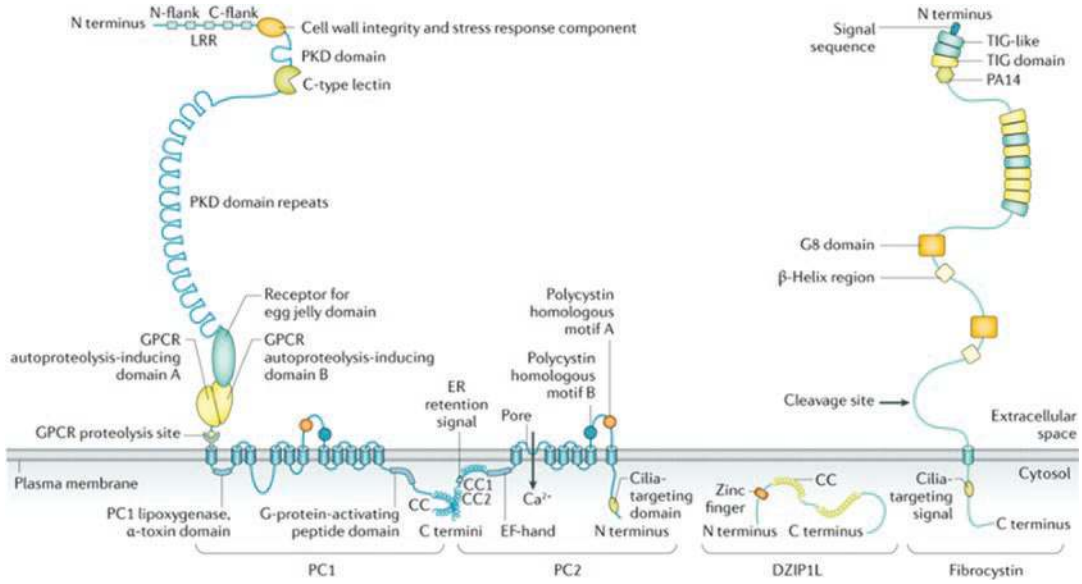

Fig. 10.2 Structures of polycystin-1, polycystin-2, polyductin/fibrocystin and DZIP1L. (From Bergmann et al. [1] with permission)

cystin-2 and the ARPKD protein fibrocystin among others, which may point to a role in paracrine signalling [86].

PKD1/*PKD2* Variant Spectrum and Routine Diagnostic Testing

Genotype-Phenotype Correlations

The wide range of age at attainment of ESKD observed within families illustrates the limitations of simple genotype-phenotype correlation analysis. There is no evidence for any sex influence in *PKD1*, however, females affected by *PKD2* were found to have a significantly longer median survival (71.0 vs. 67.3 years) than males. Another study corroborated these findings by a later mean age of onset of ESKD (76.0 vs. 68.1 years) in *PKD2* females [87, 88].

While no genotype-phenotype correlations have been identified for *PKD2* [8, 89], certain associations have been established in *PKD1*. Patients with a truncating variant have a more severe course than patients carrying a missense variant [90, 91]. *PKD1* missense variants however still go along with a more severe phenotype than *PKD2* variants. In addition, the presence of variants in multiple PKD genes can result in a more severe phenotype [92]. These patients were found to carry variants in PKD genes including *HNF1B* or a variant *in trans* affecting the other *PKD1* allele in addition to the expected germline variant [92]. However, this mechanism is unlikely to explain all cases with early-onset ADPKD. Variants 5′ to the median are associated with a slightly earlier age at onset of ESKD (53 vs. 56 years) [93]. Moreover, the median position of the *PKD1* variant was found to be located further 5′ in families with a vascular phenotype of intracranial aneurysms and subarachnoid haemorrhage [94]. At the population level phenotype findings do predict eGFR endpoints and ESKD, with genotype improving the predictive value of imaging findings and vice versa [95]. However, for genetic counselling and the prediction of the outcome of an individual patient, these genotype-phenotype correlations are only of limited value [96].

Data on families with early-manifesting offspring support a common familial modifying background for early and severe disease expression, but the underlying mechanisms are still controversial and may include anticipation, imprinting, and the segregation of modifying genes. Segregation of modifying alleles inherited

from the unaffected parent is an intriguing possibility [97] further supported by the low incidence of *in utero* presentation of ADPKD in second degree relatives. The mechanisms underlying early-onset ADPKD still require further examination [98].

Tuberous Sclerosis Complex and Contiguous TSC2–PKD1 Gene Deletion Syndrome: Important Differential Diagnoses of Cystic Kidney Disease

Tuberous sclerosis complex (TSC) is an autosomal dominant genetic syndrome characterized by marked variable expressivity and hamartomatous lesions in the brain, heart, lung, skin and kidney. Around 90% of patients suffer from epilepsy, and half exhibit cognitive impairment, autism or other behavioural disorders. Additional clinical manifestations of TSC may include sclerotic bone lesions, renal cell carcinoma (RCC) and neuroendocrine tumours [99]. Cutaneous manifestations also frequently occur with TSC and include, for example, "white spots", facial angiofibromas and peri-/subungual fibromas. About 80–90% of TSC patients have renal manifestations by adulthood, representing the second most significant cause of morbidity and mortality in all ages combined, and the most common cause of mortality after the age of 30 years. Renal lesions in TSC consist of renal cysts, angiomyolipomas (AML), fat-poor lesions, and RCC, causing CKD. There is a clear correlation between the presence of renal abnormalities (AML and renal cysts) and age. Renal lesions are observed in 38–55% of affected children at preschool age, increasing to 75–80% at school age and reaching 86–100% in adults [100]. Renal AML develop in up to 80% of individuals with TSC and appear typically first in childhood and then tend to grow during adolescence and into adulthood. The main complication of AML is retroperitoneal hemorrhage, which can be fatal due to the associated blood loss, pain, renal insufficiency, and arterial hypertension. Approximately 35–50% of patients with TSC develop multiple renal cysts [100, 101].

The incidence of TSC is 1 in 6000–10,000 live births, and its prevalence is independent of population, ethnicity and sex. TSC is caused by germline loss-of-function variants in *TSC1* (OMIM 605284; located on chromosome 9q34) or *TSC2* (OMIM 191092; located on chromosome 16p13.3), genes that encode hamartin and tuberin, respectively [101]. In the majority of patients, variants in these two genes arise *de novo*, however, the patients themselves who carry a pathogenic *de novo* variant in *TSC1* or *TSC2* bear a 50% risk to transmit the variant to their offspring. The two proteins hamartin and tuberin form a complex that negatively regulates mechanistic target of rapamycin (mTOR) complex 1 (mTORC1), a master regulator of cellular biosynthesis. Loss of the hamartin–tuberin complex results in aberrant activation of mTORC1, which promotes cell proliferation and differentiation. TSC-related cysts are thought to arise as a result of a second-hit variant in *TSC1* or *TSC2* in the renal epithelium, although genetic studies have yet to confirm this hypothesis [102].

A subset of patients carries a deletion encompassing the adjacent *TSC2* and *PKD1* genes (OMIM 600273, on chromosome 16p13) ("contiguous *TSC2–PKD1* gene deletion syndrome"). This disease was first reported by Brook–Carter et al. in 1994 with a variety of phenotypes dominated by severe, very early-onset of ADPKD and occurs in ~2–5% of TSC patients, resulting in significant kidney insufficiency already from childhood [102, 103]. Data on children diagnosed with the contiguous gene syndrome (*TSC2-PKD1*) and their long-term out-

come are scarce and currently no data on the epidemiology nor recommendations for the diagnostics, follow-up and treatment of *TSC2-PKD1* are available. The global ADPedKD initiative (www.ADPedKD.org) aims to remedy this paucity of information by collecting information on this subgroup [104].

The expansion of knowledge of the pathogenesis of TSC and the identification of the *TSC1* and *TSC2* genes and their proteins, the hamartin–tuberin complex led to the approval of mTORC1 inhibitors (also termed rapalogues), such as sirolimus and everolimus, for the treatment of the progression of subependymal giant cell astrocytomas, angiomyolipomas, skin lesions, epilepsy and lymphangioleiomyomatosis. In contrast, the efficacy of these agents in the treatment of TSC-associated renal cystic disease has not yet been fully evaluated [102].

Autosomal Recessive Polycystic Kidney Disease (ARPKD)

Epidemiology and Morphology

ARPKD is much rarer than its dominant counterpart with a proposed incidence among Caucasians of about 1 in 20,000 live births corresponding to a carrier frequency of approximately 1:70 in non-isolated populations [1, 105]. The exact incidence is unknown since published studies vary in the cohorts of patients examined (e.g., autopsied patients vs. moderately affected patients followed by pediatricians), and some severely affected babies may die perinatally without a definitive diagnosis. Isolated populations may have a higher prevalence. A recent study calculated an estimated annualized incidence of ARPKD in the USA to be 1:26,485 live births [106].

Macroscopically, the cut surface of ARPKD kidneys demonstrates the cortical extension of fusiform or cylindrical spaces arranged radially throughout the kidney parenchyma from medulla to cortex (Fig. 10.1). Histologic changes in ARPKD can vary depending on the age of presentation and the extent of cystic involvement. Invariable histological manifestations are fusiform dilations of renal collecting ducts and distal tubuli lined by columnar or cuboidal epithelium that usually remain in contact with the urinary system (unlike ADPKD), whereas glomerular cysts or dysplastic elements (e.g., cartilage, etc.) are usually not evident in ARPKD kidneys (Fig. 10.3). During early fetal development, a transient phase of proximal tubular cyst formation has been identified that can also be observed in various mouse models but is largely absent by birth [1, 107, 108]. With advancing clinical course and development of larger kidney cysts accompanied by interstitial fibrosis, ARPKD kidney structure may increasingly resemble the pattern observed in ADPKD.

Liver changes are obligatory for ARPKD and characterized by dysgenesis of the hepatic portal triad attributable to defective remodelling of the ductal plate with hyperplastic biliary ducts and congenital hepatic fibrosis (CHF) (Fig. 10.4). These hepatobiliary changes, subsumed as ductal plate malformation (DPM), are present from early embryonic development (first trimester) on and lead to progressive portal fibrosis [109]. At later stages, fibrous septa may link different portal tracts by intersecting the hepatic parenchyma often leading to portal hypertension. However, the remaining liver parenchyma usually develops normally and hepatocellular function initially often remains stable [110, 111]. As discussed for ADPKD, liver cysts usually arise from DPM and biliary ectasia, with transitions to extensive dilations of both intra- and extrahepatic bile ducts resembling Caroli's disease/syndrome.

Although ARPKD can be reliably diagnosed pathoanatomically, histology has lost relevance for daily clinical work with the increasing availability of genetic workup [11, 112, 113].

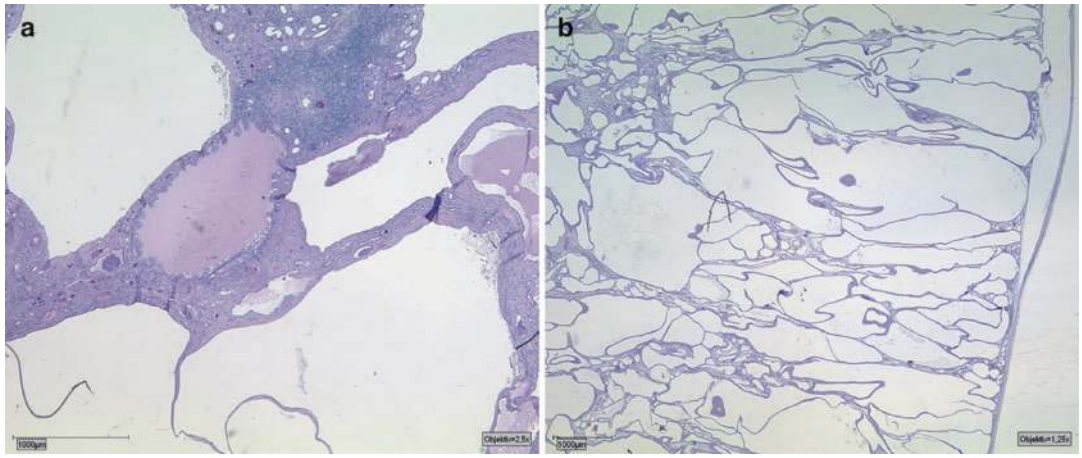

Fig. 10.3 Histological findings in ADPKD (**a**) and ARPKD (**b**) kidneys. ADPKD kidneys show a higher degree of tubuolinterstitial fibrosis. Fusiform dilations of renal collecting ducts and distal tubuli lined by columnar or cuboidal epithelium can be observed in ARPKD. These dilated collecting ducts run perpendicular to the renal capsule

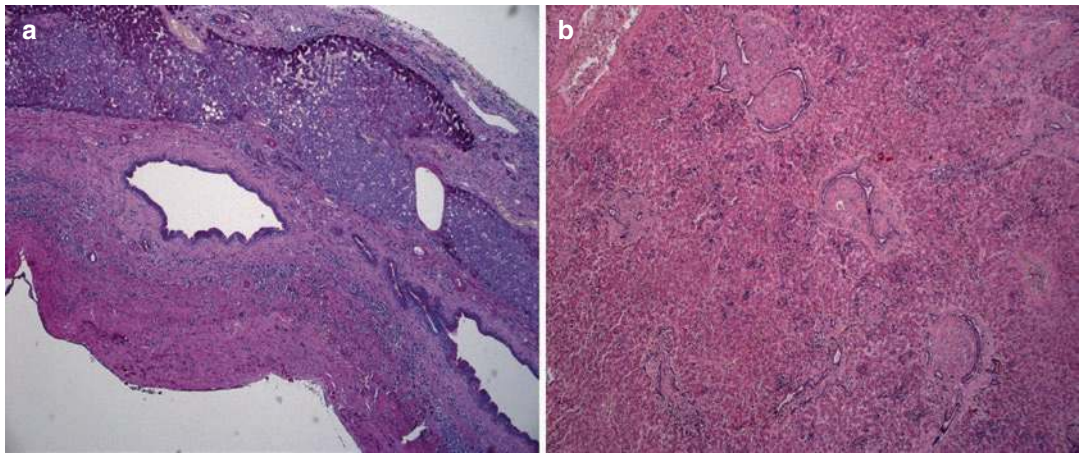

Fig. 10.4 Histological liver findings. Hepatic cysts (**a**) and the obligatory hepatobiliary changes in ARPKD subsumed as ductal plate malformation (DPM, **b**) characterized by dysgenesis of the hepatic portal triad with hyperplastic biliary ducts and congenital hepatic fibrosis (CHF)

Clinical Course and Prenatal Diagnosis

ARPKD is typically an infantile disease characterized by hepatorenal fibrocystic changes. Patients frequently present with pre- or perinatal detection of massively enlarged kidneys (Fig. 10.1, Table 10.2). Parents are typically not affected. In severely affected fetuses, oligo- or anhydramnios may develop that can result in pulmonary hypoplasia with severe perinatal respiratory disease. Despite dramatic advances in neonatal and intensive care over the past decades, the short-term mortality of ARPKD remains substantial. In less-severely affected children, progression of CKD is slow, resulting in an overall need for KRT in about 40–50% of patients in young adulthood [3, 114]. There is obligatory hepatic involvement in ARPKD resulting in portal hypertension and a risk of cholangitis.

Table 10.2 Clinical manifestations of ARPKD and ADPKD

	ARPKD	ADPKD
Incidence	1:20,000	1:500–1:1000
Macroscopic renal findings	Symmetrical, massively enlarged, reniform kidneys	Symmetrical, enlarged, reniform kidneys
Localization of renal cysts	Mainly dilated collecting ducts and distal tubuli	Cysts derived from all parts of nephron
Ultrasound and diameter of renal cysts	Increased echogenicity of renal parenchyma. "Salt-and-pepper"-pattern. Small, sometimes invisible cysts (<2 mm). More ADPKD-like pattern with avancing age	Cysts of different sizes in cortex and medulla. Usually several large cysts
Hepatic pathology	Mandatory: Ductal plate malformation/ congenital hepatic fibrosis with hyperplastic biliary ducts and portal fibrosis (may impress as Caroli syndrome)	"Liver cysts". Common in adults, rare in children. Occasionally ductal plate malformation/congenital hepatic fibrosis
Associated anomalies	Lung hypoplasia. Rarely pancreatic cysts. Single case reports of intracranial aneurysms	Pancreatic cysts and/or cysts in other epithelial organs. Familiarly clustered intracranial aneurysms, abdominal aorta aneurysms. Diverticula. Hernia. Bronchiectasis
Main clinical manifestations	Neonatal respiratory distress/failure due to pulmonary hypoplasia. Renal insufficiency. Portal hypertension. Hyponatremia. Hypertension	Arterial hypertension. Proteinuria. Hematuria. Arterial hypertension. Renal insufficiency. Pain
Risk for siblings	25%	50% (except in rare cases of spontaneous mutation with virtually no risk)
Risk for own children	<1% (unless unaffected parent is related to affected partner, or ARPKD is known in the unaffected partner's family)	50% (also for patients with spontaneous mutations)
Manifestation in affected family members	About 20% gross intrafamilial variability	Often similar within the same family
Parental kidneys	No alterations	Usually one affected parent (unless parents are <30 years or in case of spontaneous mutation)
Prognosis	Substantial mortality in patients with neonatal respiratory distress. Severe complications due to portal hypertension	Median age of end stage renal disease: 58.1 years (PKD1) vs. 79.9 years (PKD2)

Overall, the clinical spectrum is variable [115]. Notably, early studies differed widely by their selection criteria and their mode of data analysis, thus hampering direct comparison. Longitudinal registry studies with detailed characterization of patient courses have recently been established and provided important insights [106, 116]. Treatment in ARPKD remains largely symptomatic and opinion-based, although first consensus expert recommendations have been established [112].

Diagnosis by Imaging

Ultrasound remains the method of choice to diagnose children with ARPKD. Bilateral large and hyperechogenic kidneys with poor corticomedullary differentiation are the typical finding. Cysts are usually fusiform and tiny (<2 mm in diameter) and may impose as a 'pepper-salt' pattern on ultrasound. Macrocysts are uncommon in small infants, although they may be observed in advanced disease stages when ARPKD and ADPKD may become hard to differentiate by their sonographic appearance [7, 117–119]. In a large collection of ARPKD patients, 92% of ARPKD patients had a kidney length at or above the 97th centile for age [120]. In no case the kidney size was decreased and SD scores ranged from 0 to 17. In contrast to ADPKD, clear correlations were neither observed between kidney

length and kidney function nor between kidney length and duration of the disease. Various studies have found a relative decrease of kidney length during the course of the disease but numbers overall remain small and methods to quantify and standardize kidney size differed [121–124]. A recent study on 456 ARPKD patients from the international ARPKD registry study ARegPKD (www.ARegPKD.org) confirmed this finding but also revealed that height-adjusted estimated TKV remained rather stable over time [125]. Overall, there was an inverse relationship of height-adjusted estimated TKV with kidney function but with substantial variability. However, there was a clear correlation of early height-adjusted TKV (in the first 18 months) with poor kidney prognosis in childhood and adolescence (94% 10-year kidney survival for lowest quartile vs. 20% for highest quartile; Fig. 10.5) [125].

Typical ultrasound findings of the liver and portal hypertension in ARPKD include hepatomegaly, increased liver echogenicity, inhomogeneous liver parenchyma, liver cysts and dilated bile ducts, enlarged left liver lobes and splenomegaly [3, 111]. Some studies suggest that liver elastography, either by, e.g. magnet resonance techniques or by ultrasound Acoustic Radiation Force Impulse especially of the left liver lobe may be helpful in identifying liver fibrosis in patients with ARPKD [126–129]. In one cross-sectional study liver magnetic resonance elastography and ultrasound elastography measurement were strongly correlated and magnetic resonance elastography showed 78% sensitivity and 93% specificity to distinguish ARPKD and control groups at a proposed cut-off of 2.48 kPa [128]. Longitudinal data will be required to evaluate the prognostic value of these measurements for liver outcome.

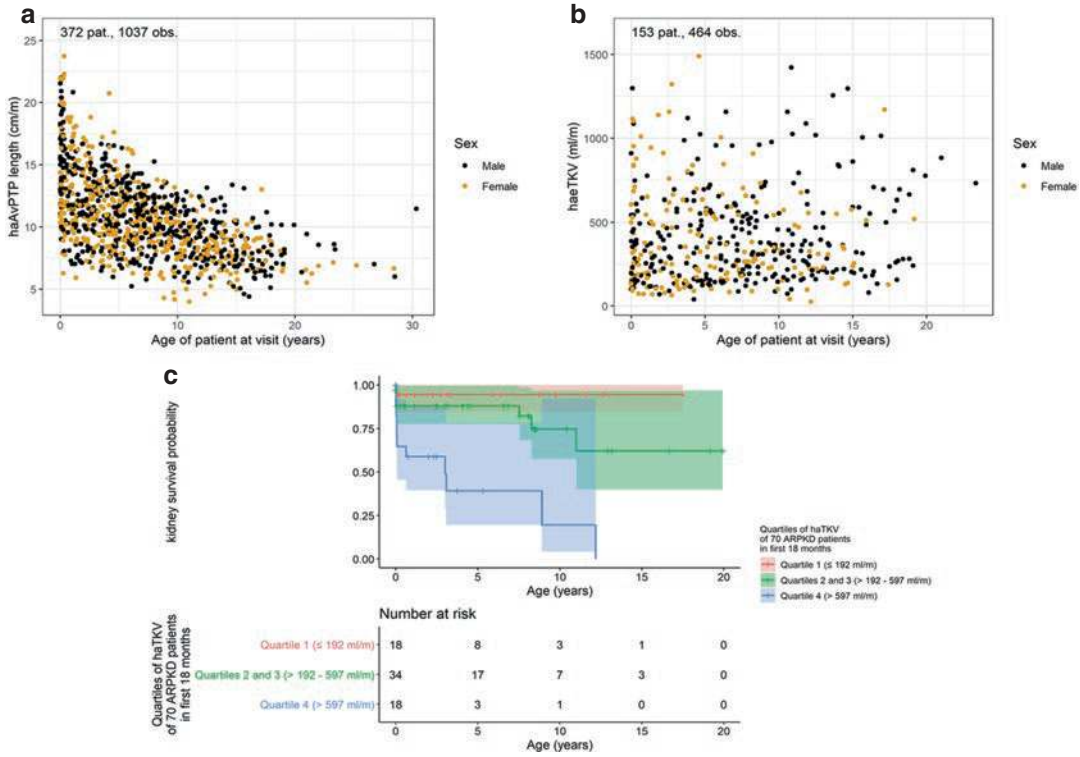

Fig. 10.5 Development of height-adjusted pole-to-pole length (**a**) and height-adjusted total kidney volume (**b**) in relation to age and kidney survival in patients in the different quartiles of height-adjusted total kidney volume (**c**). (Modified from [125])

Perinatal Disease and Mortality

Overall, the majority of cases are identified late in pregnancy or at birth. As many as 30% of affected neonates have been reported to die shortly after birth from respiratory insufficiency. Early detection of oligohydramnios seems to be associated with worse outcome [130]. In severe cases fetuses may display a "Potter" oligohydramnios phenotype with pulmonary hypoplasia (Fig. 10.6), a characteristic facies, and contracted limbs with club feet. A study performed from 2010 to 2014 in the ultrasound estimated neonatal survival to be almost 80% [106]. Advances in neonatal intensive care and improvements in kidney replacement therapies have increased the survival rates of ARPKD patients, with most of them reaching adult age. In a large study of almost 200 ARPKD patients with known *PKHD1* variant status, survival rates among those patients who survived the first month of life were 94% at 5 years and 92% at 10 years of age [120]. Kidney failure is rarely a cause of neonatal demise. In non-oliguric ARPKD neonates hyponatremia, considered to be related to defective urine concentration, is often present in the newborn period, but this complication usually resolves over time [4, 120, 131].

Kidney Disease

In a recent European study on 304 patients with ARPKD KRT was required in 25–30% of patients by the age of 15 years [114]. In another analysis 40% of young adult patients with ARPKD were on KRT [3].

Several antenatal and perinatal risk factors for dialysis dependency in early life have been identified. The presence of oligohydramnios or anhydramnios, prenatal kidney enlargement, a low Apgar score, and the need for postnatal breathing support were independently associated with an increased risk of requiring chronic dialysis [132]. The likelihood of early dialysis varied from 1.5% for patients without prenatal sonographic anomalies to 32% in patients with documented oligohydramnios or anhydramnios, kidney cysts, and enlarged kidneys (Table 10.3).

CKD with an eGFR <75% of age-adjusted normal ranges was first detected at a mean age of 4 years in a previous survey among pediatric nephrology units [120]. Infants with ARPKD may have a transient improvement in their glomerular filtration rate (GFR) due to kidney maturation in the first months of life. Subsequently, a progressive but highly variable decrease in kidney function occurs. In a small cohort of children with

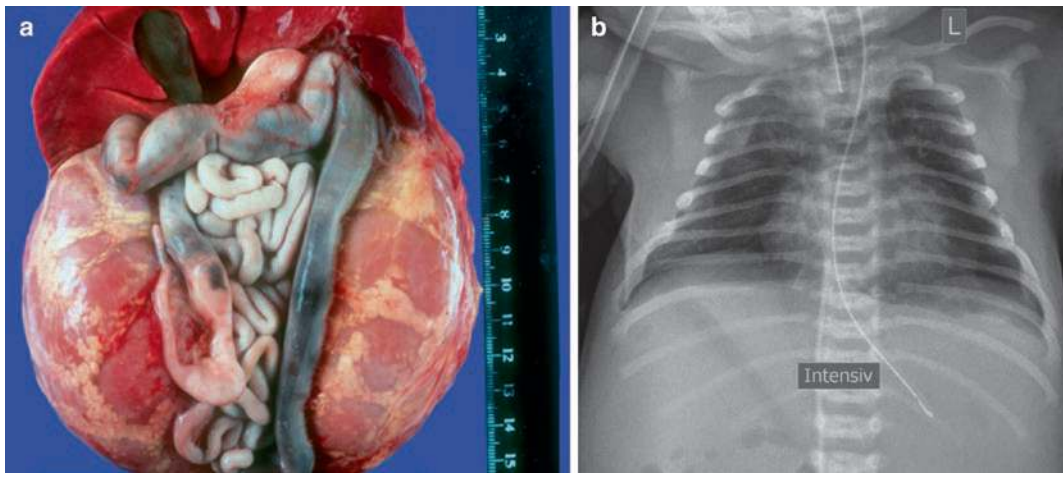

Fig. 10.6 Abdominal situs of an ARPKD patient with symmetrically enlarged kidneys that maintain their reniform configuration (**a**) and chest X-ray of a patient with pulmonary hypoplasia due to ARPKD (**b**)

Table 10.3 Multivariate cox model of pre-, peri- and postnatal predictors of necessity of kidney replacement therapy within the first year of life. Model based predicted probabilities for kidney replacement therapy within 12 and months after birth (modified from [132])

Parameter	Hazard ratio	95% CI		*p*
		Lower	Upper	
Sex	0.925	0.462	1.850	0.825
Oligo/anhydramnios	4.473	1.295	15.449	0.018
Prenatal enlarged kidneys	3.177	1.087	9.282	0.035
Vaginal delivery	1.271	0.584	2.765	0.545
Gestational age at birth (weeks)	1.121	0.917	1.371	0.265
Birth weight SDS	1.291	1.031	1.618	0.026
Apgar 10′	0.748	0.564	0.991	0.043
Assisted breathing and/or ventilation	6.994	1.536	31.845	0.012
Prenatal symptom combination	**Probability of dialysis within 12 months after birth (95% confidence interval)**			
No prenatal abnormalities	0.015 (0.005–0.041)			
Enlarged kidneys	0.033 (0.006–0.155)			
Renal cysts	0.034 (0.008–0.135)			
Enlarged kidneys and renal cysts	0.071 (0.021–0.215)			
Oligo/anhydramnios	0.087 (0.032–0.214)			
Oligo/anhydramnios and enlarged kidneys	0.174 (0.055–0.431)			
Oligo/anhydramnios and renal cysts	0.178 (0.047–0.486)			
Oligo/anhydramnios and enlarged kidneys and renal cysts	0.323 (0.222–0.445)			

ARPKD the annual GFR decline was 1.4 mL/min/1.73 m^2 (−6%) and rates of GFR decline in ARPKD patients were not significantly different from children with other causes of CKD [133].

In a cohort in the US, patients with perinatal presentation showed worse kidney follow-up than those with non-perinatal presentation [121]. While 25% of the perinatally symptomatic patients required kidney replacement therapy by the age of 11 years, 25% of the non-perinatal patients required kidney transplantation by age 32 years. Also, corticomedullary involvement on high resolution ultrasound was associated with worse kidney function in comparison with medullary involvement only in this specific cohort. Kidney volume correlated inversely with function, although with wide variability [121].

Liver Disease

In keeping with the generally prolonged survival in ARPKD, for many patients the hepatobiliary complications come to dominate the clinical picture [3, 124]. Interdisciplinary management by pediatric nephrologists and hepatologists is essential for optimal management of ARPKD from early life onward [4].

While hepatocellular function is usually preserved, these individuals develop sequelae of portal hypertension and may present with hematemesis or melena due to bleeding oesophageal varices and/or hypersplenism with consequent pancytopenia. Typical hepatic manifestations usually present later than classic kidney involvement [3, 110, 131], although complications of portal hypertension may occur early in life [134].

A serious, potentially lethal complication in ARPKD especially after kidney transplantation is ascending suppurative cholangitis that may cause fulminant hepatic failure [135–137]. Cholangitis always requires diligent evaluation with aggressive antimicrobial treatment. Noteworthy, ARPKD patients may not display the full picture of clinical and laboratory findings of cholangitis; thus, every patient with unexplained recurrent sepsis, particularly with gram-negative organisms, should be critically evaluated for this diagnosis [110, 138, 139].

A recent study on 49 young adults with ARPKD characterized the clinical phenotype in this age group and identified prominent liver involvement. ARPKD should also be considered as a differential diagnosis in adults with cystic kidney disease [3].

Adult ARPKD patients beyond the age of 40 years may have a slightly increased risk to develop hepatic tumors, especially cholangiocarcinoma [140, 141].

Additional Extra-Renal Manifestations

Studies have also described additional extra-renal clinical aspects. Arterial hypertension is a very common finding, affects up to 80% of children with ARPKD and usually develops in the first few months of life (Table 10.2). Arterial hypertension may be very pronounced in ARPKD. It was recently shown that children with ARPKD show high rates of abnormal left ventricular geometry with systolic mechanical dysfunction [142].

There have been various reports of patients with both intracranial and extracranial aneurysms [143].

Neurocognitive function and growth in ARPKD children appear to be comparable to children with other causes of CKD [144, 145].

Pulmonary tests in the follow-up of a subgroup were within normal references suggesting good long-term pulmonary outcome [146].

Genetics

PKHD1 is the major, but not the only gene for ARPKD. Variants in an increasing number of other genes such as *PKD1, PKD2, HNF1B, DZIP1L, CYS1* can mimic the disease and lead to an ARPKD-like phenotype [64]. *PKHD1* variant analysis is characterized by vast allelic heterogeneity and a huge number of private variants and missense changes.

PKHD1 Gene and Polyductin/ Fibrocystin Protein

The *PKHD1* gene is highly expressed in fetal and adult kidney, at lower levels in liver, and weakly in other tissues including pancreas, arterial walls and the lung [147]. *PKHD1* is amongst the largest disease genes in the human genome, extending over a genomic segment of at least 470 kb and including a minimum of 86 exons. *PKHD1* undergoes a complex and extensive pattern of alternative splicing, generating transcripts of highly variable size. The longest *PKHD1* transcript contains 67 exons encoding a protein of 4074 amino acids.

The predicted full-length protein (termed fibrocystin or polyductin) represents an integral membrane protein with a single transmembrane (TM)-spanning segment, and a short cytoplasmic C-terminal tail (Fig. 10.7). Based on the structural features of the deduced protein and on the human ARPKD phenotype, fibrocystin might be involved in cellular adhesion, repulsion and proliferation. In addition, the domain and structural analyses suggest that the potential *PKHD1* gene products may be involved in intercellular signalling and function as receptor, ligand and/or membrane-associated enzyme [1].

In common with most other cystoproteins, fibrocystin has been identified at primary cilia with concentration in the basal body area [148–151]. As part of acquisition of epithelial polarity during kidney development, fibrocystin becomes localized to the apical zone of nephron precursor cells and subsequently to the basal bodies at the origin of primary cilia in fully differentiated epithelial cells. Its peculiar subcellular localization and suggested proteins interactions point to a role of fibrocystin in centrosomal, mitochondrial or cilia-associated function. There is evidence for overlapping functions with, e.g. polycystin-1 [1, 152–154]. In addition, there is preliminary evidence of fibrocystin isoproteins which may be secreted in exosomes and undergo posttranslational processing [86, 152, 155, 156].

The number of alternative *PKHD1* transcripts that are actually translated into protein is as yet unknown. The distribution of variants over the entire *PKHD1* gene suggests that the longest transcript is necessary for proper fibrocystin function in kidney and liver. Thus, it might be proposed that a critical amount of the full-length protein is required for normal function.

Genotype-Phenotype Correlations

The analysis of genotype-phenotype correlations for *PKHD1* is hampered by multiple allelism and the high rate of different compound heterozygotes. Until recently, genotype-phenotype correlations were largely limited to the type (truncating/non-truncating) and allelic distribution (homozygous/compound heterozygous) of the variants.

Almost all patients carrying two truncating variants display a very severe phenotype although more recently it was shown that some patients may survive [114, 132, 157, 158]. Patients with at least one missense variant tend to be less severely affected and are more likely to survive the neonatal period. However, missense changes can clinically impress as severe as truncating variants. No significant clinical differences could be observed between patients with two missense variants and those patients harboring a truncating variant *in trans*; thus, the milder variant obviously defines the phenotype. Loss of function probably explains the rather uniform phenotype and early demise of many patients with two null alleles. Phenotypic diversity also reflects the variable extent to which different *PKHD1* missense variants might compromise the function and/or abundance of the mutant protein. Complex transcriptional profiles may play a further role in defining the patient's phenotype [158, 159].

Recently, the localization of a specific missense variant was shown to be of additional relevance for the phenotype. In a cohort of 304 patients, patients with two missense variants affecting amino acids 709-1837 of fibrocystin or a missense variant in this region and a null variant less frequently developed chronic kidney failure than other subgroups. Missense variants affecting amino acids 1838-2624 showed better hepatic outcome. Variants affecting amino acids 2625-4074 of fibrocystin were associated with a more severe hepatic phenotype [114].

The aspect of renal-hepatobiliary morbidity patterns in ARPKD has repetitively been discussed [111, 120, 131]. Many patients show uniform disease progression, but individual ARPKD patients present with an organ-specific phenotype. The observations that different variant localizations are associated with specific kidney and liver disease courses opens novel paths to understand the underlying pathophysiology [114].

Phenotypes cannot yet be fully explained on the basis of the *PKHD1* genotype but likely depend on mutational load for an individual patient, epigenetic factors (e.g., alternative splicing), and environmental influences [1]. Such modifiers will probably have their greatest impact on the phenotype in the setting of hypomorphic missense changes and may explain some of the clinical variability.

Phenotypic Variability Among Affected Siblings

For about 20% of pedigrees gross intrafamilial phenotypic variability with peri-/neonatal demise in one and survival into childhood or even adulthood in another affected sibling have been described [160]. An even higher proportion of 20 out of 48 sibships (42%) was observed among families with at least one neonatal survivor per family [120]. Likewise, data from the ARegPKD dataset suggest a rather limited variability of kidney function decline and hepatic symptoms in the clinical courses of patients surviving the neonatal period. With regards to genetic counselling this rate is alarming [161]. Of course, phenotype categorization into 'severe' and 'moderate' is a simplified and artificial view given the considerably better prognosis for patients surviving the most critical neonatal period. Also, the survival chance of an affected neonate might depend on available intensive care facilities and parental awareness of ARPKD risk. Hence, caution should be warranted in predicting the clinical outcome of a further affected child.

Prenatal Diagnosis

As previously pointed out the diagnosis is frequently identified antenatally. In view of the recurrence risk of 25%, the oftentimes devastating course of early manifestations of ARPKD and a usually similar clinical course among affected siblings, many parents of ARPKD children seek early and reliable prenatal diagnosis (PND) to guide future family planning. Frequently, ARPKD patients are identified by ultrasound only late in pregnancy or at birth. Therefore, an early and reliable PND for ARPKD in "at risk" families is feasible by molecular genetic analysis [147, 162, 163] as the basis for PND and genetic counselling according to local regulations.

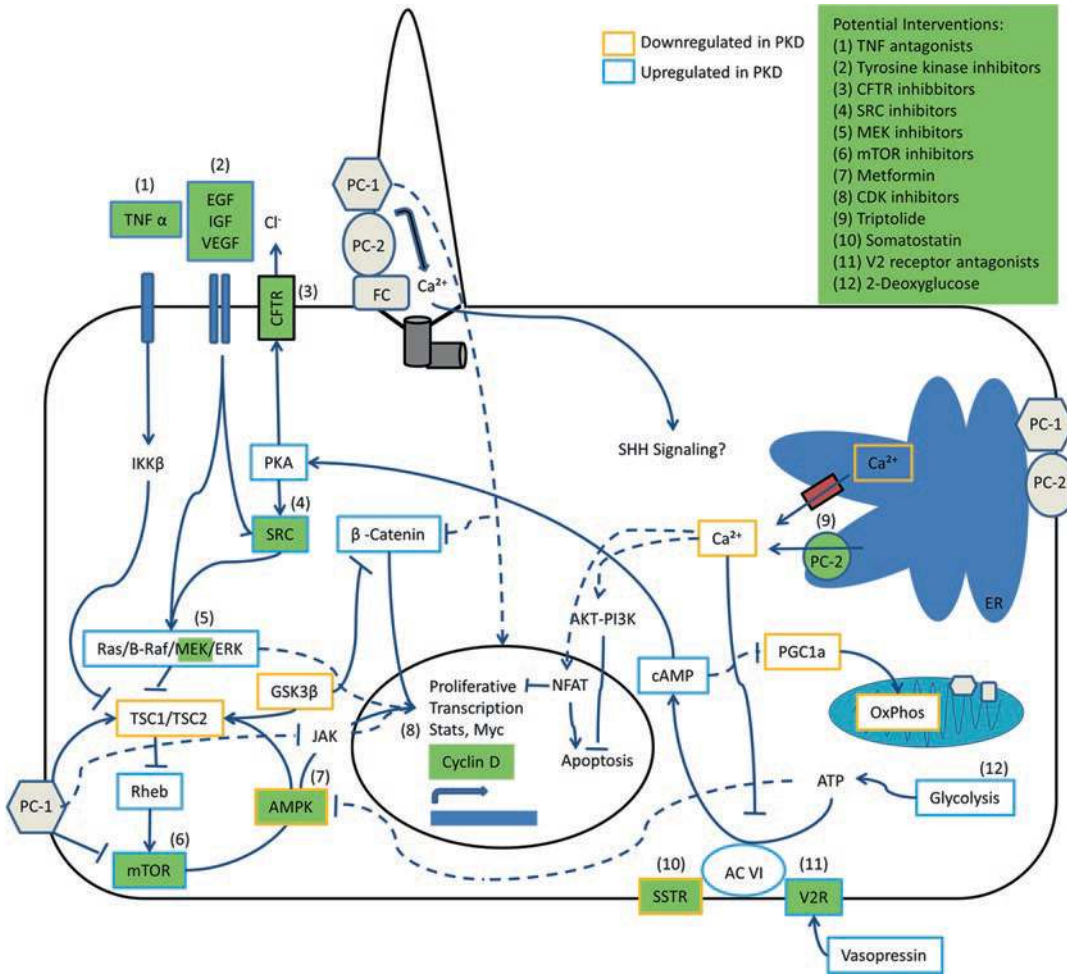

Fig. 10.7 Schematic presentation of polycystin-associated signalling pathways and potential pharmacological targets. Polycystin-1 (PC-1), polycystin-2 (PC-2), and fibrocystin/polyductin (FC) are located in primary cilia. PC2 is also located in the endoplasmic reticulum. The ciliary polycystin complex regulates ciliary calcium signals that may lead to calcium-induced calcium release from the endoplasmic reticulum. Reduction of calcium enhances cAMP accumulation, that is furthermore supported by activation of V2-receptors (V2R) but may be inhibited by activation of the somatostatin receptor (SSTR). cAMP stimulates chloride-driven fluid secretion. In PKD cAMP also stimulates cell proliferation in an src-, Ras-, and B-raf–dependent manner. Finally, Mammalian target of rapamycin (mTOR) is activated in cyst-lining PKD epithelia. *AC-VI* adenylyl cyclase type VI, *ATP* adenosine triphosphate, *CFTR* cystic fibrosis transmembrane conductance regulator, *ER* endoplasmic reticulum, *ERK* extracellular signal–regulated kinase, *MEK* mitogen-activated protein kinase kinase, *PKA* protein kinase A, *EGF* epidermal growth factor, *IGF* insulin-like growth factors, *VEGF* vascular endothelial growth factor, *CFTR* cystic fibrosis transmembrane conductance regulator, *AMPK* AMP-activated protein kinase, *SHH* sonic hedgehog signaling

Therapeutic Management

Currently, there is no curative treatment option for patients affected by PKDs to ameliorate or even regress the clinical course. However, given the insights into cell biology and dysregulated intracellular signaling pathways in different PKDs, novel treatment approaches in ADPKD have been suggested [164]. Using different rodent models, various research groups have published

promising data on treatment of PKD. Interestingly, the severity and dynamics of cystic kidney disease in orthologous mouse models varies widely. Choosing preclinical models that mimic human disease is utmost relevant when testing potential therapeutic approaches [165]. Multiple therapeutic approaches have been identified in preclinical models. As a consequence, several clinical trial programs for ADPKD have been established. Recent work summarizes the pathophysiological considerations underlying those novel treatment approaches [1] and current state-of-the-art management in adult patients with ADPKD [164]. We will therefore only provide a brief overview here, focusing on pediatric aspects of available and emerging therapeutic options.

Lifestyle Measures, Pediatric Treatment of CKD and Treatment of Co-morbidities

The basis for treatment of PKD and CKD lies in the strict application of symptomatic measures [164]. **Dietary sodium restriction, sufficient physical activity and normalization of the body mass index** should be implemented as initial approach for patients diagnosed or at-risk for ADPKD. Based on the role of vasopressin in ADPKD, **increased water intake** has been suggested in order to suppress endogenous vasopressin concentration and thereby reduce kidney cyst growth targeting a goal urine osmolality below 280 mOsm/kg H_2O [166]. Ongoing controlled trials to evaluate the impact of high water intake on TKV are in progress [167]. In addition, high fluid intake might also be generally beneficial to reduce the risk of nephrolithiasis and UTI in ADPKD. Low salt intake and maintenance of a normal body mass index would be also recommended in view of the increased risk of hypertension and cardiovascular comorbidities in children with ADPKD [14, 164]. Last but not least, **avoidance of smoking and potentially nephrotoxic drugs** including nonsteroidal anti-inflammatory drugs is also appropriate. Extra-renal co-morbidities require adequate treatment. For this chapter we will focus on aspects concerning liver disease.

Blood Pressure Control

Blood pressure control is essential in PKD patients. While blood pressure control is a critical aspect of CKD management in general in both children and adults, hypertension is a key modifiable risk factor in children with ADPKD [14]. Increased TKV and fractional cyst volume is associated with hypertension and decreased eGFR over time [27, 28]. When lifestyle measures remain insufficient, the first line pharmacologic treatment will be the inhibition of RAAS.

The HALT-PKD trials compared the combination of ACE inhibition (ACEI) with an angiotensin receptor blocker to ACEI alone in adult patients with an eGFR of either >60 or 25–60 mL/min/1.73 m^2 as well as standard blood pressure with low blood pressure targets in early-stage patients. Altogether more than 1000 ADPKD patients were enrolled [168, 169]. In these trials rigorous blood pressure control showed benefits over standard blood pressure control in early ADPKD in terms of a reduced increase in total kidney volume, a greater decline in left ventricular mass index and less albuminuria. eGFR was not different among the groups. The international consensus guidelines for pediatric ADPKD recommend at least annual blood pressure measurements for children at-risk of or diagnosed with ADPKD, with a preference for periodic 24-h ambulatory blood pressure monitoring due to the increased risk of masked hypertension. Blood pressure should be maintained below the 75th percentile (or <125/72 mmHg from age 16 years) [14]. It was suggested that lowering blood pressure even below the 50th percentile (or <120/70 mmHg from age 16 years) might provide additional long-term benefit in hypertensive children with ADPKD. Targeting the 50th–75th percentile blood pressure would be the ideal goals for hypertensive children with ADPKD [14, 16].

While formal recommendations for children with ARPKD are lacking, it is reasonable to apply the same treatment targets in the ARPKD population. Hypertension can be difficult to control in ARPKD children and may require multi-drug treatment. Hypertension needs early and aggressive treatment with careful blood pres-

sure monitoring to prevent sequelae of hypertension (e.g., cardiac hypertrophy, congestive heart failure) and deterioration of kidney function [110]. The pathophysiology of hypertension in ARPKD is not clearly understood but may involve dysregulation of renal sodium transport and activation of the RAS [170, 171] leading to increased intravascular volume [172]. It is therefore important to point out that hyponatremia in ARPKD can be considered to be an effect of excess water retention rather than sodium loss. Thus, general principles of handling hyponatremia apply. Feeding may need to be concentrated to minimize fluid intake [11, 112]. Sodium supplementation may increase hypertension. RAS antagonists (ACE inhibitors or AT1 receptor blockers) are regarded the treatment of choice [112]. Moreover, it has been proposed that the epithelial sodium channel blocker amiloride can be used to decrease intracellular cAMP concentrations that may result in Pseudo-Liddle syndrome [173].

Management of CKD and ESKD in Pediatric PKD Patients

The management of children with PKD with declining kidney function should follow the standard guidelines established for chronic kidney failure in other pediatric patients. Both peritoneal dialysis (PD) and hemodialysis are successfully used in ARPKD neonates and infants. PD has been recommended as the chronic modality of choice for infants with ESKD as nutrition can be optimized and vascular access can be preserved for later use [174]. Maintenance PD can be used in young ARPKD children with only minor modifications compared to other inborn kidney diseases [175]. Kidney transplantation is the treatment of choice for individuals with ESKD. In case of massively enlarged kidneys, native nephrectomies may be warranted to allow allograft placement.

ARPKD is one of the two major indications for combined liver and kidney transplantation (CLKTx) during childhood, next to primary hyperoxaluria that is discussed in detail elsewhere in this book [176, 177]. The best timing and strategy for combined transplantation is still a matter of debate and usually requires individualized decision-making. Recurrent cholangitis and evidence of severe portal hypertension may be indications for combined liver and kidney transplantation in ARPKD [137, 140, 176, 178]. Organ survival after CLKTx in ARPKD is good, but data from the ESPN-ERA/EDTA registry suggested higher mortality in ARPKD patients undergoing CLKTx in comparison to patients undergoing isolated kidney transplantation [137].

Functional consequences of CKD are rarely a clinical problem in children with ADPKD. In cases with VEO-ADPKD the same general principles apply as in ARPKD and other causes of pediatric CKD.

Management of Liver Disease

Liver cysts in ADPKD only rarely result in clinical problems and complications appear much less common than complications of kidney cysts. Usually, hepatic, pancreatic, or ovarian cysts are not observed before puberty [47]. Massive liver enlargement secondary to hepatic cysts may result in disabling discomfort. These individuals may benefit from percutaneous sclerotherapy when one or a few large cysts are present. Occasionally, more aggressive surgical intervention with fenestration, partial hepatectomy or even liver transplantation may be required [179]. Furthermore, as discussed below somatostatin analogues are under investigation for hepatic cysts.

Cholangitis is a prominent co-morbidity in ARPKD and always requires diligent evaluation with aggressive antimicrobial treatment.

Primary management of variceal bleeding in ARPKD may include endoscopic approaches, such as sclerotherapy or variceal banding. In some patients, portosystemic shunting or liver-kidney transplantation (sequential or combined) can be considered as a viable therapeutic option; however, since ammonia is cleared by the kidneys, impaired kidney function in ARPKD patients makes portosystemic shunting less attractive. Transjugular intrahepatic portosystemic shunt (TIPS) has been used in pediatric patients as a feasible and effective treatment alternative for surgical shunting but hepatic encephalopathy may be a problem [180, 181].

The risk of portal hypertension in ARPKD has led to concerns about the use of gastrostomy insertions in patients with ARPKD. Yet, in an international survey amongst physicians taking care of 196 ARPKD children the benefits outweighed the complications and risks of gastrostomy insertion [182].

Management of Perinatal Disease in ARPKD

During the first days of life, the pulmonary situation is the crucial factor for the overall prognosis in ARPKD [4, 11, 112]. Perinatal treatment can be highly complex and delivery in a hospital with high expertise in neonatology and pediatric nephrology has been recommended for patients with suspected ARPKD [112]. Highly sophisticated methods of ventilation and/or surfactant application have developed in neonatal intensive care, but neonatal mortality remains substantial in severely affected children. The defined use of antenatal corticosteroids to support lung maturation in late preterms may be helpful.

Nephrectomy has been proposed for pulmonary indications, to facilitate feeding and to improve blood pressure control [183–187]. However, supportive evidence for beneficial effects of nephrectomy is very limited and very careful consideration of the potential benefits against the risk of potentially accelerated loss of kidney function and subsequent kidney replacement therapy early in life are required. Furthermore, in the ARegPKD registry study bilateral nephrectomies in the first 3 months of life were associated with severe neurological sequelae (Fig. 10.8) [188]. After unilateral nephrectomy the remaining kidney may start to grow very rapidly, thus making a second nephrectomy necessary within a short time frame. Very careful indication of nephrectomies therefore seems appropriate, especially in patients with remaining kidney function.

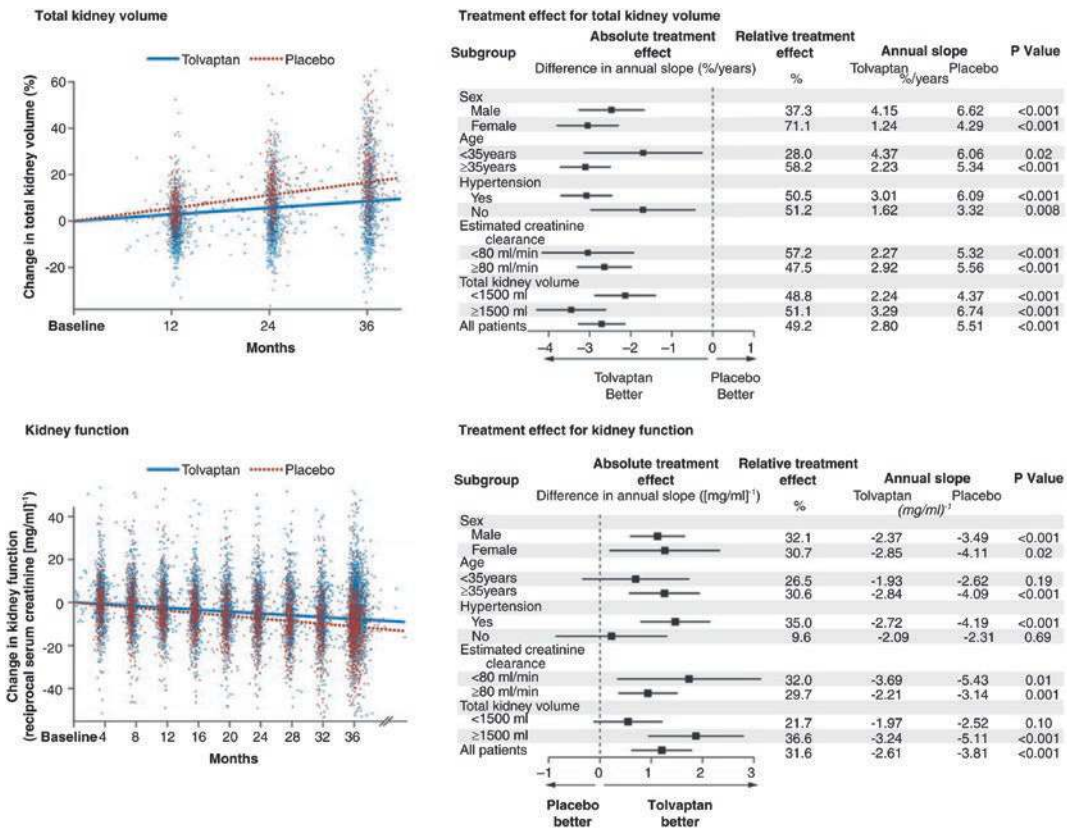

Fig. 10.8 Main results of the TEMPO 3/4 trial: Tolvaptan significantly reduced kidney growth rate and showed lower rates of renal function decline. (From Torres et al. [189], with permission)

Emerging Targeted Therapies

Vasopressin V_2 Receptor Antagonists

The rationale for the use of V2 receptor antagonists is based on the observation that tubular cells of ADPKD patients contain high cAMP concentrations and that PKD patients show high levels of circulating vasopressin. Vasopressin induces an intracellular increase of cAMP via the V2 receptor to increase fluid secretion into cysts via the CFTR channel. After promising results in preclinical studies [1], the V2 receptor antagonist tolvaptan was investigated in the TEMPO 3/4 trial (Tolvaptan Efficacy and Safety in Management of PKD and Outcomes), a large phase 3, placebo-controlled, double-blind study in 18–50-year-old patients with a GFR of >60 mL/min but very large kidneys (>750 mL) as an indicator of progressive disease. After 3 years, Tolvaptan had significantly reduced kidney growth rate (2.8%; vs. 5.5% in the control group) and shown lower rates of kidney function decline or ADPKD-related adverse events (Fig. 10.9) [189]. Later studies confirmed the long-term safety and efficacy [190] and demonstrated beneficial effects of Tolvaptan also in late-stage ADPKD [191].

A phase 3 study assessing the pharmacodynamic properties, safety and efficacy of tolvaptan over 3 years in 91 ADPKD children and adolescents has been conducted [192]. After 1 year of treatment, the mean percent increase of height-adjusted TKV from baseline in 12–17 year-old children was 2.3% for Tolvaptan and 6.1% for placebo [193]. No elevated transaminase or cases of drug-induced liver injury were reported.

The clinical overlap of ARPKD and ADPKD and ample experimental evidence suggests that Tolvaptan might also be efficacious in ARPKD. This has led to the recent initiation of a clinical trial program for Tolvaptan in children with ARPKD.

Somatostatin Analogues

Another way to decrease intracellular cAMP is the use of a long-acting analog of somatostatin. Various randomized controlled trials have been performed to evaluate somtatostatin analogues in ADPKD and ADPLD, which have recently been summarized in a systematic review and meta-analysis. The data show improvement in total liver volume but no benefit on TKV and eGFR in patients with ADPKD while there were various side effects [194]. Future work will most likely

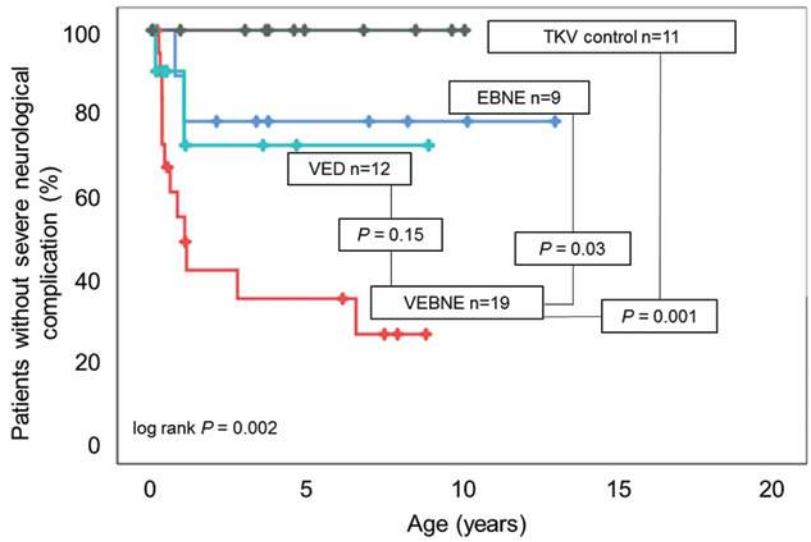

Fig. 10.9 Kaplan–Meier survival curve of ARPKD patients without severe neurological complications stratified according to nephrectomy status. Censored observations (last documented follow-up or death) are marked with a cross. P values were derived using log rank tests. EBNE early bilateral nephrectomies (second nephrectomy in months 4–15), TKV total kidney volume, VEBNE very early bilateral nephrectomies (in first 3 months), VED very early dialysis. (From [188])

focus on a potential role of somatostatin analogues for the treatment of polycystic liver disease.

mTOR Inhibition and Other Targeted Therapy of Cellular Metabolism

mTOR activation has been observed in the cyst epithelium of ADPKD kidneys. After promising results in animal models [1], different trials studied mTOR inhibition in ADPKD. The two large trials on mTOR inhibition in ADPKD did not deliver a therapeutic breakthrough [195, 196]. mTOR inhibitors might still be an option in selected patients with ADPKD or if administered in combination with other agents or in a tubule-specific way.

In addition to mTOR inhibitors, inhibition of glycolysis, activation of AMPK through Metformin, PPAR-α activation or Sirtuin inhibition are additional pharmacological strategies that are currently under investigation [85].

Dietary Intervention

Dietary interventions are an interesting emerging therapeutic strategy in addition to pharmacological approaches based on observations that PKD proteins may be involved in the regulation of cellular metabolism [85]. Moderate food or caloric restriction has shown positive effects on kidney volume in preclinical models. Importantly, obesity is a known risk factor for rapid disease progression in ADPKD. In addition to caloric restriction, time restricted feeding and ketogenic diet as well as the addition of β-hydroxybutyrate to normal chow have shown promising effects in preclinical mouse, rat and cat models of PKD [197]. Pilot clinical trials have recently been initiated.

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11 Nephronophthisis and Autosomal Dominant Tubulointerstitial Kidney Disease (ADTKD)

Jens König and Heymut Omran

The Nephronophthisis complex

The nephronophthisis complex comprises a clinically and genetically heterogeneous group of tubulointerstitial cystic disorders with an autosomal recessive inheritance pattern. It represents the most frequent genetic cause of end-stage kidney disease (ESKD) in children and young adults. Nephronophthisis can be accompanied by anomalies in other organs, e.g. liver, pancreas, central nervous system, eyes and bones. There are several well described complex clinical syndromes that can feature the renal picture of nephronophthisis, including Senior-Løken-syndrome, Joubert syndrome, COACH syndrome, Jeune syndrome, Sensenbrenner syndrome, Meckel-Gruber syndrome and others. Because of the extended clinical as well as genetic overlap, the term *nephronophthisis complex* has been introduced. However, phenotypic variability as well as the polygenic background complicate the timely establishment of the correct diagnosis.

Nephronophthisis

Nephronophthisis literally means "damage of the nephrons". 1951 Fanconi et al. introduced the term *familial juvenile nephronophthisis* to describe a disease characterized by autosomal recessive inheritance, a defect in urinary concentrating capacity, severe anaemia and progressive renal failure that leads to death before puberty [1, 2]. The incidence ranges from 1:1,000,000 in the US to 1:50,000 in Europe. To date, variants have been identified in 25 genes (Fig. 11.1) encoding nephrocystin proteins that localize to primary cilia, basal bodies and centrosomes. Thus, nephronophthisis (NPH) belongs to a group of rare hereditary disorders referred to as ciliopathies. Mutations in these genes lead to renal as well as extrarenal disease manifestations [3].

The clinical hallmarks of NPH are a reduced urinary concentrating capacity presenting as polyuria and polydipsia with regular fluid intake at night and slowly progressive chronic kidney failure. Other clinical manifestations comprise growth retardation, anaemia and persisting primary or secondary enuresis. However, these features are only facultative findings. Urine analyses usually do not show any characteristic abnormalities. Proteinuria and arterial hypertension are no typical findings before the onset of end-stage kidney failure. On ultrasound, patients generally show normal or small-sized kidneys with increased echogenicity and a loss of cortico-

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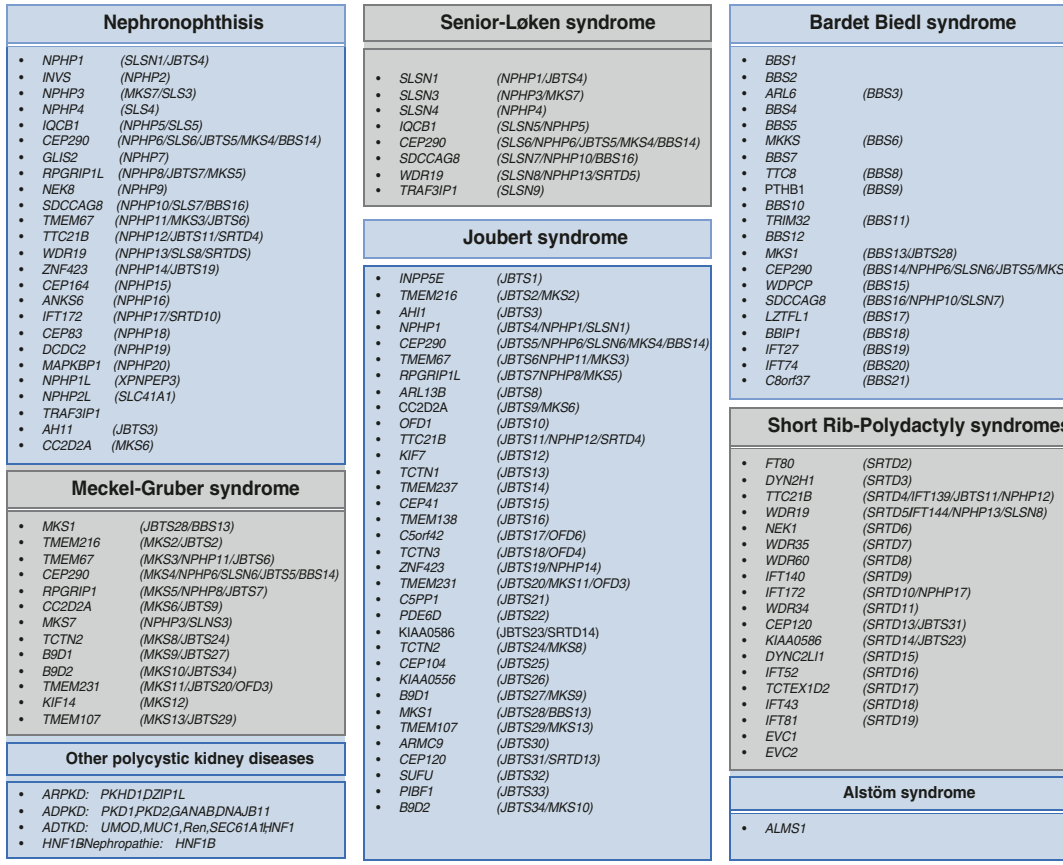

Fig. 11.1 Genetic overview on hereditary cystic kidney diseases

medullary differentiation. Cysts, which are typically located at the cortico-medullary junction, often only occur late in the disease process [4] and are not an obligatory finding (Fig. 11.2). In contrast to other hereditary cystic kidney diseases such as polycystic kidney diseases (ADPKD and ARPKD), HNF1B nephropathy or Bardet-Biedl syndrome, prenatal cystic presentation is untypical and limited to infantile NPH [5]. Because of the lack of disease-specific clinical features and the slow progress, still about 15% of patients are only diagnosed when ESKD has already been reached.

In the past, kidney biopsy has been used for the diagnosis of NPH. Histology is characterized by disintegrated tubular basement membranes, tubular atrophy and cyst formation, as well as a sclerosing tubulointerstitial nephropathy accompanied by lymphocyte infiltrates [6, 7]. Since cortico-medullary cysts only occur late in the disease process [8], they are no obligatory finding and thus no hallmark of the disease. Nowadays, against the backdrop of the availability of rapid and targeted molecular genetic analysis, kidney biopsy for the diagnosis of NPH is largely obsolete and should be limited to cases in which his-

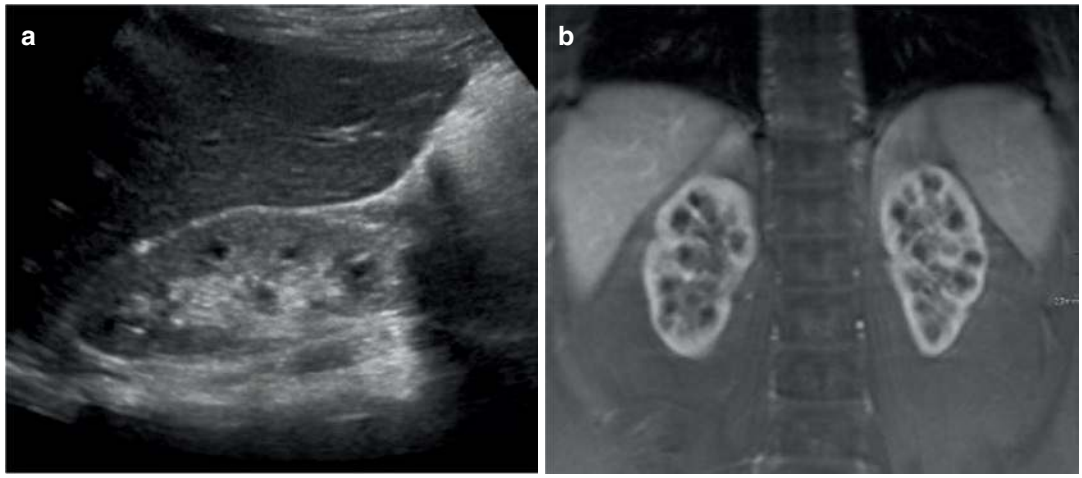

Fig. 11.2 Ultrasound (**a**) and MRI (**b**) presentation of the kidneys in a patient with classical juvenile nephronophthisis. Please note that despite the prominent cysts at the cortico-medullary junction, these are no obligatory finding

tology is needed to rule out other differential diagnoses (e.g. tubulointerstitial nephritis).

Genetics

To date, 25 genes have been identified to be associated with NPH (Fig. 11.1). Yet, about 40% of all cases still remain genetically unsolved. While *NPHP1* defects account for 20–60% of NPH cases, each of the remaining genes makes up for 1% or less of all cases [9].

Most gene products (so called nephrocystins) affected by *NPHP* gene mutations are located at the ciliary base and transition zone of primary cilia excerpting a kind of gate keeper function controlling transport processes from the cytoplasm into the cilium and vice versa [10]. Primary cilia are evolutionarily conserved, membrane-bound, microtubular projections protruding from the cell surface. They are found on virtually all cell types in the human body and play an essential role in transducing signaling information from the extracellular milieu into the cell. Only recently mutations in *NPHP1L* und *NPHP2L* have been identified, genes that encode mitochondrial rather than ciliary proteins [11].

Based on functional interaction studies, currently four distinct nephrocystin modules have been described: the NPHP 1-4-8 module, the NPHP 2-3-9-ANKS6 module, the NPHP5-6 module and the MKS module. These nephrocystin modules are related to different signaling pathways including the Wnt pathway, Hedgehog pathway, Hippo pathway, DNA damage response (DDR) pathway, mTOR pathway, the intracellular calcium and the cAMP signaling pathway [9].

Genotype-Phenotype Correlation

Depending on the onset of ESKD, three clinical subtypes of NPH have been defined: Infantile, juvenile, and adolescent NPH. The so called "classical juvenile NPH" is by far the most common entity, leading to ESKD at a median age of 13 years [4, 12]. In infantile NPH ESKD is reached very early in life (median age 8 months) whereas in adolescent forms kidney function is usually preserved until adulthood (median age 19 years).

Large homozygous deletions of approximately 290 kb involving the *NPHP1* locus on chromosome 2q12-q13 are the main genetic cause of the classical juvenile NPH accounting for up to 60% of patients. Only some patients carry point mutations or compound heterozygous variants [13, 14]. Homozygous *NPHP1* deletions have also been reported in adults who developed ESKD between 27 and 60 years of age [15].

Other genetic defects primarily associated with adolescent NPH comprise *NPHP3, NPHP4* and *NPHP9/NEK8*. While the kidney phenotype and clinical course described above is very similar for most NPH variants, this does not apply to infantile NPH, which typically already presents in utero or early infancy with enlarged cystic kidneys, arterial hypertension and ESKD before the age of 4 years. Histologically, infantile NPH differs from juvenile NPH by a cortical rather than medullary cyst location and the absence of typical tubular basement membrane changes [16]. Mutations in *NPHP2/INV* (9q22-q31) were the first molecular defects identified in infantile NPH, often associated with laterality defects like *situs inversus* and congenital heart defects [17]. Subsequently, mutations in various other genes were identified to cause infantile NPH, i.e. *NPHP3*, *NPHP12/TTC21B*, *NPHP14/ZNF423* and *NPHP18/CEP83* [9].

Detailed information about all NPH-related genes identified so far and their clinical implications is given in Table 11.1.

Life table analyses demonstrate the impact of the underlying genetic defect on renal survival. While loss-of-function mutations in *NPHP3* go along with a >50% chance of ESKD before the age of 4 years, so called hypomorphic mutations in the same gene were found to be responsible for an adolescent type of NPH. Kidney failure in the vast majority of *NPHP1* deletion carriers occurs in a narrow time slot between 7 to 16 years of age. Renal survival curves of individuals suffering from *NPHP4* variants resemble those of *NPHP1* carriers, yet with a slower progression and a fraction of 25% patients with preserved renal function at 30 years of age [4].

Extra-renal Disease Manifestations

Owing to the ubiquitous presence of primary cilia and depending on the underlying molecular defect, the clinical presentation of NPH is not necessarily limited to the kidneys but can be associated with extra-renal disease manifestations. Standardized phenotypic surveys revealed extrarenal manifestations in approx. 20–40% of affected individuals, exceeding previous incidence estimates [4]. The clinical spectrum encompasses ophthalmological abnormalities (oculomotor apraxia, retinitis pigmentosa, Leber amaurosis), hepatic fibrosis, neurologic disorders, chronic lung and upper airway infections as well as skeletal defects (Fig. 11.3). Less frequently, laterality defects, genital anomalies, congenital heart defects and endocrine dysfunctions (e.g. hypogonadism, short stature, obesity) are observed. In addition, NPH resembles the kidney phenotype of various mulivisceral ciliopathies described in detail below:

Senior-Løken Syndrome

The term Senior-Løken syndrome denotes the association of NPH and retinal degeneration [18, 19]. Two variants of retinal disorders have been described:

Leber congenital amaurosis (LCA), the most severe variant, is a clinically and genetically heterogeneous retinal disorder that occurs in infancy and is accompanied by profound visual loss, nystagmus, poor pupillary reflexes, and either a normal retina or varying degrees of atrophy and pigmentary changes [19–21]. Affected children exhibit the so-called oculodigital sign characterized by poking, rubbing and pressing of the eyes in order to mechanically stimulate the retina. The electroretinogram is extinguished or severely reduced [22]. All but one form of Leber congenital amaurosis is inherited as an autosomal recessive trait. LCA is a disorder of photoreceptors, caused by failed transport of rhodopsin and a loss of outer segments of the photoreceptor resulting in its ultimate cell death.

Although LCA is a clinical diagnosis, molecular testing is currently available for many different genes, including several genes that can be associated with NPH such as *IQCB1/NPHP5* and *CEP290/NPHP6* [23] (Fig. 11.1). So far, all reported Senior-Løken patients with underlying *IQCB1/NPHP5* mutations presented with early severe retinal degeneration (Leber congenital amaurosis) [23]. Conversely, genetic testing for *IQCB1/NPHP5* variations in patients with a late or mild ocular manifestation does not appear to be very promising. The onset of ESKD in this

Table 11.1 Clinical presentation of genetic defects associated with NPH and NPH-related ciliopathies

				NPH associated with extrarenal manifestations								
Gene	OMIM entry	Age at ESRD	Isolated NPH	Joubert syndrome	Severe retinal degeneration/ LCA	Tapeto-retinal degeneration	OMA type Cogan II	Liver fibrosis	*Situs inversus*	Meckel-Gruber syndrome	Skeletal anomalies	Other symptoms
NPHP1/ JBTS4/ SLSN1	256100	Juvenile	+	+ (2%)	–	+	+(2%)	–	–	–	–	–
NPHP2/INVS	602088	Infantile	+	–	+ (10%)	–	–	+	+	–	–	VSD, HT, OH
NPHP3/ SLNS3	604387	Infantile/ adolescent	+	+	+ (10%)	+	–	+	+	+	–	congenital heart defects
NPHP4	606966	Adolescent	+	–	–	+	+	+	–	–	–	
NPHP5/ IQCB1	609254	Adolescent	–	–	+ (100%)	–	–	–	–	–	–	
NPHP6/ CEP290	610188	Juvenile	–	+	+ (100%)	+	+	+	–	+	–	congenital heart defects, COACH syndrome
NPHP7/Glis2	611498	Juvenile	+	–	–	–	–	–	–	–	–	–
NPHP8/ RPGRIP1L/ MKS5	611560	Juvenile	+	+	+ (10%)	+	+	+	–	+	–	COACH syndrome, RHYNS-syndrome
NPHP9/ NEK8	613824	Infantile	+	–	+ (30%)	–	–	–	–	+	–	
NPHP10/ SDCC8GA	613615	Juvenile	+	–	+	+	–	–	–		–	BBS-like
NPHP11/ TMEM67/ MKS3	613550	Infantile	+	+	–	–	+	+	–	+	–	COACH syndrome; RHYNS-syndrome
NPHP13/ WDR19	614377	Juvenile	+	+	+	+	–	+	–	–	+	
NPHP14/ ZNF423	614844	Infantile	–	+	+	–	–	–	+	–	–	

(continued)

Table 11.1 (continued)

Gene	OMIM entry	Age at ESRD	Isolated NPH	NPH associated with extrarenal manifestations								
				Joubert syndrome	Severe retinal degeneration/ LCA	Tapeto-retinal degeneration	OMA type Cogan II	Liver fibrosis	*Situs inversus*	Meckel-Gruber syndrome	Skeletal anomalies	Other symptoms
NPHP15/ CEP164	614845	Juvenile	–	+	+	–	–	+	–	–	+	bronchiectasis
NPHP16/ ANKS6	615382	Infantile, juvenile	+	–	–	–	–	+	+	–	–	congenital heart defects
NPHP17/ IFT172	615630	Juvenile	–	+	+	+	–	+	–	–	+	Pituitary hypoplasia
NPHP18/ CEP83	615862	Infantile	+	–	–	+	–	+	–	+	–	Mental retardation, hydrocephalus
NPHP19/ DCDC2	616217	Juvenile	+	–	–	–	–	+	–	–	–	
NPHP20/ MAPKBP1	617271	Juvenile	+	–	–	–	–	–	–	–	–	
NPHP1L/ XPNPEP3	613159	Adult	+	–	–	–	–	–	–	–	–	Myocardiosis, epilepsy
NPHP2L/ SLC41A1	610801	Juvenile	+	–	–	–	–	–	–	–	–	bronchiectasis
TRAF3IP1	616629	Infantil/ juvenile	+	+	+	+	–	+	–	–	+	1 patient BBS-like
AHI1/JBTS3	608629	Juvenile, adult	(+)	+	(+)	(+)	+	–	–	–	+	
CC2D2A/ MKS6	612284	? (no)	no	+	–	–	(+)			+	+	COACH syndrome

BBS Bardet Biedl syndrome; *COACH* (cerebellar vermis hypoplasia, oligophrenia [kognitive dysfunction], ataxia, coloboma, hypotonia) syndrome; *OMA* oculomotor apraxia; *LCA* Leber congenital amaurosis; *OH* oligohydramnious; *OMA* okulomotor apraxia type Cogan II

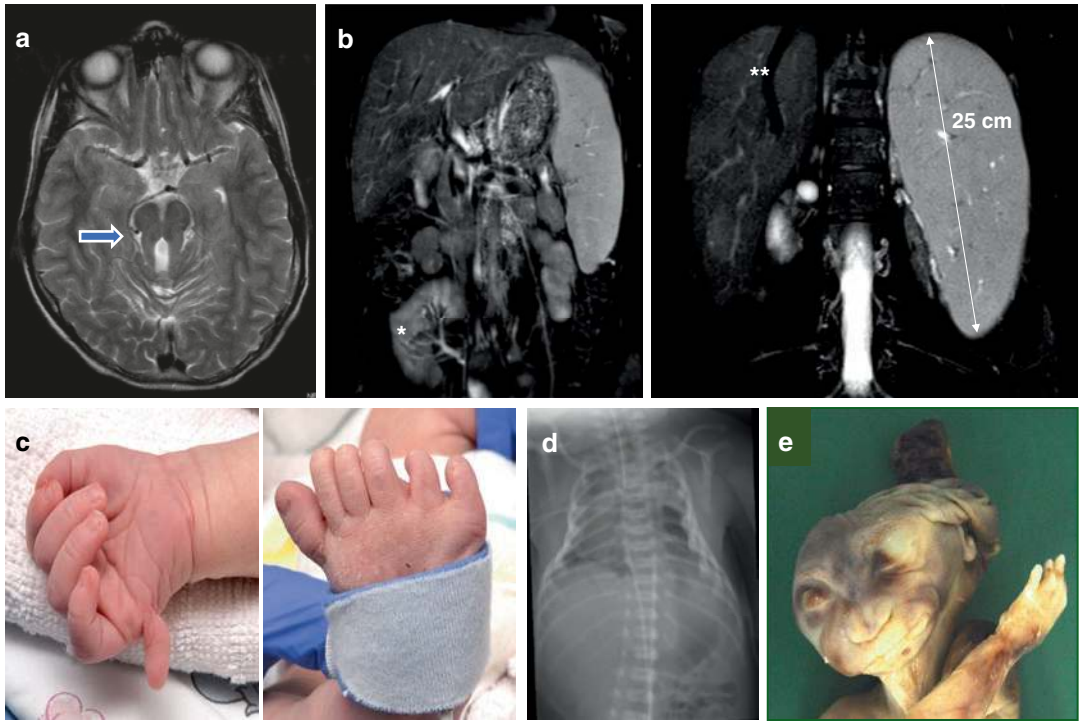

Fig. 11.3 Examples for extra-renal disease manifestations: (**a**) Molar tooth sign in a patient with Joubert syndrome (arrow). (**b**) MRI image showing massive hepatosplenomagly based on congenital hepatic fibrosis in a patient with COACH syndrome; *: transplant kidney; **: transjugular intrahepatic portosystemic shunt (TIPS). (**c**) Postaxial hexadactyly (both feet and hands) in a patient with short-rib polydactyly syndrome. (**d**) Thoracic Hypoplasia in a patient with Jeune syndrome/Jeune Thoracic hypoplasia (JTA). (**e**) Occipital meningoencephalocele and a massively malformed brain resembling anencephaly. (Used with permission of Springer Science + Business Media from Bergmann [33])

cohort however ranges from 6 to 32 years with a median age of 15 years.

A milder retinopathy variant that can also be associated with NPH is referred to as **tapetoretinal degeneration**. Usually patients suffer from slowly progressive severe tube-like restriction of the visual fields and night blindness. Fundoscopy reveals various degrees of atrophic and pigmentary retinal alterations.

Mutations associated with this milder form of Senior-Løken syndrome have been identified in the following genes: *NPHP1, NPHP4, SDCC8G8, WDR19/IFT144* and *TRAF3IP1*. While *SDCC8G8* localizes to centrosomes and cell-cell-junctions in mammalian renal epithelial cell lines and shows interactions with *NPHP5* and *OFD1, WDR19/IFT144* and *TRAF3IP1* are closely linked to intraflagellar transport processes in cilia. Mutations of all three genes cause kidney cysts in zebrafish models [24].

Joubert Syndrome

Joubert syndrome (JS) is an autosomal recessive multisystem ciliopathy with a predicted incidence of 1:55,000–200,000. Clinically JS is characterized by muscular hypotonia, cerebellar ataxia, unusual eye movements, hyperpnea/apnea in infancy, variable degrees of cognitive impairment, speech ataxia and kidney cyst formation. Additionally, certain dysmorphic features have been described such as hypertelorism, broad forehead and unilateral or bilateral ptosis [25]. There is high phenotypic variability even among family members. Neuroimaging (MRI) plays an essential role in the diagnosis of JS since the "molar tooth sign" is pathognomonic and by defi-

nition an essential feature for the diagnosis of JS. It reflects a complex malformation of the midhin brain encompassing cerebellar vermis hypoplasia, increased interpeduncular distance at the pontomesencephalic junction and elongated superior cerebellar peduncles (Fig. 11.3a) [26].

Neurocognitive functioning may range from extremely impaired to almost normal with relative strengths in verbal comprehension and reasoning abilities. Diffuse background slowing in EEG can be found in about one third of patients and is associated with a poorer neurological outcome; however clinical seizures occur in less than 10% of JS patients [27]. A systemic evaluation of the MRI presentation of 110 JS patients revealed the severity of the cerebellar vermis hypoplasia to be associated with the neurodevelopmental outcome [28].

Only about 25% of the patients present an isolated neurological phenotype; the majority of cases is characterized by various phenotypic combinations of additional ocular, kidney and hepatic disease manifestations.

The **ophthalmologic** involvement comprises oculomotor apraxia (78%) with jerking head thrusting and a rotating nystagmus (67%) [29], strabismus (72%) and ptosis (31%). Retinopathy may be present in a subset of patients depending on the underlying genetic defect, with severe forms been associated with *CEP290/NPHP6* and *AHI1* mutations, while in patients with *TMEM67*, *C5orf52* and *KIAA0586* defects no retinal degeneration has been observed so far [25].

Liver disease is observed in 15–45% of JS patients, usually characterized by elevated liver enzymes, increased liver echogenicity and parenchymal stiffness on ultrasound/elastography or splenomegaly). Histopathology resembles the findings seen in ARPKD with ductal plate malformation and portal fibrosis. 15% of patients develop portal hypertension with a need for transjugular intrahepatic portosystemic shunting (TIPS; Fig. 11.3) or liver transplantation. Most patients with severe liver disease carry mutations in the *TMEM67* gene [30].

Kidney involvement is found in 25–30% of JS patients, particularly in those carrying *CEP290/NPHP6*, *TMEM67* and *AHI1* mutations. Most patients display an NPH phenotype with reduced urinary concentrating capacity and slowly progressing GFR decline. Prenatal ultrasound is usually normal and a poor predictor for kidney disease in JS. However, in some patients an ARPKD-like phenotype with enlarged cystic kidneys and early onset hypertension can be observed) [31]. The onset of ESKD ranges from 6–24 years (mean 11.3 ± 4.8 years) and is not necessarily associated with the genotype [32].

So far mutations in 35 different genes are implicated to be causative for JS, which altogether explain 62–94% of the clinical cases [31]. Almost all of these genes show a genetic overlap with other ciliopathy disorders, particularly with Senior-Løken syndrome, COACH syndrome and Meckel-Gruber syndrome. To simplify terminology, the term Joubert syndrome is now used to refer to all patients displaying a molar tooth sign including SLS and COACH syndrome.

For most genes it has proven difficult to identify clear-cut genotype-phenotype correlations. However, for individual genes, such as *CEP290/NPHP6*, the severity of the genetic defect does predict clinical disease severity, with two truncating mutations causing a severe early-onset disorder mimicking a Meckel-Gruber phenotype whereas the presence of at least one missense mutation leads to a milder, late-onset phenotype with limited organ involvement as in NPH [33]. Since *NPHP1* mutations) have also been reported to be a rare cause of JS, the *NPHP1* gene has been referred to as *JBTS4* [29]. However, screening of 117 Joubert syndrome patients revealed *NPHP1* abnormalities only in 2% of cases indicating that this gene is only a minor contributor in the pathogenesis of this disorder [34].

COACH Syndrome

The acronym COACH stands for the clinical key features **c**erebellar vermis hypoplasia, **o**ligophrenia [neurocognitive impairment], **a**taxia, **c**oloboma and congenital **h**epatic fibrosis. COACH syndrome is a rare autosomal recessive disorder that shows substantial overlap with Joubert syndrome and is almost exclusively caused by mutations in the *TMEM67* gene [35]. In contrast to Joubert syndrome, the pathognomonic feature of

this syndrome is an obligatory liver involvement caused by the malformation of the embryonic ductal plate resulting in fibrosis of the liver. Elevated liver enzymes, reduced blood flow in the portal vein and splenomegaly secondary to portal hypertension regularly develop during progression of the disease. Liver transplantation is necessary in most cases in the long run.

Chorioretinal colobomas found in the majority of *TMEM67* patients are typically located inferior to the optic nerve and do not impair vision in most patients. Retinal degeneration seems to be no problem in this particular cohort. Kidney involvement however is observed in 50% of the patients presenting with an NPH phenotype. Just recently, an impaired sense of smell (hyposmia) has been described as a so far underdiagnosed clinical feature associated with *TMEM67* mutations that can further compromise the quality of life of affected patients [36].

Meckel Gruber Syndrome

Meckel Gruber syndrome (MKS) is a neonatally lethal dysmorphic disorder affecting multiple organ systems. It follows an autosomal recessive inheritance with a global incidence of approximately 1:135.000 births. Typical clinical features comprise occipital encephalocele (Fig. 11.3e), bilateral cystic kidney dysplasia, hepatobiliary ductal plate malformation and postaxial polydactyly. Associated features might include severe cardiac anomalies, lung hypoplasia, situs inversus, severe malformations of the central nervous system, hydrocephalus, cleft palate, microphthalmia, retinal colobomas, genital disorders and skeletal deformities [37–39]. Survival beyond the neonatal period is unusual, most affected individuals die *in utero* [40]. However, owing to its allelic overlap with Joubert syndrome, rare cases have survived the first few years of life [25]. Prenatal ultrasound documenting the combination of occipital encephalocele, grossly enlarged hyperechogenic kidneys and polydactyly as well as elevated levels of alpha-fetoprotein in amniotic fluid may lead to early diagnosis. Oligohydramnios is common and often results in Potter's sequence featuring typical facial deformities and joint contractures. Prenatal MRI may be helpful to confirm typical additional malformations. Prenatal genetic testing is only useful when positive because the associated genes identified so far only account for 50–60% of cases [25].

Mutations in 13 genes responsible for MKS-like phenotypes have been reported to date: *MKS1, MKS2/TMEM216, MKS3/TMEM67, MKS4/CEP290, MKS5/RPGRIP1L, MKS6/CC2D2A, MKS7/NPHP3, MKS8/TCTN2, MKS9/B9D1, MKS10/B9D2, MKS11/TMEM231, MKS12/KIF14, MKS13/TMEM107.* Besides genetic heterogeneity, there is significant clinical overlap with NPH and Joubert syndrome (Fig. 11.4). Current data suggest that severe truncating mutations cause MKS whereas milder, non-truncating mutations cause Joubert or isolated NPH syndromes [33]. In fact, there are even some families in which one child is diagnosed with Joubert syndrome and another with MKS, indicating that genetic modifiers also influence the clinical phenotype [41].

Congenital Oculomotor Apraxia Type Cogan II

Congenital oculomotor apraxia (COMA) type Cogan II is characterized by the impairment of horizontal voluntary eye movements, ocular attraction movements, and optokinetic nystagmus [42]. Compensation for the defective horizontal eye movements is accomplished by jerky movements of the head. The disease is not progressive, and most older patients compensate the impaired eye movements by an over-shooting thrust of the eyeballs rather than by head jerks. The condition can improve with age. Some individuals with COMA type Cogan II have an increased risk to develop chronic kidney failure due to NPH. Hence, kidney function should be analysed at regular intervals in this condition.

Recently, genetic variants in the *SUFU* gene have been reported as the first disease-specific genetic cause of COMA without associated kidney disease [43]. In addition, genetic variants in different NPHP genes have been reported in association with COMA type Cogan II including *NPHP1, NPHP4, NPHP6/CEP290* and *NPHP8* [44]. Particularly, deletions in *NPHP1* have been

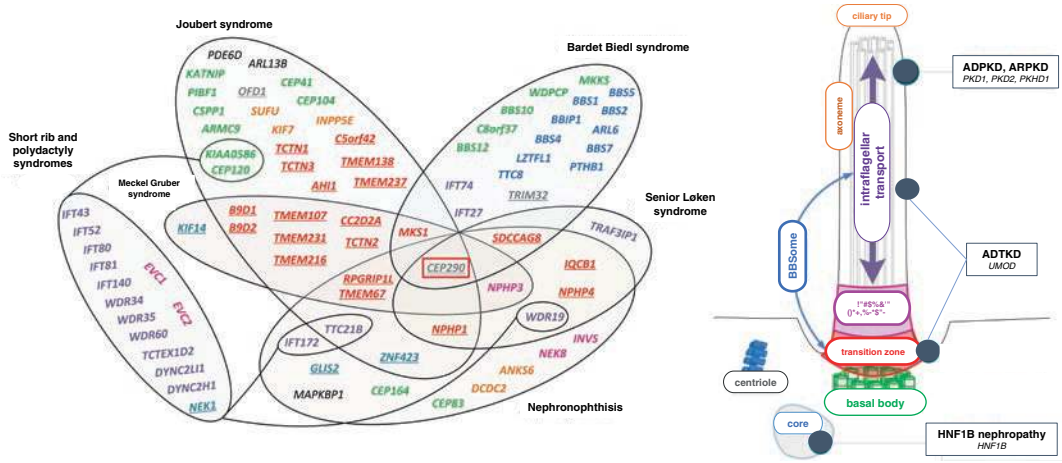

Fig. 11.4 Venn diagram illustrating the genetic and clinical overlap in hereditary renal ciliopathies. Colours indicate the ciliary location of corresponding gene products

described in quite a few affected individuals [4]. However, the vast majority of clinical cases remain genetically unsolved.

Since usually oculomotor apraxia precedes the kidney phenotype by many years but all *NPHP1* deletion carriers developed ESRD in the long run, genetic testing appears to be justified in all patients presenting COMA in early infancy, even though it will only be positive in a minority of patients.

RHYNS Syndrome

RHYNS syndrome was defined in 1997 as the combination of Retinitis pigmentosa, Hypopituitarism, Nephronophthisis and mild Skeletal dysplasia in a 17-year-old man [45]. Four years later, two brothers with a similar clinical picture were reported [46]. Just recently a whole exome sequencing approach revealed biallelic *TMEM67* variants in the index patient reported 20 years before [47].

Previously, growth hormone deficiency associated with an absent, small or ectopic pituitary gland was observed in individual JS patients and associated with variants in the ciliary genes *KIAA0753* and *CELSR2* [48, 49].

Skeletal Ciliopathies

Skeletal ciliopathies represent a distinct group of hereditary disorders clinically characterized by abnormal bone growth resulting in a long narrow chest with short ribs, short stature associated with disproportionately short limbs and polydactyly. Specific disorders in this group include the asphyxiating thoracic dystrophy (Jeune's syndrome), cranioectodermal dysplasia (Sensenbrenner syndrome), Ellis-van Creveld syndrome (EVC) and various types of short rib-polydactyly syndromes (e.g. Mainzer-Saldino syndrome) [50]. Molecular defects in 26 genes have been identified so far mostly encoding components of the ciliary transport machinery [51, 52].

Bone disorders can be accompanied by non-skeletal features including NPH leading to ESKD, cystic kidney disease, congenital hepatic fibrosis, retinal degeneration, neurological abnormalities, cardiac anomalies, cleft lip/palate and other oral defects. Other rare features include fibrocystic changes in the pancreas, ambiguous genitalia, anal atresia, polyhydramnios, malrotation, and hydrops fetalis [25]. Owing to the severity of pulmonary insufficiency associated with asphyxiating thoracic dystrophy, high levels of

perinatal lethality are observed. In cranioectodermal dysplasia (Sensenbrenner syndrome) most affected children develop early-onset NPH leading to ESKD in infancy or early childhood [51].

Respiratory Phenotype

Typically, defects of motile cilia are responsible for chronic respiratory problems subsumed under the term primary ciliary dyskinesia (PCD), a clinical entity characterized by impaired mucociliary clearance, chronic respiratory infections and distinctive low nasal nitric oxide (nNO) levels [53]. However, functional assessment of patients with renal ciliopathies revealed that molecular defects classically associated with non-motile ciliopathies can also have an impact on motile ciliary function.

Future Challenges: Genetic and Phenotypic Heterogeneity

Although the clinical characteristics of the different hereditary disease entities appear quite discriminative, there is significant genetic as well as phenotypic overlap that hampers an early diagnosis and individual management [50] (Fig. 11.4). No doubt, the tremendous progress of genetics in the last two decades had major impact on the molecular understanding of NPH-related ciliopathies. However, the new insights also further complicated the clinical situation for physicians dealing with affected individuals: It has become increasingly evident that so far well-defined clinical entities can be caused by mutations in multiple genes. Vice versa, mutations in the same gene can cause very different phenotypes that range from lethal early embryonic multivisceral manifestations to single organ involvement starting in adolescence [33] (Fig. 11.4). This complexity has been attributed to allelic heterogeneity, locus heterogeneity, reduced penetrance, variable expressivity, modifier genes, and/or environmental factors [54]. In clinical terms, this implies that the traditional approach using textbook signs and symptoms to guide diagnosis and management is no longer sufficient [56]. From the patient's perspective, the "diagnostic odyssey" does not necessarily end with the identification of a disease-causing gene defect [55]. Novel approaches are needed that should combine a deep and comprehensive clinical characterization with careful genotyping in order to discover non-obvious phenotypes and to determine precise diagnoses.

Large-scale collaborative efforts linking comprehensive clinical data collection with translational research will be fundamental to identify novel therapeutic targets and develop disease specific treatment approaches. Several national initiatives have started to address these questions, including the German NEOCYST consortium and the Dutch KOUNCIL initiative [57].

Therapy

So far, there is no specific therapy correcting the genetic or functional defects in NPH or NPH-related ciliopathies. Thus, in the early disease stage without kidney impairment, the main goal is the correction of water and electrolyte imbalances by replacing ongoing losses of water and salt due to the reduced urinary concentrating capacity.

The recent molecular insights into the pathogenesis of NPH have lead to potential novel therapeutic strategies addressing various intracellular signalling pathways. Yet, despite promising preclinical results, no clinical trials including NPH patients have been initiated to date.

Once the disease has progressed to ESKD, renal replacement therapy is required and early, if possible pre-emptive transplantation is the therapy of choice. NPH patients are not at risk of recurrence of the primary disease and the outcome of transplantation is excellent.

Autosomal Dominant Tubulointerstitial Kidney Disease (ADTKD)

Autosomal dominant tubulointerstitial kidney diseases (ADTKD) encompass a group of rare disease entities characterized by autosomal dom-

inant inheritance, tubular damage, interstitial fibrosis, the absence of glomerular lesions and a slow progression towards ESKD in late adulthood [58]. Although the clinical and histological presentation may show significant overlap with NPH [59, 60], there are two major differences: NPH refers to autosomal recessive conditions that typically present in childhood and in most cases lead to ESKD before adulthood whereas ADTKD is inherited in an autosomal dominant fashion and typically presents later in life. However, as an exception to this rule even children younger than 10 years can occasionally present clinical symptoms from ADTKD.

Recent data suggest that ADTKD qualifies to be one of the most common genetic disorders in adulthood, accounting for about 5% of genetically caused ESKD cases. So far five genes have been identified in which variants lead to ADTKD: *UMOD*; *REN*; *MUC*; *HNF1B* and *SEC61A1* [61].

Terminology and Classification

In the past, the terminology for this heterogeneous disease complex has been inconsistent and confusing. The term "medullary cystic kidney disease (MCKD)" was commonly used but fell out of fashion because the occurrence of medullary cysts is not an obligatory feature—in fact most patients with ADTKD show no cysts at all. Another term usually used by paediatric nephrologists was "familial juvenile hyperuricaemic nephropathy (FJHN)", describing a condition that is synonymous to what adult nephrologists called MCKD.

Only recently, the introduction of a gene-based, unifying terminology simplified the communication between disciplines and resulted in an increasing number of reported cases. Of note, following the KDIGO terminology, when an affected gene is identified it should be added as a suffix to the term ADTKD for further subclassification [58, 61]. To date, at least five subtypes can be distinguished (Table 11.2). Yet, about 50–60% of clinical ADTKD cases remain genetically unsolved, pointing to more causative genes to be identified in the future.

Pathophysiology

UMOD encodes uromodulin (previously known as Tamm-Horsfall protein), which is expressed

Table 11.2 Classification of ADTKD

Gene (OMIM-ID; chromosome)	KDIGO terminology	Former terminology	Protein	Protein function
UMOD (*191845; 16q12)	ADTKD-*UMOD*	MCKD2 FJHN	Uromodulin	• Regulates transport and blood pressure • Protection against urinary tract infections • Protection against kidney stones • Regulation of innate immunity
MUC1 (*158340; 1q22)	ADTKD-*MUC1*	MCKD1	Mucin 1	• Protection of epithelial mucus barrier • Immunomodulatory propertiesSignal transduction
HNF1B (*189907; 17q12)	ADTKD-*HNF1B*	–	Hepatocyte nuclear factor 1β	• Transcription factor involved in the development of neural tube, pancreas, gut, liver, lung, kidney and genital tract
REN (*179820; 1q32)	ADTKD-*REN*	–	Preprorenin	• Protease, cleavage of angiotensinogen (renin–angiotensin–aldosterone axis) • Role in nephrogenesis
*SEC61A1 (*609213; 3q21.3)*	ADTKD-*SEC61A1*	–	Alpha1 subunit of *SEC61*	• Alpha1 subunit of SEC61channel forming translocon complex that mediates transport of signal peptide-containing precursor polypeptides across the ER

Modified from Devuyst O, Olinger E, Weber S, Eckardt KU, Kmoch S, Rampoldi L, Bleyer AJ. Autosomal dominant tubulointerstitial kidney disease Nat Rev. Dis Primers. 2019 Sep 5;5(1):60

exclusively at the luminal side of renal epithelial cells of the thick ascending limb of the loop of Henle. Uromodulin is the most abundant protein in the urine of humans [62]. Studies in *UMOD*-knockout mice suggest that uromodulin has multiple physiological roles, including protection against urinary tract infections and kidney stones by direct binding of either fimbria from Escherichia coli bacteria and preventing the adhesion of these bacteria to urothelial cells or direct binding of calcium oxalate crystals [63, 64]. Furthermore, uromodulin plays an important role in maintaining the water-tight integrity of the thick ascending limb. At the same time, it appears to facilitate the transport of the NaK2Cl-Cotransporter [65] and the ROMK potassium channel to the surface of epithelial cells in the thick ascending limb [66]. Subsequently, in the scenario of defective uromodulin, restricted urinary concentration and mild volume depletion evoke an increased reabsorption of uric acid in the proximal tubule potentially explaining hyperuricemia and gout observed in 25–75% of affected patients [67]. Mutated uromodulin proteins are unable to leave the endoplasmic reticulum, resulting in accumulation of abnormal uromodulin within the epithelial cells, followed by cellular atrophy and death. This might explain the progressive chronic kidney failure of *UMOD* patients.

Mutations in the ***MUC1*** gene on chromosome 1q21 have been identified as causative of what in the past was referred to as medullary cystic kidney disease 1 (MCKD1). The majority of affected individuals show a single cytosine insertion into one variable-number tandem repeat sequence within the *MUC1* coding region. Mucin 1 is expressed intracellularly in the secretory epithelia of multiple organs, e.g. the lungs, the stomach, the intestine and the distal tubule and collecting duct of the kidneys. For so far unexplained reasons, clinical sequelae are limited to the kidneys [61]. Mutations in the *MUC1* gene result in an abnormal mucin 1 protein. The mucin 1 protein seems to play an essential role for the protection of the epithelial mucus barrier but also in signal transduction and immunomodulation. Since knockout studies in mice indicated that mucin 1 is not an essential protein, a dominant negative or gain-of function effect of *MUC1* mutations is discussed [68].

The **hepatocyte nuclear factor 1B** (HNF1B) is a ubiquitous transcription factor mainly involved in the early development of multiple organs, primarily of the neural tube, the pancreas, the gut, the liver, the lungs, the kidneys and the genital tract. Variants in the *HNF1B* gene represent the most important genetic cause of congenital anomalies of the kidney and urinary tract (CAKUT) in children. Its precise prevalence as a cause of ADTKD is hard to determine owing to a high rate of assumed undetected cases and numbers range from 5 to 31% depending on the study cohort [69].

Mutations in the ***REN*** gene leading to ADTKD are extremely rare and have been identified only in about 20 families worldwide. It appears that these mutations result in a disrupted translocation of preprorenin into the endoplasmic reticulum of renin expressing cells. Subsequently, the cleavage of pre-prorenin into prorenin and further into renin is blocked. Renin as part of the renin-angiotensin-aldosterone system (RAAS) has a high impact on nephrogenesis, blood pressure control, thirst regulation and erythropoiesis. As a consequence, ADTKD-*REN* is characterized by early onset low-erythropoietin anaemia and slowly progressive CKD accompanied by arterial hypotension and hyperkalaemia. Anaemia in ADTKD-*REN* resembles that observed in haemodialysis and patients receiving long-term angiotensin converting enzyme (ACE) inhibition, since the RAAS contributes to erythropoietin production [70]. At the same time the accumulation of preprorenin in renal tubular cells leads to apoptosis of these cells [71]. Because renin is essential for nephrogenesis, homozygous mutations of renin cause recessive renal tubular dysgenesis and are almost always incompatible with life [72]. In ADTKD-*REN* however, production of wildtype renin occurs via one allele allowing kidney development [61].

Mutations in the ***SEC61A1*** gene are even rarer and have so far only been reported in two families characterized by congenital anaemia, neutropenia and tubulointerstitial kidney disease as well

as another two families with a defect in plasma cell development, recurrent infections and an unknown kidney phenotype [73, 74]. *SEC61A* encodes the alpha 1 subunit of the heterotrimer SEC61, that is part of the ER translocon responsible for the transport of newly synthesized secretory proteins into the endoplasmatic reticulum. Mutations of *SEC61A* lead to aggregation of the altered protein in the ER and by affecting the translocon pore it appears to have a functional impact on post-translational modifications, folding and sorting of various proteins, including renin, uromodulin and mucin1. In addition, alterations in the Ca^{2+} homeostasis and energy metabolism have also been reported [75].

Clinical Presentation

Clinical symptoms are similar in all disease variants and may resemble the phenotype of NPH. Usual findings include slowly progressing chronic kidney disease, bland urine sediment, polyuria due to reduced urinary concentrating capacity and a family history compatible with an autosomal dominant inheritance pattern. If significant proteinuria or hematuria is observed, alternative causes should be ruled out. On ultrasound small cortico-medullary cysts might develop during the course of the disease but these are not a prerequisite for the diagnosis. Kidney size is normal or slightly reduced. Histological findings comprise tubular basement membrane disintegration, tubular atrophy with cyst development, and interstitial round cell infiltration associated with fibrosis resembling the findings observed in "classical" NPH. Thus, imaging and histological findings cannot confirm the diagnosis, and analysis of the clinical and pedigree data is mandatory. A definite diagnosis can be achieved by genetic testing (Table 11.3). However, for economic reasons as well as in view of the methodological difficulties associated with the genetic evaluation of *MUC1* in particular, genetic confirmation is still heavily restricted in adults.

Clinical Presentation of ADTKD-*UMOD*

Patients affected by *UMOD* mutations are typically characterized by juvenile onset of hyperuricemia, gout, and progressive kidney failure. Clinical features of both conditions vary in presence and severity. As mentioned above, the demonstration of kidney cysts is not obligatory and may differ within the same pedigree [76].

In 2004, Scolari et al. reported 205 patients from 31 families with *UMOD* mutations of whom 75% showed hyperuricemia and 65% gout, although in a subset of families neither symptom was observed. Chronic kidney disease was present in 70% of patients, leading to ESKD in 80% of these between 20 and 70 years of age [77]. Comparable findings were reported in a series of 109 patients from 45 families and in an Italian kindred with ten affected individuals, with median ages at ESKD of 54 (range 25–70) [78] and 31 (16–54) years [79], respectively. The clinical course appeared more severe in homozygotes from consanguineous families. In a large Spanish kindred with *UMOD* mutations one individual with recessive (bi-allelic) *UMOD* mutations was identified. The homozygous individual survived to adulthood and presented with an earlier onset of hyperuricemia and faster progression to ESKD than heterozygous individuals [80].

Mild urinary concentrating defects are common and may manifest by persisting enuresis [77]. MRI and ultrasound-based imaging studies on 12 individuals with *UMOD* mutations revealed small kidneys, decreased parenchyma, or cysts in all families. Kidney histology showed microcysts in 4 out of 12 cases and in the others dilated or atrophic tubules, global sclerosis, extensive tubulo-interstitial atrophy with fibrosis, and signs of chronic diffuse inflammation [62].

Clinical Presentation of ADTKD-*MUC1*

In contrast to patients with *UMOD* or *REN* gene mutations, slowly progressive chronic kidney disease is the first and main symptom in patients with *MUC1* mutations. Hyperuricemia and gout, if present at all, develop later in the course of the

Table 11.3 Clinical and laboratory findings in autosomal dominant tubulointerstitial kidney disease (ADTKD)

ADTKD type	OMIM entry	Clinical features	Age at ESRD	Laboratory findings	Renal Imaging	Other findings
ADTKD-*UMOD*	603860	• Teenage onset gout • Polyuria/polydipsia • Enuresis	16–70 years. (median 42)	• Hyperuricemia • Low urinary levels of uromodelin	Normal (50%) or small kidneys, corticomedullary cysts (40%), echogenicity ↑ (10%)	Intracellular deposits of uromodelin in thick ascending limb (TAL) of Henle
ADTKD-*MUC1*	174000	• Gout	50–76 years (median 62)	• Hyperuricemia	Normal (50%) or small kidneys, corticomedullary cysts (40%)	Intracellular deposits of MUC1fs in TAL and extra-renal tissue
ADTKD-*REN*	613092	• Childhood anaemia • Mild hypotension • Gout in adolescence	43–68 years (median 57)	• Anaemia • Mild hyperkalaemia • Hyperuricemia • Low to low-normal plasma renin levels	Normal or small echogenic kidneys, no cysts	Reduced renin-staining in juxtraglomerular apparatus
ADTKD-*HNF1B*		• CAKUT • Childhood CKD • Genital abnor-malities in girls • Syndromic features in those with 17q12 deletion	0-?	• Hypomagnesemia • Hyperuricemia • Hypokalaemia • Elevated liver enzymes • MODY	Small to normal echogenic kidneys with cortical and medullary cysts	–
ADTKD-*SEC61A1*		• Intrauterine and postnatal growth retardation • Recurrent abcess formation • Polydactyly	NA	• Congenital anaemia • Leuco−/neutropenia	Normal or small echogenic kidneys, with cortical and medullary cysts	• Cleft palate • Bifid uvula • Mild cognitive impairment

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CAKUT Congintal Anomalies of the Kidney and Urinary Tract; *MODY* Maturity Onset Diabetes of the Young

disease. Other clinical manifestations are uncommon. This was illustrated by the examination of six large Cypriot families including 72 affected individuals, in whom *MUC1* was first localized as the causative gene [81]. The disease led to ESKD at a mean age of 54 years, ranging from 36 to 80 years. The annual loss of estimated GFR is comparable in ADTKD-*UMOD* and ADTKD-*MUC1*, amounting to 3–4 mL/min/1.73 m^2 on average [67]. Arterial hypertension was found in 51% of affected individuals, and strictly related to renal function. Hyperuricemia and gout were no early findings, although the prevalence of hyperuricemia increased at approximately the same rate as in other causes of chronic kidney disease. Cysts were detected sonographically in 40% of tested gene defect carriers. Mainly corticomedullary or medullary, but also cortical cysts were reported. Approximately half of the affected individuals had normal sized kidneys with no cysts, while 11% had small echogenic kidneys without cysts.

In another 23 kindreds with 128 affected individuals, ESKD was reached at a median age of 32 years [82], ranging from 5 to 76 years. Kidney biopsy revealed histological findings identical to those found in "classical" NPH. Hyperuricemia was not a consistent finding and only reported in eight families. Hypertension was present in affected individuals from 13 families.

Clinical Presentation of ADTKD-*Ren*

Patients with *REN* gene variants present a very similar phenotype as *UMOD* patients with early onset gout and development of chronic kidney disease. However, *REN* patients are somewhat older when presenting with gout for the first time (20–30 years) and kidney failure progression is slower. ESKD usually occurs after the age of 40 years. Unlike *UMOD* patients, *REN* mutations lead to additional clinical symptoms caused by the low serum levels of renin and angiotensin. A nearly universal manifestation is the presence of hypoproliferative anaemia (haemoglobin range 7–11 g/dL) in early childhood that often resolves in adolescents, likely due to the increased sex steroid and subsequent erythropoietin production [71]. Additionally, some patients present with low blood pressure, mild hyperkalemia responsive to fludrocortisone and an increased risk of acute kidney injury in a setting of volume depletion or application of non-steroidal inflammatory drugs.

Clinical Presentation of ADTKD-*HNF1B*

Variants in the *HNF1B* gene represent the most prevalent genetic cause of congenital anomalies of the kidney and urinary tract (CAKUT). Inheritance follows an autosomal dominant trait, but about 50% of patients carry *de novo* mutations with no positive family history. The clinical spectrum is highly heterogeneous and can vary significantly even between family members [83–86].

Initially, *HNF1B* variants have been described in the context of familial forms of maturity onset diabetes mellitus (MODY diabetes). Since many of the affected patients also presented malformations of the kidneys and the urinary tract, the term Renal Cyst and Diabetes Syndrome (RCDS) was introduced to refer to this clinical association [83]. The spectrum of kidney malformations observed in this context is highly variable: The majority of patients present bilateral kidney dysplasia in association with cortically located cysts. However, isolated kidney dysplasia, unilateral multicystic dysplastic kidneys, glomerulocystic changes, various urinary tract malformations and unilateral agenesis of the kidney have also been described. Prenatal ultrasound often reveals hyperechogenic kidneys with or without the evidence of cysts [84–86]. In contrast to ARPKD, kidneys usually are not enlarged, and amniotic fluid is preserved in most cases. Noteworthy, in many girls there is an association with malformations of the female genital tract [89, 90]. Thus, abdominal ultrasound should include a statement on potential genital abnormalities. Vice versa, the additional detection of such malformations increases the probability of an underlying *HNF1B* gene defect significantly.

The term HNF1B nephropathy can be misleading since many patients present a systemic disease rather than an isolated kidney phenotype [87, 88]. In addition to MODY-type diabetes and genital malformations, this primarily concerns

hypomagnesemia caused by tubular magnesium loss, hyperuricemia, pancreatic changes and concomitant hepatopathy with elevated liver enzymes. Furthermore, autism spectrum disorders have been observed in some patients [91, 92]. However, extra-renal manifestations are not obligatory findings and might only develop late in the disease process, if at all. Nevertheless, regular screening is recommended. Additionally, owing to its dominant inheritance, genetic counseling and the clinical examination of other family members might be indicated [93].

Regarding kidney function, only a minority of patients experience severe forms with the need of renal replacement therapy in infancy or early childhood. In most children a stable or only slowly declining GFR is observed for many years with preserved kidney function until adolescence at least [94]. However, HNF1B gene defects may be a relevant contributor to chronic kidney disease in adulthood, as has only recently been elucidated [94].

Clinical Presentation of ADTKD-*SEC61A1*

So far only two families with ADTKD-*SEC61A1* have been reported. The phenotype of the first family was characterized by congenital anaemia in conjunction with intrauterine and postnatal growth retardation, cleft palate, bifid uvula, preaxial polydactyly and mild cognitive impairment [73]. In the second family, congenital anaemia was accompanied by neutropenia and recurrent cutaneous abscess formation during childhood. Gout was also a common feature in affected family members; however, growth and cognitive development were normal in this family [74].

Diagnostic Work-Up and Genetic Testing

In the presence of a tubulointerstitial disease with slowly progressive CKD, the first diagnostic step is to rule out disease entities with a higher prevalence by simple measures like urinalysis and ultrasound: While the detection of haematuria and proteinuria indicate glomerular rather than tubular disease, enlarged kidney volume in combination with cystic lesions should guide the diagnosis to ADPKD in place of ADTKD. Next, family history—if positive at all—should be consistent with dominant inheritance to rule out NPH. Finally, while teenage-onset hyperuricemia and a medical history for gout hint towards underlying *UMOD* mutations, childhood anaemia and arterial hypotension may be clinical indicators for ADTKD-*REN* [61].

Yet, clinical phenotyping will only be able to provide diagnostic clues and neither extensive imaging nor a comprehensive histological work-up are sufficient to pin down a final diagnosis. Interestingly, measurement of reduced urinary uromodulin concentrations as well as negative MUC1 staining in uroepithelial bladder cells have been used to facilitate diagnosis in ADTKD-*UMOD* and ADTKD-*MUC1* individuals, respectively [95, 96]. However, both methods are not routinely accessible.

Thus, for the confirmation of the diagnosis ADTKD, the identification of the underlying genetic defect is mandatory. Although expensive, methodically difficult and so far without clear-cut therapeutic consequences, the confirmation of a molecular diagnosis helps to avoid unnecessary invasive procedures, to guide further extra-renal diagnostics, to provide personal counselling and to evaluate family members as potential kidney donors [61]. However, it should be pointed out that genetic testing of asymptomatic children for conditions in which there is no specific treatment is generally discouraged [97].

Therapy

As in NPH, there is no specific therapy correcting the genetic or functional defects in ADTKD so far. Symptomatic treatment strategies include the management of hyperuricemia, gout and progressive kidney failure. Hyperuricemia should be treated with allopurinol or other urate-lowering medication to avoid tophus development. However, whether this treatment also slows down the decline of kidney function is still under debate [98].

CKD management should include blood pressure control, correction of electrolyte abnormalities, free water supply to compensate for urinary concentration defects, cautious use of diuretics due to the associated risk of salt loss and hyperuricemia as well as a general avoidance of NSAIDs. Whether or not angiotensin receptor inhibition can be nephroprotective in this particular non-proteinuric cohort still needs to be elucidated. However, if angiotensin receptor blockers are used, losartan is the drug of choice in patients with hyperuricaemia since this compound increases urinary urate excretion [99].

Children with ADTKD-*HNF1B* and ADTKD-*REN* disease are likely to benefit from early management by neonatologists and paediatric nephrologists, including postnatal ventilation and circulation management as well as neonatal dialysis when required [58].

In ESKD, kidney transplantation is the method of choice for ADTKD patients since the disease does not recur in transplanted kidneys. Genetic testing for family members of patients with ADTKD is mandatory before a living donation.

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Part IV

Glomerular Disorders

Hematuria and Proteinuria

12

Hui-Kim Yap and Perry Yew-Weng Lau

Introduction

The presence of blood or protein in the urine may be just a normal transient finding in children, usually accompanying a non-specific viral infection. More importantly, these findings may be an indicator of a kidney or urinary tract disorder. Macroscopic hematuria or the incidental finding of hematuria or proteinuria on urine dipstick examination is often an alarming occurrence to parents, bringing the child to medical attention. The etiology of hematuria and proteinuria includes a long list of conditions. Workup can be extensive, expensive and unnecessary as most children with isolated hematuria or isolated proteinuria do not have significant kidney disease and abnormal urine findings usually resolve on repeated testing. Conversely, persistent proteinuria or even persistent microscopic hematuria can be an indicator of significant glomerular disease, as well as associated with an increased risk for end-stage kidney disease (ESKD).

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Hematuria

In a normal person, very few red blood cells are excreted into the urine. The passage of red blood cells (diameter 6–8 μm) through the glomerulus into the urinary space is mostly prevented by endothelial fenestrations (50–100 nm) of the glomerular filtration barrier. Increased passage of red blood cells into the urinary space can be due to glomerular diseases or conditions affecting the lower urinary tract. Glomerular hematuria can be due to structural modification of components of the glomerular filtration barrier (e.g. α-chain of type IV collagen in thin basement membrane disease and Alport syndrome) or increased inflammatory response leading to damage of capillary endothelium and glomerular basement membrane (e.g. in primary glomerulonephritis and autoimmune conditions).

Macroscopic hematuria is visible while microscopic hematuria is usually detected by a urine dipstick test during a routine examination or by microscopic examination of the urine sediment. As little as 1 mL of blood per liter of urine can produce a visible change in the urine color. If fresh blood is present in the urine, the urine will be pink or red. If left standing, even in the bladder, the urine will develop a hazy smoky or brown color. The brown color comes from the metheme derivative of the oxidized heme pigment. Some pigments and crystals, when present at a significant concentration, will cause color changes in

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Table 12.1 Causes of discoloration of urine

Dark yellow or orange urine	Normal concentrated urine Rifampicin Carotene Pyridium Warfarin
Dark brown or black urine	Bile pigments Methemoglobinemia Alanine, resorcinol Laxatives containing cascara or senna Alkaptonuria, homogentisic acid, melanin, Tyrosinosis Thymol Methyldopa metabolite Copper Phenol poisoning
Red or pink urine	Red blood cells (hematuria) Free hemoglobin (hemoglobinuria) Myoglobin (myoglobinuria) Porphyrins Urates in high concentration (may produce a pinkish tinge) Foods (e.g. beetroot, rhubarb, blackberries, red dyes) Drugs (e.g. benzene, chloroquine, desferoxamine, phenazopyridine, phenolphthalein)

the urine that can be misinterpreted as hematuria. Discoloration of urine can be due to intravascular hemolysis, rhabdomyolysis, metabolic disorders and a number of foods and drugs (Table 12.1).

Definition

The definition of hematuria is based on urine microscopic examination findings of red blood cells and it varies according to the method of quantification. The most commonly accepted upper limits of normal for urinary red blood cells are three red blood cells per high power field in fresh centrifuged urine [1]. There is some controversy as to the amount of red blood cells required for the diagnosis of microscopic hematuria. A population-based study of over 12,000 children in Texas by Dodge et al. [2] recommended five or more red blood cells per high power field in at least two of three consecutive fresh centrifuged urine obtained at least 1 week apart, as the definition of hematuria to capture children with significant disease. A study of 8954 Finnish school children by Vehaskari et al. [3] defined hematuria as more than 5 red blood cells/0.9 mm^3 in a fresh uncentrifuged midstream urine specimen and this identified all children with kidney disease if the urine sample was positive twice in a 6-month period. A study using more stringent criteria has greater positive predictive value with regard to presence of disease, but loses some negative predictive value. Regardless of the criterion used, important cofactors to consider when a child has microscopic hematuria include the presence of proteinuria, urinary casts, hypertension, a family history of kidney disease and other clinical or laboratory findings suggestive of kidney or urinary tract disease.

Urine Dipstick

The urine dipstick utilizes the peroxidase-like activity of hemoglobin present in the urine. Hemoglobin peroxidase activity converts the chromogen tetramethyl benzidine incorporated in the dipstick into an oxidized form, resulting in a green-blue color. The test depends on the presence of free hemoglobin, which comes from hemolysis of the red blood cells in the urine. It is assumed that when there is significant hematuria, some of the red blood cells will always lyse and there will be sufficient free hemoglobin released to cause a positive test. The test is very sensitive, capable of detecting as little as 150 μg/L of free hemoglobin. As few as two to three red blood cells per high power field can make the urine dipstick positive.

It is important to follow the manufacturer's instructions of the dipstick. Delayed reading may produce false positive results. Positive results can also occur in hemoglobinuria following intravascular hemolysis or in myoglobinuria after rhabdomyolysis. Positive results can also be due to the presence of oxidizing agents in the urine such as hypochlorite (cleaning solution) and microbial peroxidases associated with microbial contamination, including urinary tract infection (UTI).

Conversely, false negative results can be due to the presence of large amounts of reducing

agents such as ascorbic acid or urine with high specific gravity, in which the dipstick test is less sensitive.

Due to the very sensitive nature of the urine dipstick test, it is unwise to investigate based on a "trace" reading on the dipstick. Similarly, a child with dipstick reading of "1+" on one occasion and negative readings on subsequent dipstick testing is unlikely to benefit from further investigations. Only if the urine dipstick reading for blood is persistently greater than "trace" is further evaluation warranted. In clinical practice, it is important to confirm hematuria with urine microscopic examination. An absence of red blood cells in the urine with a positive dipstick reaction in a child with red or brown urine may suggest hemoglobinuria or myoglobinuria.

Urine Microscopy

Microscopic examination of the urine sediment is important in diagnosing and evaluating hematuria. When abundant, red blood cells are easy to identify by their characteristic biconcave disc appearance under microscopy. When scanty, red blood cells become distorted in the urine and it is difficult to differentiate them from other unidentified small objects.

Urine centrifugation is one way to solve this problem. After centrifugation and removal of supernatant, the deposit is resuspended in the remaining urine and examined under the microscope. Urine microscopic examination can have false negative results when the urine is of low specific gravity or has an alkaline pH. These conditions result in red blood cells hemolysing rapidly in standing urine, resulting in a positive urine dipstick test due to the free hemoglobin, but without the characteristic red blood cells seen by microscopy.

The morphology of the red blood cells may help identify the origin of the bleeding [4, 5]. Red blood cells from the lower urinary tract maintain their morphology whereas red blood cells from the glomeruli show great variation in shape, size and hemoglobin content due to sheering stresses on their surface in their passage from the capillary lumen through gaps in the glomerular filtration barrier into the urinary space [6]. Phase-contrast microscopy on freshly voided urine allows this differentiation. Red blood cells that are more than 90–95% isomorphic (i.e., of normal size and shape) are most commonly from the lower urinary tract. If more than 30% of dysmorphic red blood cells (blebs, budding and segmental loss of membrane with reduction in red cell volume) are present, the hematuria is more likely to be of glomerular origin [7].

The presence of casts, other cells and crystals in the urine can be helpful. Red blood cell casts are always pathological and usually suggest glomerulonephritis. Identification of red blood cell casts should be done on fresh urine or acidic urine stored at 4 °C, as red blood cell casts disintegrate readily in alkaline urine, taking on a granular appearance. Hence the finding of granular casts in association with hematuria may indicate that the blood has originated from the kidneys. The low rate of red blood cell cast identification using conventional microscopy is probably related to centrifugation of the urine specimen at a low speed of 400 g. A study using high speed centrifugation at 2000 g for microscopy was able to increase the red blood cell cast yield in the urine [8]. Hyaline casts are associated with proteinuria, and a few such casts may be found in concentrated early morning samples from healthy people. When white blood cells are also present in the urine, infection and interstitial or glomerular inflammatory disorders should be considered. Interstitial nephritis is even more likely if Wright stain of the urine shows the presence of eosinophils. Infections and poststreptococcal nephritis often have neutrophils on urinalysis. If the child has other findings suggestive of nephrolithiasis, the shape of the crystals may help to identify the chemical nature of the calculi. Calcium oxalate crystals may point to hypercalciuria.

Etiology

A practical approach is to determine whether the hematuria is of glomerular or non-glomerular origin. Non-glomerular bleeding occurs from the urinary tract, which includes the collecting sys-

Table 12.2 Causes of hematuria in children

Glomerular		Nonglomerular
Familial hematuria disorders • Thin basement membrane disease • Alport syndrome • MYH9-related disease • CFHR5 nephropathy • Giant fibronectin glomerulopathy	Glomerulonephritis • Acute post-infectious GN • Membranoproliferative GN • Membranous nephropathy • Rapidly progressive GN • Systemic lupus erythematosus • IgA nephropathy • IgA vasculitis nephritis • Polyarteritis nodosa • ANCA positive vasculitis Hemolytic uremic syndrome	Urinary tract infection Adenovirus hemorrhagic cystitis Urinary schistosomiasis Hypercalciuria Renal calculi Exercise-induced Trauma or instrumentation Chemical cystitis such as cyclophosphamide Coagulopathy Sickle cell trait Cystic kidney disease Structural abnormalities of kidney, ureter, bladder (e.g. vesicoureteric junction obstruction) Vascular malformations Nutcracker syndrome Renal vein thrombosis Tumors • Renal: Wilms tumor, renal cell carcinoma, mesoblastic nephroma • Bladder: rhabdomyosarcoma Menarche Factitious

tems, ureters, bladder and urethra. The various causes of hematuria in children are listed in Table 12.2. In children, the source of bleeding is more often from the glomeruli than from the urinary tract.

There are four different clinical presentations of hematuria:

1. Child with red or dark-colored urine
2. Child with lower urinary tract symptoms
3. Child with clinical features of acute glomerulonephritis
4. Asymptomatic child with incidental finding of microscopic hematuria on urine dipstick

These four clinical presentations will be considered separately as the approach is different in each of these scenarios, though there is an overlap in the causes.

Child with Red or Dark-Colored Urine

The first step in the evaluation is to exclude red discoloration of urine due to certain foods, drugs, hemoglobinuria or myoglobinuria (Table 12.1). A urine microscopic examination is essential to confirm that the discoloration is due to red blood cells.

The causes of gross hematuria in children include:

1. Acute glomerulonephritis, especially if edema and hypertension are also present.
2. UTI, adenovirus hemorrhagic cystitis, schistosomiasis, urethritis, perineal irritation, urolithiasis, or hypercalciuria. These conditions are usually accompanied by voiding symptoms such as dysuria, frequency and urgency.
3. Exercise-induced hematuria
4. Trauma
5. Coagulopathy
6. Renal vein thrombosis
7. Urinary tract tumors
8. Recurrent gross hematuria which is seen in IgA nephropathy, nutcracker syndrome, Alport syndrome, CFHR5 nephropathy.

Exercise-induced hematuria is a transient hematuria that appears immediately after pro-

longed, vigorous exercise such as long-distance running, and usually disappears within 48 h. This is benign and results from relative higher vasoconstriction of the efferent glomerular arterioles compared with the afferent vessels, resulting in increased filtration pressure and excessive increase in red cell excretion into the urine [9].

Trauma sufficient to cause hematuria is usually associated with an obvious history such as urethral catheterization or abdominal injury. Mild trauma may cause hematuria in a patient with a previously unsuspected obstructed urinary tract, such as ureteropelvic junction stenosis.

Children with bleeding disorders such as hemophilia or thrombocytopenia commonly have microscopic hematuria, but may also develop gross hematuria following minor trauma. Sickle cell hemoglobinopathy can cause hematuria by causing infarction of the renal collecting systems [10]. Renal vein thrombosis is rare, but should be strongly considered when gross hematuria presents in nephrotic children, neonates with umbilical lines, severe dehydration, polycythemia or prothrombotic conditions.

Urinary tract tumors are rare in children. Children with Wilms tumor can have microscopic hematuria (rarely macroscopic hematuria), but are more commonly discovered following evaluation of abdominal distension or abdominal masses. Rhabdomyosarcoma of the bladder is extremely rare, and usually presents with voiding symptoms in addition to macroscopic hematuria.

The nutcracker phenomenon refers to compression of the left renal vein between the aorta and superior mesenteric artery before the left renal vein joins the inferior vena cava. This leads to left renal vein hypertension, which may result in rupture of the thin-walled vein into the renal calyceal fornix, with the clinical presentation of intermittent gross or microscopic hematuria. In addition, the increased venous pressure within the renal circulation can promote the development of varices of the renal pelvis and ureter. This phenomenon, with its associated symptoms of unilateral hematuria and left flank pain, is defined as the nutcracker syndrome. It occasionally presents as a varicocele in boys or abnormal menstruation in pubertal girls, as a result of the development of venous varicosities of the gonadal vein [11]. Orthostatic proteinuria has also been reported in nutcracker syndrome, although the exact mechanism is unknown [12]. Possible mechanisms include subtle glomerular lesions associated with hemodynamic abnormality, and an increased release in norepinephrine and angiotensin II on standing up [12]. Nutcracker syndrome appears more commonly in Asian communities. It may be one of the important causes of gross or microscopic hematuria in relatively young and previously healthy patients with a thin habitus [13].

Diagnosis of nutcracker syndrome can be made by renal ultrasound demonstrating compression of a pre-aortic left renal vein in the fork between the abdominal aorta and the proximal superior mesenteric artery, and Doppler flow scanning measuring the peak flow velocity ratio between the aorto-mesenteric portion and the hilar portion of the renal vein. The most accurate method for diagnosing the nutcracker syndrome is left renal venography, with measurement of the pressure gradient between the left renal vein and the inferior vena cava. Such invasive examination is difficult to perform in children. Magnetic resonance angiography can be used to demonstrate the dilated left renal vein after passing between the aorta and superior mesenteric artery. An alternative is multidetector computed tomography, which can detect the decrease in velocity of contrast enhancement to the parenchyma of the left kidney due to compression of left renal vein; however, the radiation risk in childhood is not negligible [14].

Controversy exists as to the treatment of nutcracker syndrome. Spontaneous resolution of hematuria in 75% of children with nutcracker syndrome followed up for 2 or more years has been reported following an increase in the body mass index (BMI) [13, 15, 16]. Surgical or radiological intervention are indicated for severe pain, significant hematuria and renal impairment, with percutaneous endovascular stent insertion being the preferred mode of therapy [17].

Children with IgA nephropathy and some of the familial hematuria syndromes (Table 12.3) can have macroscopic hematuria at the time of, or

Table 12.3 Familial hematuric disorders

Disorder	Gene(s)	Protein	Estimated risk of ESKD
X-linked Alport syndrome	*COL4A5*	α5(IV)	
	Hemizygous (male)		100%
	Heterozygous (female)		Up to 25%
Autosomal Alport syndrome	*COL4A3* or *COL4A4*	α3(IV) or α4(IV)	
	Recessive (homozygous or compound heterozygous)		100%
	Dominant		<1% if no risk factors (TBMN); ≥20% if risk factors present
Digenic Alport syndrome	*COL4A3* and *COL4A4*	α3(IV) and α4(IV)	
	Mutations in trans		Up to 100%
	Mutations in cis		Up to 20%
	Mutations in *COL4A5* and in *COL4A3* or *COL4A4*		Up to 100% (affected males)
Autosomal dominant HANAC syndrome	*COL4A1*	α1(IV)	
	Heterozygous		Unknown
Autosomal dominant MYH9 associated nephropathy	*MYH9*	Nonmuscle myosin heavy chain IIA	
	Heterozygous		30%
CFHR5 nephropathy	*CFHR5*	Complement factor H-related protein 5	
	Heterozygous (males)		80%
	Heterozygous (females)		20%
Glomerulopathy associated with fibronectin deposition	*FN1*	Fibronectin 1	>90% between second to sixth decade

ESKD end-stage kidney disease; *TBMN* thin basement membrane nephropathy

1 or 2 days following, an upper respiratory tract infection, a phenomenon known as synpharyngitic hematuria. Some degree of accompanying proteinuria, at least at the time of intercurrent illness, is common. The urine can be normal between the bouts of gross hematuria but a considerable proportion has persistent microscopic hematuria between the attacks of gross hematuria.

Alport syndrome is a genetic disease with both gross and microscopic hematuria that is associated with high risk of progression to kidney failure before the fourth decade of life. The rate of progression to kidney failure is influenced by the type of *COL4A* mutations (*COL4A5*, *COL4A3*, *COL4A4*) affecting the α-chains of type IV collagen in the glomerular basement membrane. The inheritance pattern is most commonly X-linked, but may also be autosomal recessive or dominant. A family history of relatives with hematuria, renal failure or deafness may suggest Alport syndrome, but it must be remembered that a negative family history does not exclude Alport syndrome.

A syndrome in which hereditary angiopathy, nephropathy, aneurysms and muscle cramps (HANAC) linked to heterozygous mutations of *COL4A1* gene has been described [18]. HANAC syndrome is autosomal dominant and extremely rare. The kidney disease presents with microscopic or macroscopic hematuria. Cortical cysts and mild chronic kidney disease (CKD) have been described in adults. *COL4A1* gene mutation detection should be considered if there is hematuria in a patient with cerebral abnormalities (intracerebral aneurysm, stroke), cataracts and retinal arteriolar tortuosity.

Complement Factor H-Related 5 (CFHR5) nephropathy is endemic in the Greek Cypriot population and is extremely rare in the non-Cypriot population. It is one of the group of disorders known as C3 glomerulopathy. It is caused by a mutation of the *CFHR5* gene. Which encodes proteins that regulate the alternative complement pathway. It is characterized by low serum C3 levels but normal C4 levels, and kidney biopsy invariably shows mesangial C3 deposition. Genetic testing is required for the diagnosis.

Studies from families of Cypriot descent have shown that patients with duplication of exons two to three of the *CFHR5* gene present with hematuria, proteinuria and hypertension, with up to 50% progressing to kidney failure within 10 years of diagnosis [19].

Child with Associated Lower Urinary Tract Symptoms

Hematuria with accompanying dysuria, frequency, urgency, flank or abdominal pain may suggest a diagnosis of UTI, hypercalciuria or nephrolithiasis.

One third of UTIs have associated hematuria, though this is usually microscopic. UTIs are usually caused by bacteria, but viruses, fungi and parasites are potential etiological agents. Acute hemorrhagic cystitis is characterized by gross hematuria and symptoms of bladder inflammation. It is associated with adenovirus types 11 and 21. The macroscopic hematuria usually lasts 5 days and microscopic hematuria may persist for an additional 2–3 days [20]. Schistosomiasis is an important cause of hematuria in tropical Africa, Middle Eastern countries, Turkey, India, South-East Asia and in immigrants from these areas [21]. It is caused by swimming in lakes and ponds infested with snails infected by the flatworm *Schistosoma haematobium*. The trapped eggs of the flatworm in the bladder and lower urinary tract cause an intense granulomatous inflammatory reaction resulting in hematuria. In developing countries, tuberculosis of the urinary tract is another cause of hematuria, both microscopic and macroscopic, especially in the context of a child with prolonged ill health [22].

Nephrolithiasis is rare in children. The incidence of stone disease in children has been reported to account for between 0.13 and 0.94 cases per 1000 hospital admissions [23]. It can present with hematuria alone or hematuria with colic. Pain can be due to the presence of the stone or clots of blood passing down the ureter. An association between hematuria and hypercalciuria has been reported in children with asymptomatic macroscopic or microscopic hematuria without signs of renal stones [24]. Children with hypercalciuria can also have accompanying irritative urinary symptoms such as dysuria, frequency and urgency. These children have increased urinary excretion of calcium despite normal serum calcium levels. The urinary calcium to creatinine ratio in a single urine specimen is a useful index of calcium excretion for screening and monitoring purposes. In a large study, the 97th percentile level of urinary calcium to creatinine ratio in children eating an unrestricted diet was 0.69 mmol/mmol, whereas in infancy, it can reach as high as 2.2 mmol/mmol [25].

Child with Clinical Features of Acute Glomerulonephritis

Acute glomerulonephritis is characterized by sudden onset of macroscopic hematuria, accompanied by hypertension, oliguria, edema and varying degrees of abnormal kidney function. In children, the majority of cases of acute glomerulonephritis have a post-infectious etiology, most commonly following infection with group A β-hemolytic streptococcal infection of the throat or skin. It is important to identify acute glomerulonephritis in a child with hematuria because urgent appropriate management can prevent morbidity and mortality due to uncontrolled hypertension, fluid overload and abnormal kidney function.

Asymptomatic Child with Incidental Finding of Microscopic Hematuria on Urine Dipstick

Use of urine dipstick testing to screen for UTI in a febrile child or during routine school health examination in many countries detects asymptomatic microscopic hematuria. Mass urine screening programs of school-aged children suggest that approximately 1% will have two or more urine dipsticks positive for microscopic hematuria, but this only persists at 6 months in a third of the population [2, 3, 26]. Of those chil-

dren who were subsequently referred for evaluation of persistent microscopic hematuria, a glomerular pathology was the most likely cause, occurring in between 22.2% and 52.3% based on either phase-contrast microscopy or kidney biopsy findings [27–30].

Isolated hematuria (without accompanying hypertension, significant proteinuria or abnormal kidney function) in children is traditionally regarded as benign. However, publications describing the long-term follow-up of patients who presented initially with microscopic hematuria has challenged this view. An adjusted hazard ratio of 18.5 for the development of ESKD was observed in Israeli adolescents and young adults with persistent asymptomatic isolated microscopic hematuria over a period of 22 years in a population-based retrospective cohort study [31]. A study in China involving 351 children who had undergone kidney biopsy secondary to persistent asymptomatic isolated hematuria reported increased adverse kidney events (i.e. development of proteinuria, hypertension, or abnormal kidney function) after 2–10 years of follow-up in those children with recurrent macroscopic hematuria and/or proteinuria as compared with patients with asymptomatic isolated microscopic hematuria (22.8% versus 6.0% respectively, $p < 0.001$) [32]. This finding suggests that microscopic hematuria, especially when accompanied by macroscopic hematuria and/or proteinuria, may be associated with unfavorable kidney outcome. While the clinical outcome for many children presenting with isolated hematuria is good, the lifetime risk of kidney disease is not insignificant and is dependent on the underlying pathology.

As microscopic hematuria and mild proteinuria may appear transiently during fever, illness or extreme exertion, it is therefore not cost-effective to subject every child to extensive investigations to elucidate the cause of microscopic hematuria. One practical approach is to repeat the urine dipstick and microscopic urinalysis twice within 2 weeks after the initial result. If the hematuria resolves, no further tests are required. If microscopic hematuria persists on at least two of the three consecutive samples, then further evaluation is required [33].

The common diagnoses in children with persistent microscopic hematuria without proteinuria are familial benign hematuria, idiopathic hypercalciuria, and IgA nephropathy. In addition, there is a group of genetically heterogenous monogenic conditions causing microscopic hematuria that may progress to ESKD, typically during adulthood. This group of familial hematuric disorders is caused by mutations in several genes (Table 12.3).

Benign familial hematuria, also known as thin basement membrane nephropathy (TBMN), is the most common cause of persistent microscopic hematuria in children, occurring in at least 1% of children worldwide [34]. It is autosomal dominant, and is frequently associated with heterozygous mutations of *COL4A3* or *COL4A4* genes. Absence of a family history does not exclude the diagnosis of TBMN because there may be a de novo mutation, the penetrance may not be complete, or family members may not be aware that they have microscopic hematuria [35]. The red blood cells in the urine are mainly dysmorphic and there may be red blood cell casts. Hearing deficits or eye abnormalities almost never occur in patients with TBMN or their family members. Universal thinning of glomerular basement membrane is seen on electron microscopy. A kidney biopsy is usually not indicated if TBMN is suspected, unless there are atypical features to suggest IgA nephropathy or Alport syndrome. The prognosis of TBMN was traditionally regarded as benign. However, it is now recognized that TBMN can be associated with an increased risk of kidney failure in adulthood, with up to 50% of patients developing various degrees of CKD after the age of 50 years [36]. TBMN is thought to be a spectrum of autosomal Alport syndrome, where the risk of progressive glomerulopathy is up to 20% in the presence of factors such as proteinuria, sensorineural deafness, family history of kidney failure and histological findings of focal segmental glomerulosclerosis, or glomerular basement membrane thickening and lamellation [37]. Hence, lifelong follow-up is recommended for children with persistent isolated microscopic hematuria due to suspected TBMN.

The better understanding of the genetic basis of diseases in recent years has prompted a better classification of familial hematuria into a number of rare glomerulopathies, such as CFHR5 nephropathy, MYH9-related disease and glomerulopathy with fibronectin deposition. The kidney outcome of these conditions is worse than was initially described for benign familial hematuria. Hence, genetic analysis in those children with family histories of hematuria and/or proteinuria or kidney failure may be considered for the early detection of these progressive, familial, hematuric disorders (Table 12.3).

One rare familial hematuric disorder is autosomal dominant MYH9-related disease, which includes Fechtner and Epstein syndromes [34, 38, 39]. Besides nephritis, these syndromes are associated with giant platelets, cytoplasmic leukocyte inclusions (Döhle-like bodies), sensorineural deafness and cataracts. The associated nephritis seen in 30–70% of patients presents initially with microscopic hematuria, with proteinuria developing as the disease progresses, and reaching ESKD in young adulthood.

Glomerulopathy with fibronectin deposition (GFND) is another rare autosomal dominant glomerulopathy characterized by microscopic hematuria, proteinuria, hypertension and massive fibronectin deposits in the mesangium and subendothelial space. GFND presents at different ages. The urinary abnormalities usually occur during the first decade of life, with progression to ESKD typically occurring in the second to sixth decade [40–42].

Clinical Approach

In approaching a child with hematuria, we should ensure that serious conditions are not missed, avoid unnecessary and expensive laboratory tests, reassure the family and provide guidelines for further studies if there is a change in the child's course. Obtaining a careful history and physical examination is the crucial first step in the evaluation.

History

It is helpful to determine the color of the urine and timing of color change related to the urinary stream. Macroscopic hematuria of glomerular origin is usually described as dark brown or cola-colored. In contrast, visible hematuria of lower urinary tract origin (bladder and urethra) is usually pink or red, there may be clots, and the blood may be only be visible at the beginning or end of the urinary stream. Hematuria at voiding onset is seen in urethral causes such as urethritis, whereas terminal hematuria is indicative of a bladder cause such as a bladder stone or tumor and schistosomiasis. Patients should be asked regarding history of recurrent gross hematuria, recent trauma or exercise, passage of urinary stones, recent or concurrent respiratory or skin infections, and intake of medications (including over-the-counter medications, calcium or vitamin D supplementation) or herbal compounds. In girls at peripubertal age, menarche as the cause of hematuria should be considered.

Associated symptoms may include fever, dysuria, urinary frequency and urgency, back pain, skin rashes, joint symptoms, and face and leg swelling. Predisposing illnesses such as sickle cell disease or trait should also be noted. The family history should search for documented hematuria, hypertension, intracerebral bleed, kidney stones, kidney failure, deafness, coagulopathy and polycystic kidney disease. In a sexually active teenager, the social history should consider any recent sexual activity and any known exposure to sexually transmitted diseases since cystitis and urethritis can present with hematuria.

Physical Examination

The presence of hypertension and edema, suggestive of acute glomerulonephritis, requires a more urgent and extensive evaluation. Associated rashes or arthritis may indicate hematuria due to systemic lupus erythematosus or nephritis due to IgA vasculitis. The presence of fever or loin pain

may point to pyelonephritis. A palpable and ballotable kidney mass will require radiological investigations to exclude hydronephrosis, polycystic kidney or kidney tumor. Screening for eye and hearing abnormalities may be useful if there is a suggestive family history of familial hematuric disorders associated with progression to ESKD.

Investigations

Investigations to look for the cause of hematuria can be extensive. Tailoring the evaluation according to the type of clinical presentation reduces unnecessary laboratory and radiological investigations (Fig. 12.1). The first step is to confirm hematuria with urine microscopic examination. If the child has associated edema, hypertension, oliguria or proteinuria with hematuria, evaluation for glomerular causes such as acute glomerulonephritis or hemolytic uremic syndrome is required. If the child has associated irritative urinary symptoms, evaluation for UTI and nephrolithiasis should be considered. For children with an incidental finding of microscopic hematuria during illness or after exertion, further evaluation is required only if there is persistent microscopic hematuria on at least two of three consecutive samples. If the subsequent two urine samples do not show microscopic hematuria, the hematuria is transient and further evaluation is not required.

The next step in the evaluation of persistent hematuria is to determine the site of bleeding. Two investigations that should be done once hematuria is confirmed are urine tests for protein and urine phase contrast microscopic examination to look at the red blood cell morphology. Hematuria (gross or microscopic) associated with greater than 30% dysmorphic red blood cells, in particular acanthocytes (ring forms with vesicle-shaped protrusions) [43], and proteinuria indicate glomerular bleeding. It is important to remember that some proteinuria may also be present in non-glomerular causes of macroscopic hematuria. However, the proteinuria usually does not exceed 2+ (1 g/L) on dipstick examination if the only source of protein is from bleeding due to a non-glomerular etiology. Therefore, a child with proteinuria 2+ or more should be investigated for glomerulonephritis. Similarly, red blood cell casts, if present, are highly specific for glomerulonephritis.

A serum creatinine to estimate kidney function needs to be determined in children with glomerular pathology. If there is significant proteinuria, the serum albumin should be measured. In addition, laboratory investigations to look for the cause of glomerular disease should be performed. These investigations may include serum complements C3 and C4, anti-streptolysin O titer (ASOT) or anti-DNase B, anti-factor B antibody, anti-nuclear antibodies (ANA), anti-double-stranded DNA (dsDNA) antibody, anti-neutrophil cytoplasmic antibodies (ANCA), IgA level, hepatitis B surface antigen and viral titers if appropriate. Anti-factor B antibody levels, an autoantibody targeting factor B (a component of the alternative pathway C3 convertase), is highly specific for post-infectious glomerulonephritis [44]. Serum IgA levels are increased in 30–50% of adult patients, but in only 8–16% of children with IgA nephropathy [45]. In countries where IgA nephropathy is an important cause of glomerulonephritis, 10–35% of children undergoing kidney biopsy for isolated hematuria were found to have IgA nephropathy [45, 46].

The clinical presentation is important in deciding the type of investigations required. For example, a preceding sore throat, pyoderma or impetigo and the presence of edema, hypertension and proteinuria are suggestive of post-streptococcal glomerulonephritis. Serum ASOT, anti-DNase B and complement C3 levels would suffice in this case. If these tests are not informative, then further investigations are warranted to rule out other causes of glomerulonephritis. If a familial hematuric disorder is suspected, an audiological examination may be useful to detect high frequency sensorineural hearing deficit associated with Alport syndrome. If suspicion of X-linked Alport syndrome is high, skin biopsy with immunostaining for the α5(IV) chain can be useful. The presence of macrothrombocytopenia with or without basophilic cytoplasmic leukocyte inclusion bodies (Döhle-like bodies) suggests

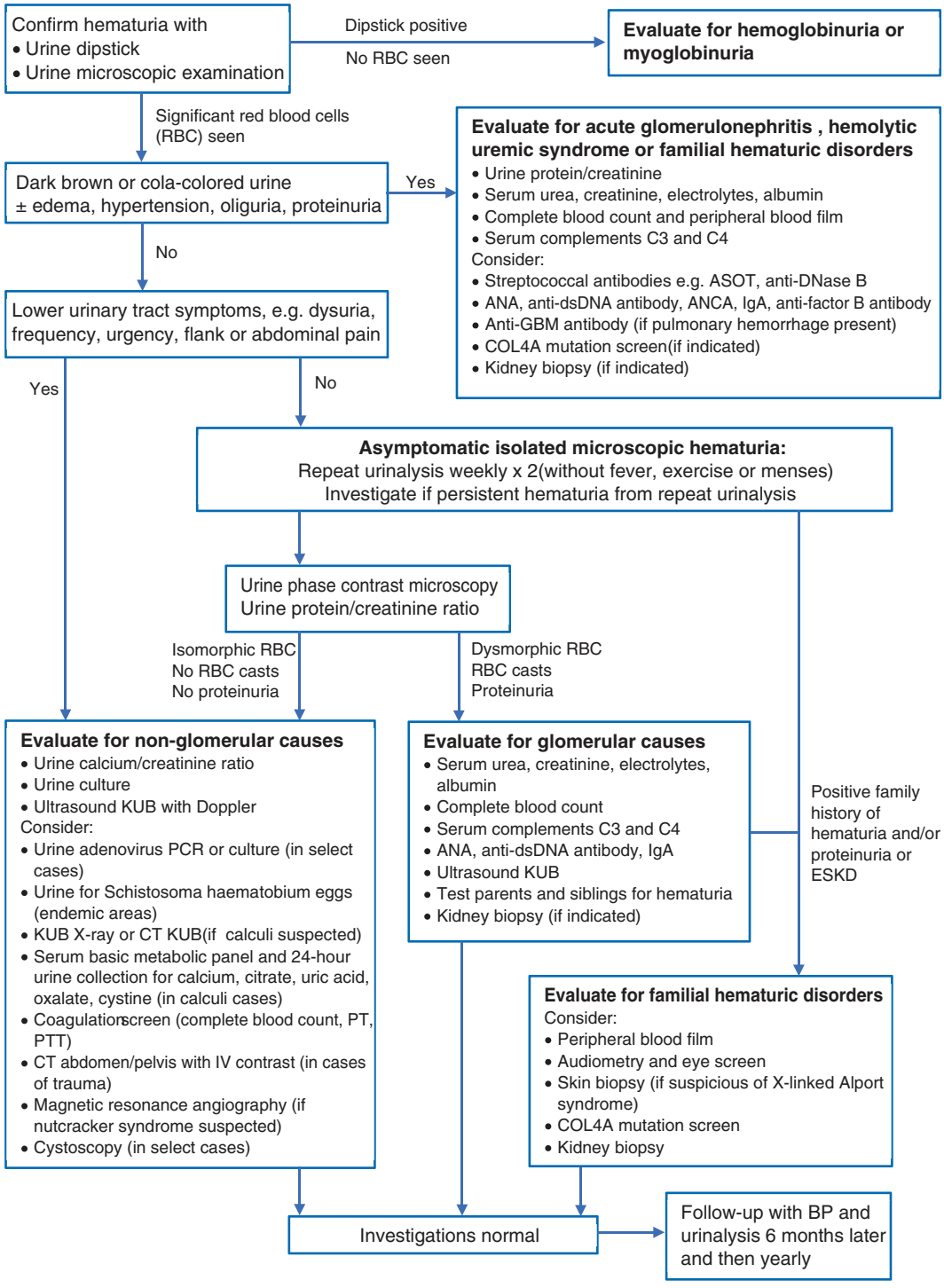

Fig. 12.1 Algorithm for investigating hematuria

MYH9-related diseases [39]. The utility of kidney ultrasonography for evaluation of the asymptomatic child with microscopic hematuria of glomerular origin remains unproven [47]. However, it may be useful to determine the size of the kidneys as a guide to chronicity in patients with evidence of progressive kidney disease, and to diagnose polycystic kidneys in the presence of a suggestive family history.

Hematuria associated with mainly isomorphic red blood cells, together with absence of red blood cell casts and proteinuria, indicate a nonglomerular cause. Urine calcium to creatinine ratio is performed to rule out hypercalciuria. In endemic areas, urine should be examined after sedimentation for *Schistosoma haematobium* eggs, especially during the day when excretion is highest. Ultrasound of the kidneys and bladder is indicated to exclude hydronephrosis, kidney calculi, tumor or cystic kidney disease. A plain abdominal x-ray may be necessary to exclude ureteric stones. If a urinary tract calculus is identified, a complete assessment of the urinary constituents associated with stone risk is needed. If the investigations reveal the presence of tumor, structural urogenital abnormality or urinary calculus, a urological referral is required. A coagulation screen may be necessary when there is a family history of bleeding diathesis. Computed tomography scan of the abdomen and pelvis may be required if there is a history of abdominal trauma followed by gross hematuria. If the nutcracker syndrome is suspected in a thin child with recurrent gross hematuria, Doppler sonography assessing the left renal vein diameter and peak velocity is a useful diagnostic tool. When Doppler renal vein ultrasonography is not diagnostic, axial imaging by computed tomography or magnetic resonance imaging may be required.

Cystoscopy may also be required in cases of children with recurrent nonglomerular macroscopic hematuria of unknown cause. Cystoscopy in children seldom reveals the cause of hematuria, but should be done when preliminary investigations have failed to find a cause, and bladder or urethral pathology is a consideration because of accompanying voiding symptoms. Vascular malformations in the bladder have been detected via cystoscopy. In the rare instance when a bladder mass is noted on ultrasound, cystoscopy is also indicated. Cystoscopy to lateralize the source of bleeding is best performed during active bleeding.

An asymptomatic child with an incidental finding of persistent microscopic hematuria often poses the greatest dilemma regarding the extent of investigations and subsequent follow-up. The most common diagnoses in children with persistent microscopic hematuria without proteinuria and hypertension are TBMN, idiopathic hypercalciuria, IgA nephropathy and Alport syndrome. It is therefore worthwhile to screen family members for microscopic hematuria. If the parents are found to have incidental asymptomatic microscopic hematuria without proteinuria and kidney failure, TBMN is the most likely cause. More extensive evaluation is then not necessary. However, it is important that these patients are followed up yearly to detect proteinuria, which is an indication of progressive kidney disease. When proteinuria is present, these patients need to undergo further evaluation, including genetic analysis for the relevant mutations associated with the familial hematuric disorders. In communities where post-infectious glomerulonephritis is common, subclinical disease is also a common cause of persistent microscopic hematuria.

Indications for Kidney Biopsy

Kidney biopsy is usually not indicated in isolated glomerular hematuria. Kidney biopsy should be considered in the following cases of hematuria associated with:

- Significant proteinuria, except in poststreptococcal glomerulonephritis
- Persistent low serum complement C3
- Unexplained azotemia
- Systemic diseases such as systemic lupus erythematosus or ANCA-associated vasculitis
- Family history of significant kidney disease suggestive of progressive forms of familial hematuric disorders including Alport syndrome

- Recurrent gross hematuria of unknown etiology where investigations are suggestive of a glomerular pathology
- Persistent glomerular hematuria where the parents are anxious about the diagnosis and prognosis.

With recent improvements in understanding the molecular genetics of the familial hematuric disorders, genetic testing, if available and affordable, can sometimes contribute useful diagnostic and prognostic information and may even obviate the need for a kidney biopsy [48].

Proteinuria

It is well-established that proteinuria is a mediator of progressive kidney insufficiency in both adults and children [49–51], as well as a risk factor for cardiovascular disease [52, 53]. Conversely, proteinuria can also be a transient finding in children, occurring during times of stress, including exercise, fever and dehydration, and does not denote kidney disease.

Renal Handling of Proteins

Plasma proteins can cross the normal glomerular filtration barrier. The ability of these proteins to cross the glomerular filtration barrier is related primarily to the molecular size and charge. The larger plasma proteins, such as globulins, are virtually excluded from the normal glomerular filtrate. Smaller proteins, mostly of low molecular weight (LMW; <40,000 Dalton), are filtered across the glomerular filtration barrier. Molecular charge plays an important role in determining glomerular permeability to macromolecules. This is due to the presence of negatively charged sialoproteins that line the surfaces of both the glomerular endothelial and epithelial cells, and glycosaminoglycans present in the glomerular basement membrane. Hence, negatively charged molecules are less able to cross the glomerulus than neutral molecules of identical size. On the other hand, positively charged molecules have enhanced clearances.

In health, albumin is the most abundant protein in serum and constitutes about 40% of the filtered urinary protein despite being anionic and molecular weight of 67,000 Dalton. The rest of the urinary proteins are immunoglobulins, peptides, enzymes, hormones and partially degraded plasma proteins. After crossing the glomerular barrier, 71% of the filtered proteins are reabsorbed by the proximal tubule, 23% by the loop of Henle and 3% by the collecting duct. The quantity of urinary proteins excreted results from a balance between the amount of these proteins filtered and the amount reabsorbed.

Under normal conditions, approximately 60% of protein in urine is filtrate of plasma protein and 40% is of kidney origin. This is a heterogeneous group of proteins, many of which are glycoproteins. Some are derived from cells lining the urinary tract and have the potential of being important diagnostic indicators. The major protein in this group is Tamm-Horsfall protein or uromodulin, which is a major constituent of urinary casts [54]. Adults excrete 30–60 mg/day. It is secreted mainly in the thick ascending limb of the loop of Henle.

Excessive urinary protein losses can be due to the following mechanisms:

- **Glomerular proteinuria:** Increased permeability of the glomerular basement membrane due to structural defects of the membrane, loss of its negative charges or damage by immune complexes or other mediators. This leads to increased filtration of macromolecules, especially albumin, across the glomerular filtration barrier
- **Tubular proteinuria:** Impaired reabsorption of normally filtered LMW proteins (e.g. α-1 microglobulin, β-2 microglobulin, retinol binding protein) by the proximal tubule
- **Secretory proteinuria:** Increased secretion of tissue proteins into the tubules, most notably Tamm-Horsfall protein in interstitial nephritis
- **Overflow proteinuria:** Marked overproduction of LMW proteins (e.g. myoglobin),

resulting in the filtered load exceeding the normal proximal reabsorptive capacity

Hence, the specific type of protein excreted in the urine, such as albumin or LMW proteins, depends on the type of kidney disease. Albuminuria is a marker of glomerular disease while urinary loss of LMW proteins suggests tubulointerstitial disease.

Albuminuria

Albuminuria refers to abnormal loss of albumin in the urine. Albumin is found in the urine in normal subjects and in larger quantities in patients with kidney disease. Recommendations for measurement of urine protein emphasize quantification of albuminuria rather than total protein, as epidemiologic data worldwide have demonstrated a strong graded relationship between the quantity of urine albumin with both kidney and cardiovascular disease risk in adults. The Kidney Disease Improving Global Outcomes (KDIGO) 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease incorporated albuminuria in the criteria and classification of CKD in adults [55]. A urinary albumin excretion rate of 30 mg/24 h or more sustained for longer than 3 months is used to indicate CKD in adults. This value is considered to be approximately equivalent to an albumin-to-creatinine ratio in a random urine sample of greater than 30 mg/g or 3 mg/mmol. On the other hand, urinary albumin excretion rates in children and adolescents vary with age. Urinary albumin excretion is lowest in children age less than 6 years, followed by an increase through the adolescent years, with a peak at age 15–16 years [56].

Microalbuminuria is below the detection threshold of a standard urine dipstick test. Albuminuria in children and adolescents has been defined as urinary albumin excretion of 30–300 mg/24 h urine collection, 20–200 μg/min in a nighttime collection and 3–30 mg/mmol creatinine (30–300 mg/g creatinine) in a first morning urine sample [57]. This range of urinary albumin excretion, although used in the pediatric population, is derived from population studies in adults.

The prevalence of albuminuria in children and adolescents is estimated to be about 5.7–7.3% in boys and 12.7–15.1% in girls from the National Health and Nutrition Examination Survey III (NHANES III) in the United States [57]. The higher prevalence of albuminuria in girls than in boys could be due to the larger muscle mass and urinary creatinine excretion of boys, resulting in a lower albumin:creatinine ratio. There is a positive association between albumin:creatinine ratio and pubertal development stage [58]. Girls a have a peak albumin:creatinine ratio at Tanner stage 4 (14 years old), whereas in boys there is no increase in albumin:creatinine ratio until Tanner stage 5 (18 years old) [58]. Estimation of the prevalence of albuminuria in children and adolescents can be difficult because a great percentage of children (30–50%) have transient albuminuria because of strenuous exercise [59] or during febrile illnesses.

The significance of albuminuria in diabetic children and adolescents is well established and urinary albumin excretion rate has been used as a screening test for the presence of diabetic nephropathy [60]. The significance of using albuminuria for other conditions in the pediatric population is less well-defined. A cross-sectional study analyzing the NHANES data for 12–19 year old adolescents [61] showed that, despite the presence of cardiovascular risk factors, overweight teenagers had a lower prevalence rate of albuminuria compared to healthy controls. The reason for this lower prevalence of albuminuria in overweight teenagers is uncertain, but one explanation is that lower exercise levels in overweight teenagers leads to less confounding influence by orthostatic proteinuria. Conversely, another study has shown that obese children (BMI 30.4 kg/m^2) compared to normal weight children (BMI 18.2 kg/m^2) were found to have a significantly higher albumin:creatinine ratio determined in a random spot urine sample [62], indicating early kidney dysfunction as a consequence of obesity. The elevated albumin:creatinine ratio in these obese children was also associated

with impaired glucose tolerance and hypercholesterolemia, two of the most important features of the metabolic syndrome [62]. The relationship between albuminuria and cardiovascular outcome in the pediatric population is not as well-studied as in adults [56]. Further longitudinal research is required to evaluate the significance of increased urinary albumin excretion rate in obese children with regards to the future development of cardiovascular morbidity in adulthood.

Definition of Abnormal Urinary Protein Excretion in Children

Normal urinary protein excretion varies across age, gender, puberty and body size. Neonates and young infants have higher urinary losses of both glomerular and tubular proteinuria due to lack of maturation in the proximal tubular reabsorption of proteins. In infants less than 6 months of age, at least one study has suggested that as much as 6–8 mg/m^2/h or up to 300 mg/1.73 m^2/day of proteinuria is normal [63]. The normal rate of protein excretion in the urine for children 6–24 months of age is less than 4 mg/m^2/h or 150 mg/1.73 m^2/day [64]. The first morning spot urine protein-to-creatinine ratio is normal when it is less than 50 mg/mmol (500 mg/g).

Children older than 24 months of age are expected to achieve normal adult urinary protein values, with the caveat of an exaggerated postural loss of glomerular protein (albumin), which can be seen in 2–5% of the adolescent population (i.e. orthostatic proteinuria) [59]. Hence, in children older than 24 months, the normal rate of protein excretion in the urine is still less than 4 mg/m^2/h or less than 150 mg/1.73 m^2/day. However, the first morning spot urine protein-to-creatinine ratio is defined as normal at less than 20 mg/mmol (200 mg/g) and the spot urine albumin-to-creatinine ratio is normal at less than 3 mg/mmol (30 mg/g) [64].

At all ages, urinary protein excretion of greater than 40 mg/m^2/h or 3 g/1.73 m^2/day is defined as nephrotic range proteinuria (Table 12.4) [64]. If albumin excretion is measured, nephrotic range proteinuria is defined as urine albumin greater

Table 12.4 Quantification of urinary protein excretion in children

Method	Abnormal proteinuria	Precautions
Urine dipstick	≥1+ in a urine specimen of specific gravity ≥1.002	False-positive if urine pH >8.0 or specific gravity >1.025 or tested within 24 h of radiocontrast study
Sulfosalicylic acid test	≥1+	False-positive with iodinated radiocontrast agents
Urine protein:creatinine ratio in spot urine	>20 mg/mmol (>0.02 g/mmol) or >200 mg/g *in children >2 years old* [51] >50 mg/mmol (>0.05 g/mmol) or >500 mg/g *in children 6 months to 2 years old* Nephrotic range: >220 mg/mmol (>0.22 g/mmol) or >2200 mg/g [55]	Protein excretion varies with child's age
Timed urine protein excretion rate [49]	>4 mg/m^2/h or >150 mg/1.73m^2/24 h *in children >6 months old* >8 mg/m^2/h or >300 mg/1.73m^2/24 h *in children <6 months old* Nephrotic range: >40 mg/m^2/h or >3 g/1.73m^2/24 h [59]	In an accurately collected 24-h urine specimen, the urine creatinine should be in the range of 0.13–0.20 mmol/kg or 16–24 mg/kg ideal body weight for females, and 0.18–0.23 mmol/kg or 21–27 mg/kg ideal body weight for males
Urine albumin:creatinine ratio in spot urine	>3 mg/mmol (>0.003 g/mmol) or >30 mg/g in children >2 years	
Timed urine albumin excretion rate [57]	>30 mg/1.73m^2/24 h	

than 2200 mg/1.73 m^2/day or albumin-to-creatinine ratio greater than 220 mg/mmol or 2200 mg/g [55].

With regards to using urinary albumin or protein excretion in the classification of children with CKD, variations in the definition of abnormal urinary albumin or protein excretion based on age must be taken into account. Abnormal urinary protein excretion in children should also consider the possibility of tubular versus glomerular proteinuria, depending on the underlying disease. Urinary albumin excretion rate may be normal in tubular proteinuria. Hence, in children, the quantification of total protein, as compared to the albumin only fraction, may be the preferred method of assigning risk in relation to the presence of urinary protein.

The KDIGO 2012 guidelines recommended a urinary total protein or albumin excretion rate above the normal value for age be used for children and adolescents [55]. Table 12.5 shows the categories of persistent albuminuria and proteinuria to be used in the classification of CKD. Albuminuria is classified into normal to mildly increased, moderately increased and severely increased. However, in children, urine protein-to-creatinine ratio is the preferred test, followed by albuminuria, and lastly by automated reagent strips for detection of proteinuria. This is because the vast majority of children have underlying congenital anomalies of the kidney and urinary tract, unlike in adults, where the etiology of CKD is more commonly an underlying glomerular disease such as diabetic nephropathy or hypertensive damage. The use of albumin excretion may therefore be less sensitive for diagnostic purposes in children, as those with underlying tubular conditions will tend to excrete more Tamm-Horsfall protein and other LMW proteins.

Urine Dipstick

The urine dipstick is an excellent screening test for the presence of proteinuria [64]. The dipstick is impregnated with the dye tetrabromophenol blue buffered to pH 3.5. At a constant pH, binding of protein to this dye results in the development of a blue color in proportion to the amount of protein present. If urine is protein-free, the dipstick is yellow. The color of the dipstick changes through yellow-green, to green, to a green-blue with increasing concentrations of protein. The dipstick can be read as negative, trace, 1+, 2+, 3+ and 4+, which corresponds to insignificant, less than 0.2 g/L, 0.3 g/L, 1 g/L, 3 g/L and greater than 20 g/L concentrations, respectively.

The dipstick test has a few limitations. Observer error can occur during interpretation of the color of the dipstick. False positive and false negative tests can occur. If the dipstick is kept in

Table 12.5 Categories of albuminuria in chronic kidney disease [55]

	Categories		
Investigation	Normal to mildly increased (A1)	Moderately increased (A2)	Severely increased (A3)
Albumin excretion rate (mg/1.73 m^2/24 h)	<30	30–300	>300
Protein excretion rate (mg/1.73 m^2/24 h)	<150	150–500	>500
Albumin:creatinine ratio (ACR)			
(g/mmol)	<0.003	0.003–0.030	>0.030
(mg/mmol)	<3	3–30	>30
(mg/g)	<30	30–300	>300
Protein:creatinine ratio (PCR)			
(g/mmol)	<0.015	0.015–0.050	>0.050
(mg/mmol)	<15	15–50	>50
(mg/g)	<150	150–500	>500
Protein reagent strip	Negative to trace	Trace to +	+ or greater

the urine too long, the buffer may leach out and a false positive test may result. False positive tests for protein can also occur in the presence of gross hematuria, pyuria and bacteriuria or if the urine is contaminated with antiseptics such as chlorhexidine or benzalkonium, which are often used for skin cleansing prior to clean catch of the urine. False positive results may occur with urine specimens after the administration of radiographic contrast or with ingestion of certain medications, including penicillins, cephalosporins, tolbutamide or sulfonamides.

The result of the dipstick test can be affected by the concentration of the urine. If the urine is very dilute, the urinary protein concentration may be reduced to a level below the sensitivity of the dipstick (0.1–0.15 g/L), even in patients excreting up to 1 g of protein per day. Hence, a negative dipstick should be interpreted with caution if the urine specific gravity is less than 1.002. In contrast, if the urine specific gravity is greater than 1.025, a healthy child can register trace of protein on the dipstick, resulting in a false positive result. The dipstick test for protein can also be affected by the pH of the urine. Very alkaline urine (pH greater than 8.0) can cause a false positive result while very acid urine (pH less than 4.5) can cause a false negative result.

False negative results occur in non-albumin proteinuria. Albumin binds better to the dye than other proteins. Hence, the urine dipstick primarily detects albumin, leaving LMW proteins undetected. The dipstick results correlate better with albuminuria than with proteinuria. However, though the dipstick is more specific for albumin, it lacks the sensitivity to detect microalbuminuria associated with early glomerular injury seen in diabetic nephropathy or cardiovascular disease. A negative dipstick test for protein does not exclude the presence in the urine of low concentrations of globulins, mucoproteins or Bence-Jones protein.

Sulfosalicylic Acid Test

An alternative method to measure urine protein in patients with questionable proteinuria by dipstick in the office is the sulfosalicylic acid precipitation of protein in urine. This technique provides a more quantitative estimate of all the proteins present in the urine, including both albumin and the LMW proteins. This test is performed by mixing one part urine supernatant with three parts 3% sulfosalicylic acid, and the resultant turbidity is then graded, as shown in Table 12.6 [65]. As with the urine dipstick, iodinated radiocontrast agents can cause a false positive result; hence, the urine should not be tested for at least 24 h after a contrast study.

Table 12.6 Sulfosalicylic acid test

Grade	Appearance	Protein concentration (g/L)
0	No turbidity	0
Trace	Slight turbidity	0.01–0.1
1+	Turbidity through which print can be read	0.15–0.3
2+	White cloud without precipitate through which heavy black lines on a white background can be seen	0.4–1
3+	White cloud with precipitate through which heavy black lines cannot be seen	1.5–3.5
4+	Flocculent precipitate	>5

Quantification of Proteinuria: 24-h Urine Specimen Versus Spot Urine Specimen

The results obtained with urine dipstick and with quantitative 24-h protein excretion methods correlate fairly well in most situations. As mentioned earlier, the dipstick is more sensitive to albumin, whereas quantitative methods detect all proteins, including globulin and LMW protein. For example, in multiple myeloma, large amounts of protein are excreted and yet the urine dipstick for protein is negative. Hence, quantitative urinary protein measurement is necessary in this case. A more important reason why quantitative measurement of protein loss in the urine should be done is to determine whether the patient requires a more extensive evaluation. For example, many patients with a dipstick reading of 1+ protein

have a normal quantitative result and additional evaluation is not indicated.

Quantification of proteinuria has traditionally demanded timed urine collection. Urinary protein excretion in adults is usually measured in a 24-h urine collection. This is more accurate than a spot urine protein analysis. However, 24-h urine collection poses logistical problems with timing and volume measurements, especially in young children who have yet to achieve continence at night. In this case, a 12-h urine collection can be done, and the protein excretion rate is then extrapolated to a 24-h value by using the appropriate correction factor.

The other method is to obtain a single voided urine sample. The concentrations of both protein and creatinine are measured in the urine sample and protein levels are expressed per unit of creatinine. The advantages of this method include not requiring timed urine samples and not having to correct for body size. The assumption is creatinine excretion is directly related to body mass and is relatively constant throughout the day. Many studies have found that the amount of protein excreted in a 24-h urine correlates extremely well with the protein-to-creatinine ratio measured in random urine samples [66, 67].

What remains debatable is whether early morning urine samples or random urine samples obtained during normal activities during the day are better indicators of kidney disease. The urine protein-to-creatinine ratio is higher in urine samples obtained in a person in an upright position than in a recumbent position, a phenomenon known as orthostatic proteinuria [59]. Studies that included subjects with normal kidney function as well as those with kidney failure have shown that urine protein-to-creatinine ratios from daytime samples correlated better with 24-h urine protein excretion values than did values from early morning samples [59]. Conversely, early morning samples had better correlation when data were evaluated from normal subjects and from those with kidney disease but normal glomerular filtration rates [68]. In subjects with kidney disease and orthostatic proteinuria, daytime urine protein-to-creatinine ratios can be misleading. Hence, in the evaluation of children with possible kidney disease, the first morning urine specimen is recommended for urine protein-to-creatinine ratio quantification to eliminate the effect of posture. In general, the 24-h urine collection for protein excretion is ideal as the initial diagnostic investigation, with the exception of children who have yet to achieve continence; whereas, the first morning spot urine protein-to-creatinine ratio is useful to monitor progress of proteinuria.

An important consideration when interpreting spot urine protein-to-creatinine ratio is a falsely elevated urine protein-to-creatinine ratio, which can occur when there is low creatinine excretion in children with very low muscle mass (e.g. neuromuscular disorders). The spot urine protein-to-creatinine ratio can be underestimated when there is a very concentrated urine sample with high creatinine level in the urine. One method to avoid overestimation or underestimation is to send spot urine sample for both protein-to-creatinine ratio as well as urinalysis, with the expectation that significant proteinuria will be present in both examinations.

The spot urine protein-to-osmolality ratio has been suggested as another convenient method for estimating urine protein excretion without a 24-h urine collection and to overcome the problem of low creatinine excretion in children with very low muscle mass. Compared to urine creatinine concentration, urine osmolality, which is a direct measure of degree of urine concentration, may thus have advantages of a methodology to standardize normal protein excretion on a random sample. Data describing the normal range of urine protein-to-osmolality ratios have been published for adults [69]. However, the results in the pediatric population differ from the adult population. In the pediatric population, urine protein-to-creatinine ratio was superior to urine protein-to-osmolality ratio for predicting abnormal amounts of proteinuria in children and adolescents [70]. Dilanthi et al. reported that urine protein-to-creatinine ratio (sensitivity 100%, specificity 94%) was more sensitive than urine protein-to-osmolality ratio (sensitivity 85.7%, specificity 100%) in detecting children with mild proteinuria [71]. Hence, using spot urine protein-

to-osmolality as an alternative method to estimate 24-h urine protein excretion in children is not widely practiced. If spot urine protein-to-osmolality ratio is used, it is important to remember that high urine osmolality due to glycosuria can cause an underestimation of the protein-to-osmolality ratio. Glycosuria should be excluded prior to assessment of protein-to-osmolality ratio in a spot urine sample.

The dipstick, Multistix® PRO (Bayer, Elkhart, Ind., USA), is able to analyze concentrations of both urinary protein and creatinine semi-quantitatively in only 60 s and is commercially available. The semi-quantitative urine protein-to-creatinine ratio by Multistix® PRO correlated well with both quantitative urine protein-to-creatinine ratio and daily urinary protein excretion [72, 73], and use of the Multistix® PRO would avoid errors and difficulties associated with timed urine collection. It may become a useful tool to monitor the urinary protein excretion in children with kidney diseases in the outpatient setting.

Clinical Scenarios

Proteinuria can be symptomatic (presenting with edema) or asymptomatic, which can be intermittent or persistent.

Child with Edema

Proteinuria associated with edema can be due to nephrotic syndrome, nephritic syndrome or kidney failure. Nephrotic syndrome is defined as heavy proteinuria that is severe enough to cause hypoalbuminemia, edema and hypercholesterolemia. Nephrotic range proteinuria is defined as greater than 40 mg/m^2/h or greater than 3 g/1.73 m^2/day for timed urine collection, or random urine protein-to-creatinine ratio of greater than 0.22 g/mmol (220 mg/mmol or 2200 mg/g) [64]. The evaluation and management of a child presenting with nephrotic syndrome, nephritic syndrome or kidney failure is different from that of a child with intermittent or persistent proteinuria without edema. Nephrotic syndrome, nephritic syndrome and kidney failure are discussed elsewhere in the book.

Child with Intermittent Proteinuria

In intermittent proteinuria, protein is detectable in only some of the urine samples collected. This may be related to posture or occur at random.

Frequently, intermittent proteinuria is not related to posture. It may be found after exercise or in association with stress, seizures, dehydration or fever. It may occur on a random basis for which there is no obvious cause. This is also known as transient proteinuria, which is defined as proteinuria noted on 1 or 2 occasions, but not present on subsequent testing, especially when the inciting factor remits or is removed. A large proportion of healthy children may have an occasional urine sample with proteinuria. This is rarely associated with significant kidney disease.

Orthostatic (postural) proteinuria is defined as elevated protein excretion when the subject is upright, but normal protein excretion during recumbency. This occurs commonly in adolescents, with a prevalence of 2–5% [74]. The diagnosis is suggested by a normal protein-to-creatinine ratio in a first morning spot urine sample after the subject has been supine for the entire night, but an elevated protein-to-creatinine ratio after the subject has been upright for at least 4–6 h. Total urine protein excretion rarely exceeds 1 g/1.73 m^2/day in orthostatic proteinuria. A study of healthy Turkish children aged 6–15 years old found that the prevalence of orthostatic proteinuria was lower than previous literature if at least three random urine samples at least 2 weeks apart were taken to exclude transient proteinuria [75]. For continuing assessment of proteinuria after the third sample, the first morning urine sample was collected. In this study, the prevalence of proteinuria was 3.7%, 1.3% and 0.94% on the first, second and third samples, respectively and the prevalence of orthostatic proteinuria was 0.65% after the first morning urine collection. This study also suggested that underweight children had a tendency for orthostatic

proteinuria compared with overweight and obese children [75].

The postulated causes of orthostatic proteinuria are alterations in kidney or glomerular hemodynamics, circulating immune complexes and partial left renal vein entrapment, as may be seen in thin individuals [76]. The incidence of orthostatic proteinuria decreases with advancing age, possibly due to the gradual accumulation of retroperitoneal fat. Long-term studies where patients have been followed for up to 50 years have documented the benign nature of orthostatic proteinuria, although rare cases of glomerulosclerosis have been identified later in life in patients who were initially diagnosed to have orthostatic proteinuria [77, 78]. No treatment is required for children with orthostatic proteinuria. It has been recommended that children with orthostatic proteinuria should be followed up annually [79].

It is important to remember that patients with glomerular disease may have an orthostatic component to their proteinuria. Protein excretion in these patients is greater when they are active or upright than when they are resting. Hence, orthostatic proteinuria should not be diagnosed unless the recumbent urine sample is normal.

Child with Persistent Proteinuria

Persistent proteinuria is defined as proteinuria of 1+ or more by dipstick on multiple occasions. This should be further investigated. Subjects who have persistent proteinuria, especially if this is associated with additional evidence of kidney disease such as microscopic hematuria, are more likely to have significant pathology in the urinary tract. In the Japanese school screening study, which looked at almost five million children, the prevalence of persistent isolated proteinuria was 0.07% in the 6–11-year age group, and this rose to 0.37% in the 12–14 year age group [27].

The majority of cases of persistent proteinuria are of glomerular origin, though non-glomerular mechanisms can also cause marked proteinuria (Table 12.7).

Glomerular proteinuria may be due to the following factors:

Table 12.7 Causes of proteinuria in children

Intermittent proteinuria	Persistent proteinuria	
	Glomerular	Tubular
Non-postural Fever Exercise Seizures Emotional stress Dehydration No known cause Postural (Orthostatic)	Primary glomerulopathies Minimal change disease Focal segmental glomerulosclerosis Membranoproliferative glomerulonephritis Membranous nephropathy Rapidly progressive glomerulonephritis Congenital nephrotic syndrome Secondary glomerulopathies Post-infectious glomerulonephritis Lupus nephritis IgA nephropathy IgA vasculitis nephritis Alport syndrome Hepatitis B nephropathy Hepatitis C nephropathy Human immunodeficiency virus (HIV) nephropathy Amyloidosis Hemolytic uremic syndrome Diabetes mellitus Hypertension Hyperfiltration following nephron loss Reflux nephropathy	Hereditary Cystinosis Galactosemia Tyrosinemia Hereditary fructose intolerance Wilson disease Lowe syndrome Mitochondrial cytopathies Dent's disease Polycystic kidney disease Acquired Pyelonephritis Tubulointerstitial nephritis Acute tubular necrosis Analgesic abuse Drugs such as penicillamine Heavy metal poisoning (e.g., lead, cadmium, gold, mercury) Vitamin D intoxication

- Increase in glomerular permeability to plasma proteins in residual nephrons in cases where there is reduction in nephron mass. This mechanism probably explains the increased proteinuria seen in patients with reflux nephropathy, progressive kidney disease reaching end-stage and the increased proteinuria observed in kidney transplant donors [80].
- Loss of negative charge in the glomerular filtration barrier [81, 82]. This results in mostly albuminuria. There is little increase in glomerular permeability to globulins;hence, the proteinuria is highly selective. A typical example is minimal change disease.
- Direct injury to the glomerular filtration barrier. The glomerular capillary wall consists of three structural components that form the permselectivity barrier: the endothelial cells, glomerular basement membrane and podocytes. The podocyte is crucial for maintenance of the glomerular filter, and disruption of the epithelial slit diaphragm leads to proteinuria [83]. These changes have been demonstrated in patients with nephrotic syndrome irrespective of the primary disease. Such injury increases the "effective pore size" in the glomeruli, causing an increase in the permeability of the mechanical barriers to the filtration of proteins. Hence, there is an increase in filtration of albumin and also larger proteins such as globulins. The clearance of globulins is relatively high and the proteinuria is described as non-selective.
- Mutations of key podocyte genes. Mutations of genes involved in regulation of the slit diaphragm proteins and their interaction with the actin cytoskeleton also result in proteinuria.
- Changes in glomerular capillary pressure due to disease and resulting in increased filtration fraction [49, 50, 84]. Examples are increased filtration fraction in hyperreninemia and hyperfiltration in the early stages of diabetic nephropathy.

The increased filtered load of protein overwhelms the tubular reabsorptive mechanisms; hence, the excess protein appears in the urine. Glomerular proteinuria can be classified as selective or nonselective. In selective proteinuria, there is a predominance of LMW proteins such as albumin or transferrin, as compared to higher molecular weight proteins such as IgG. The selectivity index is expressed as the clearance ratio of IgG over albumin or transferrin. An index less than 0.1 is indicative of highly selective proteinuria [85, 86], and this is seen in steroid-sensitive nephrotic syndrome and Finnish-type congenital nephrotic syndrome. More recent studies have shown that there is a significant relationship between selectivity of proteinuria and tubulointerstitial damage in kidney disease [87]. When proteinuria is highly selective, tubulointerstitial damage is less common.

Non-glomerular mechanisms include tubular proteinuria, overflow proteinuria and secretory proteinuria. Tubular proteinuria results when there is damage to the proximal convoluted tubule, which normally reabsorbs most of the filtered protein. The amount of protein in the urine due to tubular damage is usually not large and does not exceed more than 1 g/1.73 m^2/day. Glomerular and tubular proteinuria can be distinguished by protein electrophoresis of the urine. The primary protein in glomerular proteinuria is albumin, whereas in tubular proteinuria the LMW proteins migrate primarily in the α and β regions. β2-microglobulin, α1-microglobulin and retinol-binding protein are the markers commonly used for identification of tubular proteinuria [88]. Children with proximal tubulopathies such as Lowe syndrome and Dent's disease have tubular proteinuria. Albuminuria may eventually be detected in many tubulopathies as a marker of late glomerular involvement.

Overflow proteinuria results when the concentration of filterable proteins in the glomerular filtrate exceeds the maximal tubular reabsorption capability for that protein. This can occur even with normal renal function. Examples include monoclonal gammopathy of undetermined significance or multiple myeloma in adults (immunoglobulin light chains or Bence-Jones protein), hemoglobinuria, myoglobinuria, β2-microglobulinemia, myelomonocytic leukemia and even following transfusions. After multi-

ple transfusions of either albumin or whole blood, plasma albumin concentration may increase sufficiently to cause albuminuria.

In secretory proteinuria, the increased excretion of tissue proteins into the urine may result in proteinuria. The typical example is excretion of Tamm-Horsfall protein in the neonatal period, accounting for the higher levels of protein excretion typically seen at this age. In UTIs, mild proteinuria may be detected due to irritation of the urinary tract and increased secretion of tissue proteins into the urine. Secretory proteinuria also occurs in analgesic nephropathy and inflammation of the accessory sex glands.

Clinical Approach to Proteinuria

The finding of proteinuria in a single urine specimen in children and adolescents is relatively common. In large school screening programs, the prevalence of isolated proteinuria on a single urine ranged from 1.2 to 15% [2, 28, 89]. Persistent proteinuria on repeated urine testing is much less common [75]. When proteinuria is detected, it is important to determine whether it is intermittent, especially orthostatic, or persistent. It is also important to exclude kidney failure and acute nephritic or nephrotic syndrome because these conditions demand urgent investigations and treatment.

History

It is important to ask about any recent illness. Inquire about symptoms of CKD (e.g. polyuria, nocturia, pruritus, lethargy) or glomerulonephritis (e.g. edema, hematuria, oliguria), and connective tissue disorders (e.g. rashes, joint pain). A history of recurrent UTIs may suggest reflux nephropathy. Medications that are associated with proteinuria include non-steroidal anti-inflammatory medications, antibiotics (e.g. penicillin, sulfonamides, cephalosporins, quinolones, aminoglycosides), amphotericin B, cisplatin, allopurinol, and herbal medicines. A family history of polycystic kidney disease, hematuria, proteinuria, nephrotic syndrome, kidney failure or deafness should be obtained.

Physical Examination

Examination may reveal evidence of CKD such as growth failure, pallor from anemia, and rickets from metabolic bone disease. Hypertension is common in CKD. The presence of raised jugular venous pressure, hepatomegaly and edema suggest the child may be fluid overloaded due to acute nephritic syndrome or severe kidney functional impairment, and thus require urgent diuresis. Nephrotic syndrome may cause generalized edema, ascites, pleural effusion and scrotal edema. Associated signs of systemic illnesses, such as palpable purpuric rash on the lower limbs suggesting IgA vasculitis nephritis (Henoch Schönlein purpura) and joint swelling suggesting connective tissue disorders, should be sought. Palpable flank masses may suggest hydronephrosis or polycystic kidney disease.

Investigations

Isolated proteinuria is benign in the vast majority of children and can be transient and postural; hence, it is inappropriate to extensively investigate all children found to have proteinuria. A step-by-step approach is recommended to evaluate isolated proteinuria in an asymptomatic child or a child with an incidental finding of urine dipstick protein 1+ or 2+ during an acute illness. However, if the child has signs and symptoms suggestive of kidney disease, a detailed investigation should start promptly. Similarly, if the initial urine dipstick shows the presence of hematuria and proteinuria, detailed evaluation for kidney disease should be performed. Microscopic hematuria is the most common indicator of a glomerular lesion in a proteinuric patient. The existence of hematuria with proteinuria carries a more serious connotation than isolated proteinuria. Investigations including kidney biopsy of school children with persistent hematuria and proteinuria have found that 25–60% had evidence of a

glomerulopathy [29, 90], especially in those with proteinuria greater than 1 g/L. [29]

The first step in the evaluation of a child with isolated proteinuria is to determine whether the proteinuria is persistent (Fig. 12.2). Most children who have proteinuria on screening urine dipstick do not have kidney disease, and the proteinuria will resolve on repeat testing [27]. If proteinuria of 1+ or more persists on two subsequent dipstick tests at weekly intervals, further investigations are required. If proteinuria is absent on subsequent testing, the initial proteinuria may be transient and related to fever, vigorous exercise or emotional stress, and no further investigations are required. The parents and patient should be reassured and, as a precaution, a urine dipstick test for protein can be repeated in 3–6 months. If proteinuria on dipstick recurs or is persistent, the next step is to quantify the amount of proteinuria.

There are two methods to quantify proteinuria, spot urine protein-to-creatinine ratio and 24-h urinary total protein collection. An early morning spot urine protein-to-creatinine ratio is recommended to exclude orthostatic proteinuria. In orthostatic proteinuria, the first morning urine sample is negative for protein and the later urine samples may contain varying concentrations of protein, whereas the 24-h urinary total protein is normal or mildly elevated. If orthostatic proteinuria is strongly suspected, one way to prove this is to provide the family with urine dipsticks. The child's urine is tested twice daily for 1 week. The family should test the first urine sample voided in the morning and the last urine sample voided in the evening before the child sleeps. It is important that the bladder is completely emptied before going to sleep, and the child remains supine in bed throughout the night so that the early morning urine sample on awakening consists of urine formed in the recumbent position. The evening urine sample consists of urine formed in the upright position. If the urine dipstick is persistently negative in the morning and positive in the evening, orthostatic proteinuria is likely. No further investigations are required, and the urine should be rechecked for proteinuria yearly as a precaution.

If spot urine protein-to-creatinine ratio is more than 0.02 g/mmol (20 mg/mmol or 0.2 mg/mg), it is advisable to confirm the presence of significant proteinuria with a 24-h urinary total protein collection. After excluding transient and orthostatic proteinuria, and if the 24-h urinary total protein is greater than 0.3 g/1.73 m^2/day, it is useful to evaluate for kidney disease. Urinary protein excretion less than 0.3 g/1.73 m^2/day is associated with regression of proteinuric chronic nephropathies [91], suggesting that investigations are only necessary above this level. The suggested work-up includes the following:

Urine Examination

Microscopic examination of the fresh urine sample for blood, casts and crystals is required. A clean catch urine sample for culture may be necessary to rule out occult UTI, especially if there is a history of recurrent fevers in infancy. If a tubular disorder or interstitial nephritis is suggested from the history or urinary findings of eosinophils, measurement of the urinary excretion of β2-microglobulin, α1-microglobulin and retinol-binding protein, markers of tubular proteinuria, can be helpful. Tubular proteinuria is suspected if the urinary excretion of β2-microglobulin, α1-microglobulin and retinol-binding protein exceeds 0.04, 2.2 and 0.024 mg/mmol creatinine or 4×10^{-4}, 0.022 and 2.4×10^{-4} mg/mg creatinine, respectively [88].

Blood Examination

Assess the kidney function with serum urea, creatinine and electrolytes. Creatinine clearance or application of an estimating formula such as the revised Schwartz formula [92] gives a more accurate assessment of kidney function than serum creatinine alone. A reduction in kidney function is one of the most important indications for a kidney biopsy. Serum total protein and albumin should be checked. Most proteinuric patients do not have decreased serum levels of protein or albumin unless they have nephrotic syndrome or they have heavy proteinuria for a significant period. Hypoproteinemia may be an indication for kidney biopsy. In addition, serum cholesterol

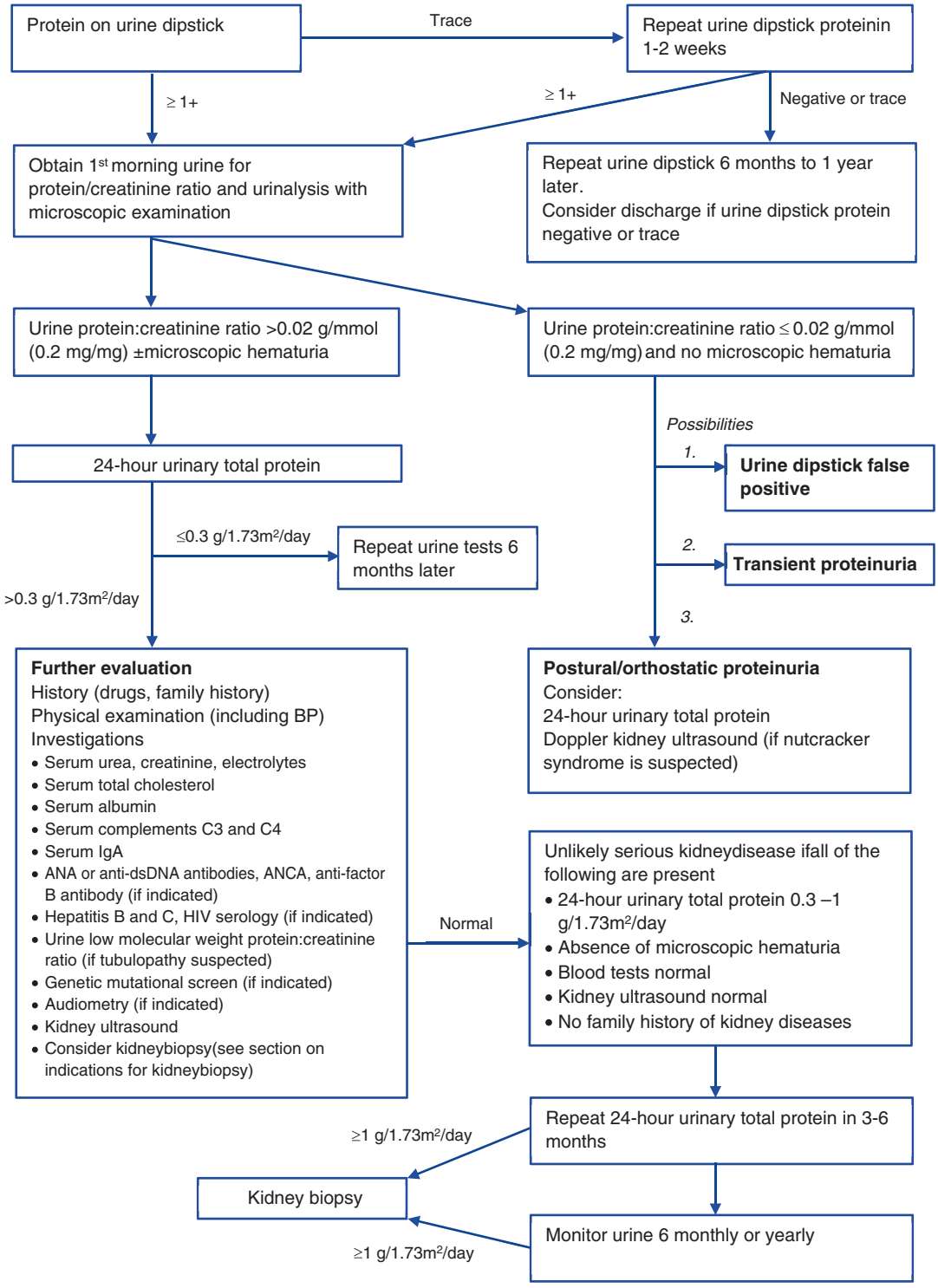

Fig. 12.2 Algorithm for investigating proteinuria

is measured as an indicator of the presence hyperlipidemia, which is suggestive of nephrotic syndrome.

Serum levels of C3 and C4 should be checked routinely as this may provide evidence of glomerulonephritis. Decreased C3 and C4 levels are seen in systemic lupus erythematosus, while decreased C3 with normal C4 levels occurs in post-infectious glomerulonephritis or C3 glomerulopathies, including membranoproliferative glomerulonephritis. Anti-factor B antibody levels, an autoantibody targeting factor B (a component of the alternative pathway C3 convertase), is highly specific for post-infectious glomerulonephritis, distinguishing it from C3 glomerulopathy [44]. ANA, anti-dsDNA antibodies, IgA levels, antistreptolysin O titers (ASOT) or anti-DNase B titers, ANCA, hepatitis B, hepatitis C and human immunodeficiency virus (HIV) serology should be considered if the clinical setting and preliminary investigations are suggestive, as these may give a clue to the underlying etiology of the proteinuria. In addition, appropriate mutational screening should be considered if a hereditary disorder for the proteinuria or nephrotic syndrome is suspected.

Kidney Imaging

Kidney ultrasonography is done routinely in the evaluation of isolated proteinuria to identify anatomical abnormalities of the kidneys or urinary tract as these can result in reduction of nephron mass. A significant difference in the sizes of the kidneys may suggest underlying reflux nephropathy or renal dysplasia. If reflux nephropathy is suspected, a DMSA scan is useful to demonstrate the existence of renal scars. Doppler kidney ultrasonography is helpful if the patient has coexisting hypertension as proteinuria can occur in hypertensive nephropathy due to renal artery stenosis. In patients with orthostatic proteinuria, Doppler sonography of the left renal vein may be a useful screening tool to exclude the nutcracker syndrome [93].

Audiometry

Audiometry is indicated when there is a family history of nephritis, kidney failure or deafness. Deafness may be detected during later childhood in Alport syndrome, and is generally associated with progressive kidney disease.

If these urine and blood tests as well as the initial kidney ultrasound are normal, and if the proteinuria is less than 1 g/1.73 m^2/day, it is unlikely that the child has a serious kidney disease. The family should therefore be reassured that the proteinuria may disappear or it may persist without any evidence of progressive kidney failure developing. As the level of proteinuria is associated with outcome in chronic nephropathies [49, 91, 94], it is also important to emphasize to the family that follow-up urine tests are necessary. The child should be reviewed in 3–6 months. If the repeat test shows that the proteinuria is not marked (i.e., less than 1 g/1.73 m^2/day), the child's urine is then monitored twice during the subsequent year and yearly thereafter. If there is persistent significant proteinuria on follow-up, a kidney biopsy may be indicated.

Indications for Kidney Biopsy

Kidney biopsy is indicated in the following situations:

- Persistent significant proteinuria of more than 1 g/1.73 m^2/day [95] or random urine protein-to-creatinine ratio of >0.05 g/mmol (>50 mg/mmol, >0.5 g/g) [96]. The heavier the proteinuria, the more likely a tissue diagnosis will be obtained from the kidney biopsy. A study on kidney biopsies in Japanese children with asymptomatic, persistent, isolated proteinuria showed that a 41.4% probability of significant glomerular changes, such as focal segmental glomerulosclerosis, when using a urine protein-to-creatinine ratio > 50 mg/mmol (0.5 g/g) as a biopsy criterion [96]. The exception is the child with typical steroid sensitive nephrotic syndrome suggestive of minimal change disease, where kidney biopsy is not indicated at presentation.
- Proteinuria associated with urinary sediment abnormalities (e.g. hematuria). Kidney biopsy is more likely to be diagnostic when proteinuria is associated with urinary sediment abnor-

malities than when there is either isolated proteinuria or isolated hematuria.
- Decreased glomerular filtration rate (GFR). A GFR less than 60 ml/1.73 m^2/min is an indication for kidney biopsy. The exception is a child who is recovering from an acute glomerulonephritis (e.g. post-infectious glomerulonephritis). In this case, the GFR should be remeasured in a month, and if the GFR remains low, a kidney biopsy is required.
- Low C3 levels for more than 3 months. A low C3 level during the acute phase of post-infectious glomerulonephritis is not an indication for biopsy. If the C3 level remains low after 3 months, a kidney biopsy is indicated.
- Evidence of a vasculitis (e.g. systemic lupus erythematosus, IgA vasculitis nephritis, ANCA-positive vasculitis), either clinically or serologically.

Potential Role of Genetic Testing in Evaluation of Proteinuria in Children

Historically, the diagnostic workup of children with persistent isolated proteinuria included urinalysis, blood examination, and radiological imaging before proceeding to histological diagnosis via kidney biopsy. The role of kidney biopsy in the investigation of asymptomatic, isolated proteinuria is controversial in children due to its invasiveness and requirement for sedation. Advances in technology has resulted in increased availability of genetic testing to establish a diagnosis. Genetic testing may be considered early in the diagnostic workup in order to reduce the need for kidney biopsy.

ADCK4-related glomerulopathy is an example of a genetic disorder presenting with proteinuria where effective intervention is possible. The PodoNet consortium found biallelic ADCK4 pathogenic variants in 1.9% of 534 patients with steroid resistant nephrotic syndrome. Most patients with ADCK4 mutations had a renal-limited phenotype with nephrotic range proteinuria or advanced CKD during adolescence [97]. Renal biopsy uniformly showed FSGS. These patients did not respond to corticosteroids or other immunosuppressive therapy and progressed to ESKD in the second decade of life. A Korean study showed that all patients with ADCK4 mutations also had medullary nephrocalcinosis [98].

Coenzyme Q10 supplementation (dose of 20–30 mg/kg/day) reduces proteinuria and preserves GFR in young patients with ADCK4 mutations when initiated in the early stage of the disease when patients have asymptomatic proteinuria and before the development of irreversible kidney damage [99–101]. Hence, there is a role of genetic testing in the diagnostic workup of patients with proteinuria. Early detection and early intervention of potentially treatable genetic kidney diseases such as ADCK4-related glomerulopathy may slow or prevent progression to kidney failure.

Treatment Options for Significant Proteinuria in the Non-nephrotic Range

It is well-recognized that glomerular proteinuria may play a role in the progression of kidney disease [102]. Proteinuria has also been identified as a risk factor for cardiovascular disease in adults and children [52, 53]. Moreover, as the severity of proteinuria increases, it is associated with metabolic disturbances such as hypercholesterolemia, hypertriglyceridemia and hypercoagulability that contribute to cardiovascular disease.

The following are postulated mechanisms whereby proteinuria may induce kidney injury: [64].

- Filtration of lipoproteins and absorption by proximal tubules may activate inflammatory pathways causing cell injury.
- Filtration of cytokines or chemokines may provoke cell proliferation, inflammatory cell infiltration, and activation of infiltrating cells.
- Filtration or generation of novel antigens may function as antigen-presenting cells and initiate a cellular immune response.
- Iron that is filtered into tubular fluid and bound to transferrin may be directly toxic or may

have indirect effects due to iron-catalyzed synthesis of reactive oxygen metabolites.
- Activation of the alternative complement pathway by proximal tubules may be harmful.
- The release of lysosomal enzymes into the cytoplasm of protein-reabsorbing tubules may cause damage.
- The release of vasoconstricting molecules may cause ischemic tubular injury.
- Interstitial fibrosis may result from the release of fibrosis-promoting factors from kidney cells activated or injured by proteinuria.
- The proteinaceous casts can obstruct kidney tubules.

Persistent significant proteinuria should be addressed. Besides determining the cause and initiating specific therapy if possible, other treatment options include dietary protein recommendations and use of anti-proteinuric medications.

Dietary Protein Recommendations

Dietary protein restriction has been proposed in adults with CKD to stabilize kidney function [91, 103]. In a small series of children with CKD, there was some benefit from dietary protein restriction [104]. However, another controlled study did not demonstrate a significant impact of protein restriction on the rate of progression of CKD [51]. High dietary protein intake may worsen proteinuria in some patients with nephrotic syndrome. Moreover, it does not result in a higher serum albumin. Hence, it is best to avoid an excess of dietary protein in children with proteinuric kidney diseases. It is recommended that children with proteinuria receive the suggested dietary intake of protein for age; they should not be protein-restricted [105].

Drugs with Anti-proteinuric Effects

The angiotensin converting enzyme inhibitors (ACEI) and the angiotensin II receptor blockers (ARBs) can reduce systemic blood pressure (BP) and exert other beneficial effects, such as decreasing urinary protein excretion and decreasing the risk of kidney fibrosis. In addition, kidney function is better preserved in children with CKD when lower systolic BPs are achieved [106]. However, the long-term benefit of ACEIs and ARBs in children and adolescents with proteinuria remains to be established. Intensive BP control with ACEI effectively delays the progression of kidney disease among children with an underlying glomerulopathy or renal hypoplasia or dysplasia, but not among children with other congenital or hereditary nephropathies. In the ESCAPE trial, ACEI was shown to reduce proteinuria by approximately 50% within the first 6 months. However, proteinuria gradually increased during ongoing ACEI therapy to levels that were no different after the third year, despite persistently good BP control [107]. A post hoc analysis of the ESCAPE trial showed that a higher degree of proteinuria reduction during the first months of ACEI treatment was independently associated with a lower risk of CKD progression [108]. Both a higher residual proteinuria level after attaining the full dose of ACEI and the total exposure to proteinuria during follow-up was associated a higher risk of CKD progression in children. These findings were independent of the underlying kidney disease, baseline proteinuria and BP control.

There are reports of infants born to mothers taking ACEI during the second and third trimesters of pregnancy who developed oligohydramnios, pulmonary hypoplasia and postnatal hypertension. Postmortem examination of these neonates who died showed severe glomerular and tubular malformations in the kidneys. Hence, ACEI are contraindicated during pregnancy [109]. The safety of ACEI and angiotensin II receptor blockers in young infants is still unknown. Cases of ACEI-induced nephrotoxicity had been reported in premature infants with cardiac failure due to congenital heart disease [110] and young infants after cardiac surgery [111]. There was no underlying kidney disease found in these infants and acute kidney injury was reversible upon discontinuation of the ACEI. Another concern of ACEIs in premature infants is that

they may impair the final stages of kidney maturation, and therefore should be avoided before a corrected post-conceptual age of 44 weeks [112].

Aliskiren, the first orally active direct renin inhibitor, has shown promising results in proteinuria reduction in adult patients with CKD [113]. In a case series of four children treated with aliskiren for refractory proteinuria, three children experienced clinically significant adverse effects, including symptomatic hypotension, hyperkalemia and accelerated loss of kidney function [114]. Hence, clinicians should exercise caution when prescribing aliskiren until appropriate pediatric trials establish dosing, efficacy and safety.

Conclusion

Hematuria or proteinuria in children is frequently encountered. Many investigations have been recommended in the workup for a child presenting with hematuria or proteinuria. Many of the cases of hematuria or proteinuria are normal transient findings. Hence, a stepwise evaluation is recommended to avoid unnecessary and expensive investigations, but identify children with serious conditions. There is an increasing role of genetic testing to establish a clinical diagnosis of hereditary causes of kidney disease. Early detection and treatment of serious conditions should hopefully delay or prevent the onset of kidney functional abnormalities. Screening programs for hematuria and proteinuria may be able to identify children at an earlier stage; however, the major disadvantage is the cost effectiveness, as well as the anxiety in parents and children when the finding is spurious or transient.

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Steroid Sensitive Nephrotic Syndrome

13

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Abbreviations

APN	Arbeitsgemeinschaft für Pädiatrische Nephrologie
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
CI	Confidence intervals
CNI	Calcineurin inhibitor
DXA	Dual energy x-ray absorptiometry
ESKD	End stage kidney disease
FRNS	Frequently relapsing steroid sensitive nephrotic syndrome
FSGS	Focal and segmental glomerulosclerosis
GFR	Glomerular filtration rate
HR	Hazard ratio
ISKDC	International Study of Kidney Disease in Children
KDIGO	Kidney Disease Improving Global Outcomes
MCD	Minimal change disease
MesPGN	Mesangial proliferative glomerulonephritis
MMF	Mycophenolate mofetil
MPA	Mycophenolic acid
RCT	Randomized controlled trial
RR	Relative risk
SDNS	Steroid dependent steroid sensitive nephrotic syndrome
SDS	Standard deviation score
SIRS	Soluble immune response suppressor
SLE	Systemic lupus erythematosus
SRNS	Steroid resistant nephrotic syndrome

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SSNS	Steroid sensitive nephrotic syndrome
VEGF	Vascular endothelial growth factor
VPF	Vascular permeability factor

Introduction

Nephrotic syndrome is characterized by massive proteinuria, hypoalbuminaemia and generalized oedema. Clinically childhood nephrotic syndrome has been classified into steroid sensitive nephrotic syndrome (SSNS), steroid resistant nephrotic syndrome (SRNS), congenital and infantile nephrotic syndrome (0–12 months) and nephrotic syndrome secondary to other diseases including IgA vasculitis, systemic lupus erythematosus (SLE) and hepatitis B nephropathy. Between 1967 and 1974, the International Study of Kidney Disease in Childhood (ISKDC) enrolled 521 children aged 12 weeks to 16 years with idiopathic nephrotic syndrome to evaluate the histopathological, clinical and laboratory characteristics of nephrotic syndrome in children. The kidney biopsy studies demonstrated that about 80% of children had either minimal change disease (MCD; 76.4%), focal and segmental glomerulosclerosis (FSGS; 6.9%) or mesangioproliferative glomerulonephritis (MesPGN: 2.3%) [1]. Subsequently the ISKDC demonstrated that the response to corticosteroids was highly predictive of kidney histology with 93% of children with MCD achieving complete remission following an 8 week course of prednisone [2]. However between 25–50% of children with MesPGN or FSGS on biopsy also responded to prednisone [2]. Since most children presenting with nephrotic syndrome now do not undergo kidney biopsy at diagnosis, children with idiopathic nephrotic syndrome are now classified according to their initial response to corticosteroids into SSNS or SRNS. Although many children with SSNS have one or more relapses, the majority continues to respond to corticosteroids throughout their subsequent course [3–5] and the long term prognosis for complete resolution with normal kidney function is good. This chapter is devoted to SSNS. Commonly used definitions for SSNS and SRNS are shown in Table 13.1.

Table 13.1 Definitions used in idiopathic nephrotic syndrome [125, 166, 311–313]

Classification	Definition
Nephrotic syndrome	Oedema, uPCR ≥200 mg/mmol (≥2000 mg/g) or ≥50 mg/kg/day or ≥3+ on urine dipstick, hypoalbuminaemia ≤25 g/L (≤2.5 mg/dL)
Complete remission	uPCR ≤20 mg/mmol (≤200 mg/g) or ≤1+ protein on urine dipstick for 3 consecutive days
Initial responder	Attainment of complete remission within initial 4 weeks of corticosteroid therapy
Initial non-responder/steroid resistance	Failure to achieve remission during initial 4 weeks of corticosteroid therapy
Relapse	uPCR ≥200 mg/mmol (≥2000 mg/g) or ≥3+ protein or more on urine dipstick for 3 consecutive days
Infrequent relapse	One relapse within 6 months of initial response or 1–3 relapses in any 12 month period
Frequent relapse	Two or more relapses within 6 months of initial response or 4 or more relapses in any 12 month period
Steroid dependence	Two consecutive relapses during corticosteroid therapy or within 14 days of ceasing therapy
Late non-responder	Persistent proteinuria after 4 weeks or more of corticosteroids following one or more remissions

uPCR urine protein-creatinine ratio

Epidemiology

Prospective studies of children with newly diagnosed idiopathic nephrotic syndrome identified through Pediatric Surveillance Units in the Netherlands, Australia and New Zealand reported incidences of idiopathic nephrotic syndrome of 1.12–1.9 per 100,000 children aged below 16 years [6–8]. However the reported incidence of idiopathic nephrotic syndrome from other countries varied between 1.8 and 16.9 per 100,000 children aged below 16 years [9]. The

incidence is highest in children of south Asian ancestry as demonstrated in studies from Canada and the United Kingdom [9].

Most children with nephrotic syndrome have SSNS [9]. The incidence of SRNS ranges from 2.1 to 27.3% and varies with country of origin with the highest rates seen in African and African-American children and the lowest rates seen in children of South Asian ancestry [9]. SSNS is more common in boys than girls with a male: female ratio of around 2:1 and a peak incidence between 1–4 years [1]. In 2007, a review of retrospective studies from tertiary services comparing the prevalence of FSGS over two time periods found that the odds of FSGS in children with nephrotic syndrome had increased twofold suggesting an increase in the number of children with FSGS [10]. However, the analyses involved fewer than 1000 children, there was significant heterogeneity in the results and bias related to selective referral to tertiary centres could not be excluded.

Aetiology and Pathogenesis

A T Cell Disease but New Thoughts on B Cells

A series of clinical observations led Dr. Shaloub in 1974 to propose that SSNS was due to an abnormality of function in T cells [11]. Nephrotic syndrome had been observed in patients with Hodgkins lymphoma, and cases of thymoma [12, 13]. The disease was noted to remit in children who had measles, which led some people to propose using measles as a therapeutic strategy [14–16]. A major effect of measles is to inhibit cell mediated immunity thereby shutting down T cell function. Further the response of nephrotic syndrome to T cell suppressive agents such as steroids or calcineurin inhibitors (CNI) also supported their role in nephrotic syndrome [11]. These features all suggested that lymphocytes are key cells in SSNS. Recent success in treating recurrent FSGS and SSNS with the CD20 B cell depleting antibody rituximab raises the possibility of either B cells influencing T cells or B cells themselves being primary players in nephrotic syndrome. It is unclear if this a function of the role of B cells as antigen presenting cells or because of antibody production.

A Circulating Factor

MCD appears to exist in a spectrum with FSGS. A proportion of children with MCD on clinical and histological grounds evolve into FSGS [17]. In both there appears to be a circulating factor with the children with FSGS being less responsive to therapeutic agents. Within this group is a subset of children where the disease resides in structural changes in the glomeruli with genetic mutations in key glomerular slit process proteins including nephrin, podocin, Actinin4 and WT-1. These are described elsewhere but in brief are associated with no response to steroids and progression to end-stage kidney disease (ESKD), and do not show evidence of a circulating factor as demonstrated by rapid recurrence of disease in a transplanted kidney. The timing of response with the return to normal function taking days to weeks is also supportive of slow podocyte recovery from an injurious cytokine. The higher rates of recurrence in children with FSGS receiving living related kidneys suggests that there may be a degree of HLA restriction of response and this is also supported by HLA linkage studies showing that increased incidence of disease is tied to certain alleles such as HLA B8, B13, DWQ2, DQB10301 and DR7 [18–21].

Various growth factors and cytokines have been proposed as pathogenic in SSNS over the years. The initial identification of the factor, vascular permeability factor (VPF) now called vascular endothelial growth factor (VEGF), was thought to have identified the key protein leading to nephrotic syndrome [22–24]. However, the identification of this protein in normal urine delayed further investigation of its role. More recently it has been noted to be increased in urine during relapses of nephrotic syndrome though circulating levels are unchanged suggesting that VEGF levels reflect the concomitant proteinuria [25, 26]. Recent tissue-specific knock-outs of VEGF in mice restricted to podocytes have dem-

onstrated a key role for local VEGF in maintaining glomerular endothelial integrity and again reinforced its importance though perhaps more locally in maintaining permeability [27, 28]. Soluble immune response suppressor (SIRS) was also identified as a potential protein mediating nephrotic syndrome but again the inability to characterize this protein despite many mechanistic observations led to its exclusion as the likely factor [23, 24, 29]. Other circulating factors have been proposed and the development of a functional assay of glomerular permeability by Dr. Savin in the late 1990s identified a proteinuric factor that was small, highly glycosylated, and hydrophobic [30]. This appeared to be likely to allow fractionation of nephrotic sera and identification of the factor. Other observations that protein A columns could remove the nephrotic factor post-transplant also seemed to point to identifying features [31]. More recently induction of proteinuria in rats with transfer of serum may allow models that can identify this factor as has the demonstration that overexpression of the Th2 cytokine IL-13 induces proteinuria in rats [32]. Recently podocyte-secreted angiopoietin-like-4 was found to mediate proteinuria in SSNS using overexpression in the podocyte in rat models though further confirmatory studies are awaited [33].

The central role of T cells in disease has led to a number of strategies to identify the underlying defect. The thought that the disease was caused by a low frequency pathogenic clone has given way to a view that there is a generalized alteration in the lymphocytes that is triggered in these individuals and then can be switched off by treatment. This has been studied in several ways including assessment of T cell derived cytokine responses either directly in plasma or by measurement of supernatants from activated mononuclear cells or measurement of RNA, assessment of T cell subsets by immuno-phenotyping or finally by functional assays of cell mediated immunity. More recently the identification of a role for microRNAs in FSGS affecting WT1 or more recently CD2AP in nephrotic syndrome suggested this may be a fruitful area of research [34, 35].

Phenotypes of Cytokine Secreting T Cells: Th1, Th2, Treg, Th17

Naïve T cells on activation become polarized into different subsets defined by their cytokine production and driven by the cytokine milieu in which they are activated. The initial division of T cells was into CD4 (originally helper) T cells, that respond to exogenous antigen presented by antigen presenting cells in the context of MHC Class II, and CD8 (originally effector) T cells that respond to internal antigens presented by all cells. CD4 T cells were further divided into Th1 and Th2 cells based on the cytokines they produced [36]. This was initially observed in mice but human Th1 cells also produce cytokines such as IFN-gamma and TNF, which are used in cell mediated immune responses. Th2 cells produce IL-4, IL-5 and IL-13 which are key to humoral immunity and are used by B cells to class switch and act as growth factors for eosinophils [37, 38]. It is now apparent that CD8 T cells can produce cytokines and can be polarized to Tc1 and Tc2 expressing similar cytokines to those in CD4 Th cell subsets [39]. The observation that allergy is more common in children with nephrotic syndrome suggested that this might be a Th2 disease [18]. A subset of T cells thought to suppress activity in other T cells was originally described as suppressor T cells and these have recently been reclassified as regulatory T cells. These are thymically derived and express regulatory cytokines such as TGF-β and IL-10 and express regulatory molecules such as CTLA-4. A key marker of these cells is the expression of the transcription factor foxp3 [40–42]. Interestingly there is now another T cell subset that is an alternative to regulatory T cells called the Th17 cell because it expresses the cytokine IL-17. Th17 cells are induced by IL-23 but can be generated by IL-6 and TGF-ß thus acting as an alternate pathway of development to regulatory T cells [42, 43]. There are now data linking Th17 cells to nephrotic syndrome and the biological effect of these cells on podocytes [44, 45].

In general, studies of cytokines have been disappointing. No clear up-regulation of Th2 type

cytokines has been demonstrated. Studies of serum of patients in remission show IL-1 unchanged, IL-2 normal or undetected in four of five studies, sIL-2R increased in four of six studies, IFN-gamma normal or not detected in three and increased in two studies, IL-4 normal or decreased, IL-8 normal, increased and decreased in four studies, IL-10, IL-12 and IL-13 either normal or not detected and TNF-β normal in three of four studies [46]. Studies of culture supernatants of stimulated mononuclear cells from children with active SSNS are also highly variable though four studies suggest elevated IL-4, two studies elevated IL-12, and five studies elevated TNF-α. RNA measurements for specific cytokines in blood have been equally unrewarding as have those using intracellular cytokine staining [46]. Urinary reports are confounded by concurrent proteinuria but there has been a recent report of IL-17 increased in the urine of patients with SSNS [22, 47]. Other non-T cell inflammatory proteins associated with SSNS include neopterin which is produced by activated macrophages and is increased in SSNS [48].

Genetic Influence in the Aetiology of SSNS

Significant research effort over recent years has identified over 50 genes linked to SRNS [49] with the majority coding for proteins in the podocytes which control function and stability of the actin cytoskeleton. In stark contrast, almost no genes have been identified that have a Mendelian influence on the development of SSNS. This is despite the known ethnic variation in prevalence, familial clustering and a history of affected first degree relatives previously reported at 3% [50, 51]. Similarly to IgA Nephropathy, any genetic influence is likely mediated through complex inheritance of risk alleles in multiple genes.

Initial investigation of complex inheritance focused solely on HLA genes and identified possible association with HLA-DQ/DR. Further studies using exome wide association [52] and then genome wide association studies (GWAS) [53–56] confirmed that association across European, African, South Asian and Japanese cohorts. Through the use of HLA imputation, HLA haplotypes that confer maximum risk and also those that confer protection can be derived. Interestingly, while these were reproducible across the European and South Asian cohorts, Japanese cohorts had differing haplotypes [53–56]. The use of GWAS to study Membranous Nephropathy enabled identification of a non-HLA locus at PLA2R1 which has been incredibly informative in understanding disease pathology. In SSNS non-HLA loci have been identified (BTNL2, CALHM6/DSE, PARM1, TNFSF15 and NPHS1) but the signals are not as strong and to date are not clearly reproducible across all ethnic groups but cohort size has been relatively small [53, 55, 56]. The pathological mechanism by which an HLA haplotype may confer risk in SSNS has not yet been clearly elucidated and while it is likely to be related to the association with CD4 positive T cells, further work utilising new molecular tools may prove highly valuable [57].

Role of the Thymus

The information above, the association of nephrotic syndrome with T cell lymphomas and thymomas, the timing of thymic involution occurring around puberty at the same time as the resolution of relapses for the majority of children with uncomplicated SSNS, and the exquisite sensitivity of thymocytes to steroids all suggest a role for early T cells or other thymically derived cells in SSNS. Further evidence of early thymic emigrants in single cell analysis of CD2 positive cells from children with FSGS also supports a role for T cells, which are early emigrants from the thymus.

Role of Infection

While there has been no clear infectious agent identified as inducing nephrotic syndrome, there is an identifiable viral prodrome in around 50% of cases of relapse. Whether this merely

reflects cytokine release with the initiation of nephrotic syndrome or whether this is initiation of the disease by a viral trigger is not clear. Some groups have postulated that inflammation through TLRs may upregulate CD80 on podocytes leading to activation though CD80 and nephrosis [58]. There has also been interest in CD80 as a therapeutic target in resistant nephrotic syndrome [59].

Summary

While the evidence supports a role for T cells activated to secrete a permeability factor, identifying the specific T cell changes or characterization of the factor remains a major challenge in SSNS. The clinical results with rituximab in depleting B cells, and the data on CD80 expression on podocytes raises other alternatives as potential causes of SSNS including circulating cells other than T cells and specific podocyte responses, but there are limited data so far on these pathways.

Histopathology

Steroid sensitive nephrotic syndrome comprises a spectrum of disease that includes MCD, MesPGN (also known as "diffuse mesangial hypercellularity"), IgM nephropathy and FSGS. Although these are readily distinguished on biopsy, the clinical significance of this distinction remains controversial with significant overlap in behaviour and variation in morphological diagnosis over time in a small proportion of cases. This confusion is reflected in the literature. Some studies suggest a difference in behaviour between those with and without mesangial hypercellularity in the absence of immune deposits [60]. Some studies suggest an increased risk of steroid resistance and/or development of focal sclerosing lesions with MesPGN/IgM nephropathy [61, 62] with some studies documenting transition of MCD, MesPGN and IgM nephropathy to FSGS in frequently relapsing patients over time [17, 63]. In addition some studies suggest that the response to therapy in cases with immune deposition is "unpredictable" [64] or variable [65], and finally a number of studies have failed to find any significant difference in outcomes between these categories [66, 67]. Histological overlap also exists with MCD, MesPGN and FSGS in patients with predominant mesangial deposition of C1q (C1q nephropathy), although representing a small minority of patients with initial presentation of clinical nephrotic syndrome [68–70]. Similarly, mesangial IgA deposition in association with nephrotic syndrome has also been described in a defined subset of IgA nephropathy patients, with either MCD like changes or MesPGN on biopsy. IgA with MCD-like changes is generally responsive to steroid therapy, although with a significant rate of relapse [71]. Regardless of histopathology, children with disease resistant to steroids generally have a poorer outcome compared with those with responsive disease [72]. The ultimate prognosis for children with primary nephrotic syndrome and frequently relapsing disease associated with mesangial hypercellularity and/or positive immunofluorescence remains difficult to predict.

Minimal Change Disease (MCD)

The defining histological feature of minimal change disease (MCD) is normal appearing glomeruli on light microscopic examination (Fig. 13.1). This assumes that the specimen has an adequate sample of glomeruli, including deep glomeruli from the juxtamedullary region of the renal cortex. Glomeruli of normal young children are generally smaller compared with adults so appear relatively hypercellular. There is no significant expansion of mesangial matrix, and no increase in mesangial cellularity (either by increased numbers of mesangial cells or infiltration by inflammatory cells). The cytoplasm of the podocytes may appear to be mildly swollen or vacuolated. Glomerular capillary loops remain patent, and in many cases may appear mildly dilated. The glomerular capillary walls are thin with no evidence of basement membrane thickening. No basement membrane reduplication or

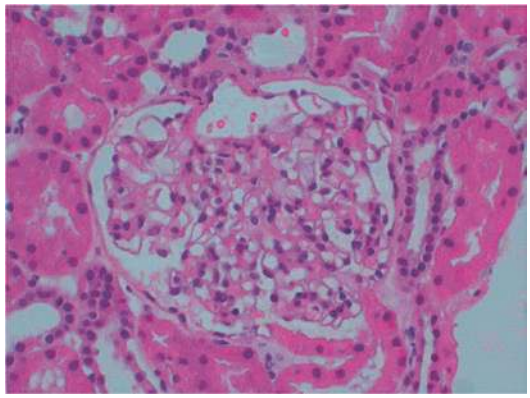

Fig. 13.1 The glomerulus appears normal to light microscopic examination, with normal mesangial matrix and cellularity. Capillary loops are dilated with normal thin capillary walls. (H&E stain, ×400)

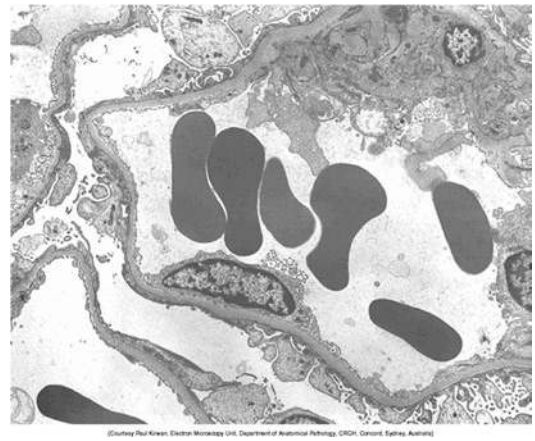

Fig. 13.2 Low power electron photomicrograph includes a capillary loop with extensive effacement of foot processes accompanied by swelling and microvillarisation of podocytes. Glomerular basement membranes are of normal appearance and no dense deposits are seen. (Courtesy of Paul Kirwan, Electron Microscopy Unit, Department of Anatomical Pathology, CRGH, Concord, Sydney, Australia)

epithelial spike formation are evident on examination of silver stained sections. The presence of an occasional glomerular "tip" lesion, defined as adhesion of the tuft to the Bowman's capsule at the site of opening of the proximal convoluted tubule, may be seen in MCD provided the glomerulus is otherwise normal in size and cellularity [73, 74]. The interstitium is normal without significant inflammation, fibrosis or tubular atrophy. Proximal tubule epithelial cells may contain hyaline droplets consistent with protein loss.

The immunofluoresence in MCD is negative. Very small amounts of IgM or C3 are considered by some to be compatible with MCD; however any significant immune reactant even in the setting of histologically normal glomeruli effectively excludes this diagnosis [64, 67, 72]. However, the clinical significance of these immune positive cases with normal histology is still controversial, and many now consider that these cases represent a spectrum of disease rather than distinct entities. Electron microscopic examination of untreated MCD shows uniform abnormality of the podocytes, with marked effacement of the foot processes over at least 50% of the glomerular capillary surface resulting in a smooth homogenous layer of epithelial cell cytoplasm which lacks the normal interdigitation. The cytoplasm of the cells may be enlarged with clear vacuoles and prominence of organelles. This is accompanied by microvillus transformation along the urinary surface of the podocytes (Fig. 13.2). The glomerular basement membrane otherwise appears normal, as do the mesangial cells and matrix. Immune deposits are absent. These changes are commonly modified with steroid treatment, and the degree of foot process effacement may be incomplete if the biopsy is taken from a partially treated patient.

Mesangial Proliferative Glomerulopathy (MesPGN)

Light microscopic examination of MesPGN shows generalized, diffuse mesangial cell hyperplasia, involving over 80% of the glomeruli. Increased numbers of mesangial cell nuclei are clearly present within mesangial matrix which is either normal or only mildly increased in amount (Fig. 13.3). There is generally no obvious lobulation of the glomerulus, and segmental sclerosis is absent. As in MCD, glomerular basement membranes remain thin and capillary loops clearly patent. By definition, spikes are not seen in silver stained sections. There is no significant intersti-

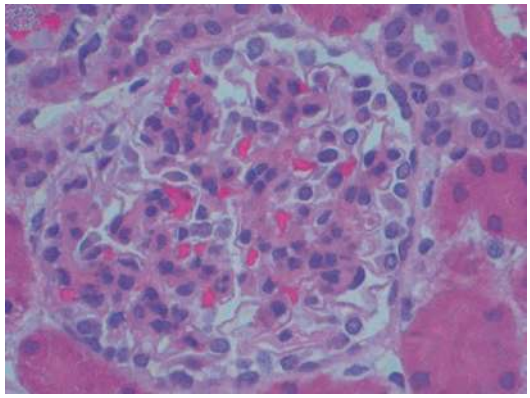

Fig. 13.3 The glomerulus shows increased numbers of mesangial cells with mildly increased matrix. The capillary loops appear normal. (H&E stain, ×400)

tial change (either tubular atrophy or fibrosis) to suggest glomerular loss. Glomerular immaturity, characterized by hypercellularity and a layer of cuboidal epithelium along the surface of the glomerular tuft, may be seen in some cases, particularly in younger children. Recent studies have suggested that these cases may have a less favourable clinical course [75].

Many cases of MesPGN show positive granular mesangial IgM ± C3 and very occasionally small amounts of C1q or IgG, although a proportion of cases have negative immunofluorescence. Some have considered these immune-positive cases as MesPGN, while others separate the positive cases into further distinct categories, most commonly IgM nephropathy. As noted earlier, these three "entities" (MCD, MesPGN, IgM Nephropathy) probably represent a spectrum rather than separate diseases. On electron microscopy there is mesangial cell hyperplasia with effacement of epithelial cell foot processes and microvillus transformation of epithelial cells. Dense deposits are not typically found, and the glomerular capillary basement membrane is normal.

IgM Nephropathy

IgM Nephropathy shows light microscopic features that may mimic those of either MCD or MesPGN. The sampled glomeruli may appear completely normal on routine stains, or may show diffuse mesangial hypercellularity. Some cases will show a combination of features, with some but not all glomeruli appearing hypercellular. As with MCD and MesPGN, segmental sclerosing lesions are not seen in an adequately sampled specimen, glomerular capillary loops remain thin walled and patent, and there is no basement membrane thickening or evidence of spike formation. Interstitial changes are absent. Granular deposits of IgM are confined to the mesangium and are generally seen in all glomeruli regardless of their histological appearance. Lesser amounts of C3 are common, and some cases may also show small amounts of C1q or IgG. In these cases, the IgM should remain as the dominant reactant. On electron microscopy there may be a mild increase in mesangial matrix. Immune deposits are often absent though some cases will show occasional small dense deposits that are located in paramesangial regions. Effacement of epithelial cell foot processes is usually seen to a varying degree, usually with microvillus transformation.

IgA Nephropathy with Nephrotic Syndrome

IgA nephropathy may present with clinical nephrotic syndrome indistinguishable from MCD in approximately 8–10% of cases. Nephrotic IgA nephropathy may show light microscopic features of MCD, MesPGN, or a focal GN (proliferative or sclerosing), however it is defined by the presence of dominant mesangial IgA deposition (frequently with some associated C3, and in approximately 50% of cases with lesser amounts of IgG and/or IgM), usually with electron microscopic evidence of immune deposits.

C1q Nephropathy

C1q nephropathy is an uncommon disorder that may also present with clinical nephrotic syndrome. Histology of these cases most commonly

shows an MCD-like picture (70–75% of cases), with MesPGN (20%) and FSGS (7–13%) seen in some cases. Distinction is made with immunofluorescence finding of predominant C1q deposition in the mesangium and electron dense deposits on electron microscopic examination. Although many of these cases are clinically steroid resistant or steroid dependent, being more likely to require chronic immunosuppression and combined therapy, overall prognosis is good in particular for those with minimal changes on light microscopy. Of note, a number of studies have shown disappearance of the C1q deposits following therapy [68, 70].

Focal Segmental Glomerulosclerosis

Although FSGS more commonly results in steroid resistant disease, a proportion of cases will respond, at least initially, to steroid therapy [76, 77], and thus brief mention of the pathological features is made here. In FSGS, segmental (involving only a portion of the tuft) and focal (involving some but not all glomeruli) sclerosis of glomeruli is present. The light microscopic changes are not specific for primary idiopathic FSGS and other causes of segmental sclerosing lesions need to be excluded [78]. The sclerosed segments show collapse of the glomerular capillary with increase in matrix material though with variable patterns of glomerular involvement [79]. The uninvolved portion of the glomerular tuft should appear essentially normal. Idiopathic FSGS typically shows early preferential involvement of the deep juxtamedullary glomeruli so that adequate sampling of this region is needed to reduce the risk of missing a focal lesion. (This risk is estimated at 35% if only 10 glomeruli are examined, falling to a 12% risk if 20 glomeruli are examined [76]). Even a single segmental sclerosing lesion away from the glomerular tip is sufficient to exclude a diagnosis of MCD. Clues to the presence of possible FSGS without diagnostic sclerosing lesions include abnormal glomerular enlargement, which appears to be an early indicator of the sclerotic process, and focal interstitial fibrosis and tubular atrophy (above that expected for age), which suggest glomerular loss [76]. Typically idiopathic primary FSGS shows negative immunofluorescence though non-specific uptake of IgM may be seen, commonly within sclerosed segments. Deposits similar to that of IgM nephropathy may also be present. On electron microscopy non-sclerosed glomeruli show epithelial cell foot process fusion though this may not be complete or as widespread as in typical untreated MCD. However, this is often not helpful in making this distinction as steroid therapy may partially restore foot processes in MCD.

Clinico-pathological Correlations at Presentation of Nephrotic Syndrome

Children with MCD cannot be separated on clinical features from those with FSGS or MesPGN though children with MCD are generally younger and less likely to have haematuria, hypertension and kidney dysfunction at presentation [1]. The ISKDC found that 80% of children with MCD were aged 6 years and under compared with 50% of children with FSGS. Systolic and diastolic blood pressures were elevated at presentation in 21% and 14% of children with MCD and 49% and 33% of children with FSGS. Haematuria occurred in 23% of children with MCD and 48% of children with FSGS.

Clinical and Laboratory Features at Onset of Nephrotic Syndrome

In 30–50% cases the onset of SSNS is preceded by an upper respiratory tract infection [80, 81]. Atopy is more common in children with SSNS compared with children without SSNS [81] and more common in SSNS than SRNS [82] but an acute allergic reaction rarely precipitates a relapse. The most common initial symptom in SSNS is periorbital oedema though the significance of this finding may not be realized till the child develops generalized oedema and ascites [83]. Frequently the periorbital oedema

is misdiagnosed as an allergy or as conjunctivitis particularly if the child is assessed initially in primary care rather than in emergency or paediatric care settings [84]. Symptoms may be present for longer than a year before diagnosis though 78% present within a month of the first symptom [84]. The degree of oedema is variable with some children having only mild periorbital and ankle oedema while others have pleural effusions and gross ascites with scrotal and penile oedema in boys and labial oedema in girls. The rapid formation of oedema with reduction in plasma volume may be associated with abdominal pain and malaise. Some children have serious infections at presentation including peritonitis [85]. Elevated systolic and diastolic blood pressures are present in 5–20% at presentation in children but generally hypertension does not persist [1, 80]. Urinalysis shows ≥3–4+ protein on urinalysis with a urine protein-creatinine ratio (uPCR) ≥ 200 mg/mmol (≥2000 mg/g). Microscopic haematuria is present at diagnosis in 20–30% of children but rarely persists and macroscopic haematuria is rare occurring in less than 1% of children with SSNS [1, 80]. Serum albumin levels usually fall below 20 g/L and may be less than 10 g/L with a concomitant reduction in total protein levels. Kidney function is generally normal though serum creatinine may be elevated at presentation in association with intravascular volume depletion and rarely acute kidney failure. Children have elevated cholesterol and triglycerides and these continue to be abnormal while the child remains nephrotic. However it is unknown whether the intermittent lipid abnormalities seen in children with SSNS during relapses increase the risk of cardiovascular disease in adult life [86]. Serum electrolytes are usually within the normal range. Total serum calcium levels are low associated with hypoalbuminaemia but ionized calcium is usually normal. Haemoglobin and haematocrit levels may be elevated at presentation in patients with reduced plasma volumes.

Outcome of Children with SSNS

Relapse

Despite a relapsing course, the long-term prognosis for most children with SSNS is for resolution of their disease and maintenance of normal kidney function. Early follow up studies of children with SSNS and MCD [3, 5] indicated that 80–90% children relapsed one or more times. Among children who relapsed, 35–50% relapsed frequently or became steroid dependent [3, 5]. A recent retrospective observational study of 631 children diagnosed with idiopathic nephrotic syndrome (589 with SSNS) between 1993 and 2006 with a median follow up of 3.9 years (IQR 2.1–6.66 years) found that 24% children had a single episode of SSNS, 43% relapsed infrequently and 33% relapsed frequently or had SDNS [87]. In the NEPHROVIR study (a prospective population-based observational study), the 5-year relapse-free rate was 22% (36/174) [88]. The first relapse in 138 children occurred at a median of 8.3 months (IQR 3.4–11.3). Forty three children (24%) relapsed frequently and 83% of these still required treatment with prednisone and/or immunosuppressive agents at 96 months.

Although numerous predictors for a frequently relapsing or steroid dependent course have been identified, there is considerable variation in significant predictors between studies. Most studies of predictors have been retrospective studies from tertiary paediatric nephrology services. Predictors include young age at presentation [8, 88, 89], male sex [8, 89, 90], a longer time to first remission after commencing prednisone [91–95], a shorter time between first remission and first relapse [5, 94, 96], the number of relapses in the first 6 months after presentation [91, 93, 96], infection at presentation or relapse [91, 95, 96] and low birth weight [97].

Most children with SSNS cease having relapses before adulthood. The International Study of Kidney Disease in Children of 344 children with SSNS with MCD on kidney biopsy found that the proportion of children without

relapses increased from 40% at 5 years to 80% at 8 years with very few children continuing to relapse at 18 years [5]. However, relapses may continue into adult life. In ten series of 705 participants with SSNS followed for 10–44 years, the proportion of people with continuing relapses varied between 9 and 50% [4, 90, 98–105]. The same series reported that the overall risk of reduced glomerular filtration rate was 0–2%. Higher numbers of relapses were reported in series with higher proportions of participants with FRNS or SDNS [4, 102]. Studies have also assessed the risk of treatment related complications in adults with childhood nephrotic syndrome. Hypertension was reported in between 0 and 32% of 569 adults (eight studies) with previous or continuing SSNS [4, 90, 100–105]. Overweight and obesity were reported in 13–46% of adults [99, 101, 102, 104, 105] though several authors pointed out that the prevalence of obesity may not differ from that in the local population [104, 105]. Short stature was reported in 2–20% of adults [99, 100, 102, 104]. Myocardial infarctions were reported in 2 of 66 participants with continuing relapses [4, 105] while a follow up study of 40 participants who had had SSNS during childhood but had no relapse for 23 years or more, identified three males who had had myocardial infarctions but all had other risk factors for cardiac disease [106]. Also the frequency of events in this group did not differ from that in the general population of the same age. No studies reported any malignancies in adults with previous nephrotic syndrome.

Kidney Function and the Development of Late Steroid Resistance

Most children with SSNS maintain normal kidney function. In a retrospective Canadian study of 78 children with SSNS followed for a median of 4.4 years (IQR 5.6), eGFR remained unchanged during follow up [107]. Development of late steroid resistance is well recognised. In European studies, most children with SSNS and biopsy proven or presumed MCD do not develop late steroid resistance and have a good prognosis for kidney function. In the ISKDC series of 334 children with SSNS and MCD, 15 (4.5%) children became transiently non-responsive to steroids but only one child (0.3%) became persistently non-responsive to therapy and developed ESKD [5]. Similarly in five series of 463 patients [4, 90, 98–100], only one patient (0.2%) developed late steroid resistance and progressed to ESKD [4]. A recent Canadian study of 589 children with initial SSNS reported that 10 (1%) children progressed to ESKD during a follow up period of up to 6.6 years [87]. Studies from the USA have identified higher risks of late steroid resistance and ESKD [108, 109]. In a retrospective analysis of 115 children with SSNS, 19 (17%) developed late steroid resistance with its development being associated with a shorter interval to first relapse and with relapse during the initial steroid therapy [109]. Although more African American children had initial steroid resistance, ethnicity did not predict for late steroid resistance [109]. A retrospective study from the USA Midwest Pediatric Nephrology Consortium investigated the outcomes in 29 children with late steroid resistance [110]. The median time to late steroid resistance was 19 months (range 2–170 months). After a mean follow up of 85 ± 47 months, 20 (70%) children were in complete or partial remission following treatment with CNI, mycophenolate mofetil (MMF) or alkylating agents. Six (21%) had persistent nephrotic range proteinuria and three (10%) had reached ESKD. Fewer African-American children responded to treatment compared with other children. These data suggest that children with late steroid resistance are more likely to respond to non-corticosteroid immunosuppressive agents and to have a better prognosis for kidney function compared with children with initial steroid resistance. Although initial steroid resistance is commonly associated with FSGS on kidney biopsy, the authors found no consistent relationship between initial or later kidney histology and late steroid resistance. Children with late steroid resistance are more likely to have recur-

rences of nephrotic syndrome following kidney transplantation [111]. Previous studies [112–115] have emphasized that progression to chronic kidney failure is not seen or is uncommon in children with FSGS if they continue to be steroid sensitive during follow up periods averaging about 10 years.

Other Complications of Steroid Sensitive Nephrotic Syndrome

Compared with earlier data, death is now uncommon in children with nephrotic syndrome. One study reported only one death (0.7%) associated with disease among 138 children with SSNS presenting between 1970 and 2003 [99] and no deaths were reported among 631 children presenting between 1993 and 2016 [87]. The death rate before corticosteroids and antibiotics were available was 40%, of whom half died from infection [83]. In the 1960s, 70s and 80s death rates of around 7% were reported among children with SSNS [80, 100, 116]. While upper respiratory tract infections are the most common infections, pneumonia, urinary tract infections, cellulitis, septicaemia and peritonitis are important causes of hospital admissions in children with nephrotic syndrome [117, 118]. Infections are more common in children receiving higher cumulative doses of corticosteroids and in those receiving non-corticosteroid immunosuppressive agents [119]. Routine screening for latent tuberculosis infection before treatment is indicated in areas with a high prevalence of tuberculosis but this is not cost effective in areas with a low prevalence [120]. Thromboembolism, most commonly venous, is a rare but potentially life threatening complication of SSNS though it is more common in children with congenital nephrotic syndrome or SRNS and in children aged over 12 years [121]. The majority of clinically evident venous thromboembolic episodes present in the first 3 months after nephrotic syndrome diagnosis [122]. Admissions for nephrotic syndrome complicated by acute kidney injury (AKI) increased significantly between 2000 and 2009 while admission rates for infection and thromboembolism were unchanged [117]. AKI developed in 59% of 336 children with nephrotic syndrome admitted to 27 paediatric nephrology services in USA; 237 children had SSNS. The risk of AKI was associated with infection, nephrotoxic drug exposure and duration of that exposure as well as steroid resistant disease [123]. Admissions complicated by infection, hypertension, thromboembolism and acute kidney injury result in longer hospital stays and increased costs [117, 118, 124].

Indications for Kidney Biopsy

Following the studies of the ISKDC, routine kidney biopsy at presentation and before corticosteroid administration has been abandoned. Biopsy is reserved for nephrotic children with unusual clinical and laboratory features (macroscopic haematuria, hypertension, persistent kidney insufficiency and low C3 component of complement) and for those with initial or secondary steroid resistance [125]. Rarely SSNS presents in the first year of life though more commonly nephrotic syndrome in this age group is resistant to corticosteroids and associated with monogenic podocyte disorders [126]. Where available, genetic studies should be carried out in children aged below 1 year before considering a kidney biopsy [127]. Originally biopsies at presentation were recommended for children aged above 8–10 years based on the ISKDC studies [128]. Now many paediatric nephrologists do not have a rigid upper age limit for treating children with idiopathic nephrotic syndrome without prior kidney biopsy and will give corticosteroids to children and adolescents if kidney function and complements are normal, persistent hypertension absent and microscopic haematuria transitory. This management is supported by retrospective studies of clinicopathological correlations in Indian children and adolescents, in which children without two or more abnormal clinical features generally demonstrated steroid sensitivity regardless of histology [129, 130]. However these data may not apply to African-American adolescent populations where the incidence of MCD at 20–30% [131] is much lower than the 40–50% seen in Indian or Northern European

adolescents [129, 132]; kidney biopsy is recommended for children aged 12 years or above in the USA [133].

Opinions differ as to whether children with SSNS should have kidney biopsies before commencing corticosteroid sparing therapies. In some centres particularly in North America, kidney biopsies have been commonly carried out before using alternative therapy while this practice has been largely abandoned in Europe and India. Respondents to surveys in North America reported that biopsies before commencing alternative therapy provided them with prognostic information or would influence therapy with the choice of steroid sparing agent varying according to histology [134–136]. Studies from North America have demonstrated that kidney pathology (FSGS, MesPGN and IgM nephropathy) with less favourable prognoses are common in children with FRNS or SDNS [137, 138], that steroid dependent patients with MesPGN, IgM nephropathy or FSGS are more likely to have one or more relapses after cyclophosphamide therapy compared with children with MCD [63, 112] and that African American children with FSGS are more likely to progress to chronic kidney failure [139]. In contrast studies from Europe and India [113, 130, 140] have demonstrated no relationship between kidney histology and the pre-biopsy or post cyclophosphamide course even though MesPGN and FSGS are more common in selected series of children with FRNS or SDNS compared with ISKDC data. Some clinicians have argued that kidney biopsies should be obtained before commencing treatment with CNIs to provide a baseline for interstitial and tubular damage due to the underlying disease particularly in children with FSGS, where CNIs are considered the treatment of choice [134, 136]. Guidelines from KDIGO recommend that kidney biopsies are not required before commencing CNIs or other non-corticosteroid agents [125]. However kidney biopsies should be considered in children who have received prolonged treatment (2–3 years) with a CNI and are indicated in children with late resistance to corticosteroids [125, 141, 142].

Before the clinician biopsies a child with FRNS or SDNS, he or she needs to consider whether the benefits of this procedure outweigh the 1% risk of significant haemorrhage requiring blood transfusion [143, 144]. In particular, the clinician needs to know whether the kidney pathology will influence the specific therapy administered and/or whether it will provide information on the likelihood of the child progressing to ESKD. Studies show that, even if the kidney biopsy shows FSGS, the most important predictor for ESKD in idiopathic nephrotic syndrome is not the kidney pathology but the achievement and maintenance of remission following any therapy [145].

Management of Steroid Sensitive Nephrotic Syndrome

Treatment of the First Episode of Nephrotic Syndrome with Corticosteroids

Corticosteroids have been used to treat idiopathic nephrotic syndrome since the early 1950s [146]. Because of the clear net benefits of corticosteroids, no placebo-controlled trials were performed in children with nephrotic syndrome. The ISKDC agreed on a standard corticosteroid regimen for the first episode of SSNS [147] and this has provided the control group against which to test other regimens of prednisone or prednisolone therapy. At presentation children received prednisone 60 mg/m^2/day in divided doses for 4 weeks followed by 40 mg/m^2/day in divided doses on 3 consecutive days out of 7 days for 4 weeks. Subsequently a randomised controlled trial (RCT) carried out by the Arbeitsgemeinschaft für Pädiatrische Nephrologie (APN) [148] demonstrated that alternate day prednisone was more effective in maintaining remission than prednisone given on 3 consecutive days out of 7 days so alternate day prednisone dosing is generally used now in the second 4 week period. Since no significant differences in the time to remission or risk for subsequent relapse between single and divided doses of prednisone have been demonstrated [149], a single daily dose may be preferred during daily therapy to achieve greater compliance. For ease of clinical use, a dose of 2 mg/kg has commonly been substituted for

60 mg/m^2/day. Dosing per kilogram results in lower dosing for patients with weights below 30 kg [150] and two retrospective studies suggested that relative underdosing may increase the likelihood of FRNS [151, 152]. However two recent RCTs comparing dosing per kilogram with dosing by surface area in children weighing under 30 kg found no differences between dosing regimens in the time to remission and the number of relapses by 6 months with some increase in corticosteroid related adverse effects in children dosed by surface area [153, 154].

Though demonstrated to be more effective than shorter durations of treatment [155], the 8 week ISDKC/APN regimen was associated with a high relapse rate so RCTs investigated longer durations of prednisone compared with the ISKDC/APN regimen to determine if longer durations of prednisone reduced the risk of relapse and reduced the number of children, who developed FRNS or SDNS. Recently four well-designed RCTs (three placebo-controlled) involving 775 children have shown no benefit of extending the duration of treatment beyond eight (4 weeks daily and 4 weeks of alternate day prednisone) or 12 weeks (6 weeks daily and 6 weeks alternate day prednisone) on reducing the number of children with relapse or with frequent relapses (Table 13.2). Therefore children presenting with their first episode of SSNS should be

Table 13.2 Outcomes of corticosteroid treatment for initial episode of steroid sensitive nephrotic syndrome in four large well designed randomised controlled trials [150–154]

Study name	Teeninga 2013 [153]		Sinha 2014 [154]		Yoshikawa 2014 [150]		PREDNOS 2019 [151]	
Duration of treatment (months)	Three	Six	Three	Six	Two	Six	Two	Four
Number analysed	62	64	88	92	124	122	109	114
Efficacy outcomes								
Number with relapse	48	51	55	49	80	83	88	91
Number with FRNS/SDNS[a]	31	38	35	36	46	45	55	60
Median time to first relapse (months)	6 (95% CI [b] 4–8)	8 (95% CI 6–10)	6.6	9.2	8.1	8.1	2.9 (IQR [c] 2.2–4.5)	4.6 (IQR 3–6)
Number of relapses per patient	Median 2.5 (IQR = 1.0–5.0)	Median 4.0 (IQR = 1.0–6.0)	1.54 ± 1.59[d]	1.26 ± 1.58	1.25	1.33	3.61 ± 3.25	3.98 ± 3.30
Number of patients with adverse effects								
Hypertension	8	10	14	18	15	9	NR	NR
Cushingoid facies	14	21	37	37	54	61	78	83
Obesity	NR[e]	NR	5	5	20	34	NR	NR
Hirsutism	NR	NR	8	10	NR	NR	41	45
Glaucoma	0	0	NR	NR	19	13	NR	NR
Cataract	1	0	NR	NR	0	0	1	1
Adrenal insufficiency	NR	NR	NR	NR	0	1	NR	NR
Infection (episodes)	6	10	15	21	1	0	NR	NR
Hyperglycaemia	NR	NR	NR	NR	2	3	NR	NR
Glycosuria	NR	NR	NR	NR	NR	NR	14	19
Aggressive behaviour	NR	NR	4	4	NR	NR	101	94

[a] FRNS/SDNS: frequently relapsing nephrotic syndrome or steroid dependent nephrotic syndrome
[b] 95% CI: 95% confidence intervals
[c] IQR: interquartile range
[d] Standard deviation
[e] NR: not reported

treated with 8 weeks [156, 157] or 12 weeks of prednisone [158, 159]. Currently there are no adequately powered well designed RCTs comparing 8 with 12 weeks of prednisone treatment. Children under 6 years may be at higher risk of developing frequently relapsing or steroid dependent nephrotic syndrome [159]. It is uncertain whether prolonging the treatment period in young children would reduce the their risk of relapse and an RCT is underway to investigate this [160]. Meta-analyses of earlier trials had previously suggested that increasing the duration of prednisone beyond 8 to 12 weeks was associated with a reduced risk of relapse [161]. However poorly designed RCTs with inadequate allocation concealment and lack of blinding can exaggerate the efficacy of therapy [162, 163]. Therefore the 21 RCTs published between 1988 and 2018, which evaluated prednisone duration in the first episode of SSNS, were stratified in meta-analyses according to risk of bias for allocation concealment or for performance/detection bias. RCTs at low risk of bias showed no benefit of increasing the duration of prednisone while studies at high risk of bias showed a benefit of increased duration of prednisone [164].

Current international and national guidelines suggest 8–12 weeks of prednisone in the initial episode of SSNS [125, 133, 165, 166] with some guidelines including tapering of prednisone dose after 12 weeks though the recent PROPINE study found no benefit in terms of efficacy and safety of tapering prednisone dose [167]. The French protocol for the initial episode of SSNS includes the administration of three doses of intravenous methylprednisolone (1 g/1.73m^2) for those children who have not achieved remission by 30 days [165] and this protocol is used in other European centres [168]. Children who require intravenous methylprednisolone to achieve their first remission are more likely to become steroid dependent, to require ciclosporin and to develop late steroid resistance with reduced kidney function [169, 170]. Surveys of pediatric nephrologists in North America and Europe have demonstrated considerable variation among respondents in their approach to the first episode of idiopathic nephrotic syndrome [134, 136, 168, 171, 172]. A survey on SSNS management in all 12 Canadian pediatric nephrology centres indicated both within-centre and between-centre variability in prescribed doses and duration of prednisone for the first presentations and relapses of SSNS irrespective of whether or not the Centre had standard protocols [173].

Treatment of Relapsing SSNS with Corticosteroids

The ISKDC defined relapse as recurrence of proteinuria for 3 consecutive days after previously achieving remission (Table 13.1). Proteinuria may remit spontaneously in 15–30% of relapses without commencing prednisone or increasing the dose [174, 175]. Therefore it is reasonable to wait for some days of mild proteinuria (≤2+ on dipstick) before commencing corticosteroids provided the child remains well and without significant oedema because spontaneous remissions may occur after several days of low grade proteinuria particularly if the child has an intercurrent viral infection. Compliance with prednisone therapy should be considered in children with multiple relapses since poor compliance could be misinterpreted as steroid dependence. If available, triamcinolone acetonide, a long acting steroid for intramuscular injection, may be used instead of oral prednisone treatment if non-compliance is suspected [176].

Paediatric nephrologists have generally treated relapses with daily prednisone (60 mg/m^2/day) till the child achieved remission and then continued alternate day therapy (40 mg/m^2) for 4 weeks or more [177]. Two observational studies and a small RCT have demonstrated that most children with relapsing SSNS achieve and maintain remission with prednisone given at a dose of 30 mg/m^2/day [178–180]. These data need confirmation in an adequately powered RCT.

In children with FRNS, observational studies have demonstrated that low-dose alternate-day prednisone (mean dose 0.48 mg/kg on alternate days) or low-dose daily prednisone (0.25 mg/kg/day) reduced the risk of relapse compared with historical controls with maintenance of growth

rates [181, 182]. Guidelines recommend low-dose alternate-day prednisone in children with FRNS and SDNS [183]. However a recent RCT [184] in 61 children with FRNS or SDNS found that children receiving daily prednisone (0.2–0.3 mg/kg/day) had significantly fewer relapses than children receiving alternate day prednisone (0.5–0.7 mg/kg/day) with no increase in adverse effects. Four small RCTs have demonstrated that, in children with FRNS, increasing the frequency to daily administration at the onset of an intercurrent infection significantly reduces the risk of relapse [185–188]. However results from a large RCT with 271 children evaluated found that giving 6 days of daily low-dose prednisolone at the time of an URTI did not reduce the risk of relapse of nephrotic syndrome in children in the United Kingdom [189].

Adverse Effects of Corticosteroids

A study from the Kidney Research Network Registry [190] provides an overview of the frequency of corticosteroid adverse effects in patients with primary proteinuric kidney disease. Among a cohort of 884 patients (393 children) with primary proteinuric kidney disease, 534 received corticosteroids. At least one steroid associated adverse event was seen in 333 (62%) of those who received corticosteroids with hypertension, diabetes, overweight and obesity, infections and short stature being the most common. There was no difference in risk of steroid associated adverse effects between children and adults. The adjusted relative risk increased overall 2.5-fold for each 1 mg/kg increase in corticosteroid dose; hypertension increased 4.5-fold, obesity increased 2.9-fold and diabetes increased 1.9-fold.

Behavioural changes are common and include anxiety, depression, emotional lability, aggressive behaviour, inattention, hyperactivity and sleep disturbance [191, 192]. The adverse effects seen in the four large RCTs evaluating the initial episode of SSNS are shown in Table 13.2. Health related quality of life over time is reduced in children with nephrotic syndrome on prednisone or steroid sparing agents, compared with children not on medications [193]. In addition, nephrotic syndrome causes significant mental and economic stress on families [194].

The current practice of using alternate day rather than daily prednisone to maintain remission results from early reports that growth was less affected by alternate day prednisone. An RCT demonstrated that children given alternate day prednisone after kidney transplantation grew better than those given daily prednisone [195]. Studies of children with FRNS or SDNS, which have evaluated the adverse effects of corticosteroids on linear growth, have shown variable results. In a study of 56 children with FRNS or SDNS, children lost 0.49 ± 0.6 of height standard deviation score (SDS) during pre-pubertal growth [196]. Prednisone therapy was the only significant variable associated with the negative delta SDS. In a second study, growth rates remained normal if prednisone doses were maintained below 1.5 mg/kg on alternate days in 41 prepubertal children [197]. A third study of 64 boys found that growth rates remained stable from diagnosis for 5 years and then deteriorated [198]. In two studies final height was significantly below target in children, who required prednisone during puberty [196, 198] though partial catch up growth occurred in pubertal children permanently withdrawn from prednisone [196]. However a third study of 60 children with SSNS found that final height SDS did not differ significantly from initial height SDS (−0.60 ± 1.0 versus −0.64 ± 0.92) though the mean final height SDS differed significantly from that expected in healthy children [199].

Derangements of bone mineral metabolism may occur in patients with nephrotic syndrome and normal kidney function. Vitamin D-binding protein and 25-hydroxyvitamin D levels are reduced in nephrotic children [200] while generally levels of calcium, 1,25-dihydroxyvitamin D and parathyroid hormone levels are normal [200–202]. 25-hydroxyvitamin D levels increase in remission but remain low compared with healthy children [202] and may be associated with continuing reduction in bone mineral density [203]. Abnormalities of bone mineral metabolism are

aggravated by treatment with corticosteroids. Corticosteroids reduce bone formation by inhibiting osteoblast activity and inhibiting bone matrix formation. In addition they increase bone resorption directly and by reducing calcium absorption via inhibition of Vitamin D activity with a secondary increased release of parathyroid hormone [204, 205]. Low bone area and trabecular thickness with focal areas of osteoid accumulation consistent with osteopenia and abnormal mineralisation have been found in children with steroid dependent SSNS [205]; bone formation rate correlated inversely with the daily prednisone dose. Serum osteocalcin and alkaline phosphatase levels fall during corticosteroid therapy consistent with reduced bone formation [202].

Corticosteroid therapy is associated with osteopenia (decrease in quantity of bone tissue) and osteoporosis (osteopenia with bone fragility). Trabecular bone is affected more severely than cortical bone. Dual energy x-ray absorptiometry (DXA) is widely used to assess bone mass in children with SSNS. DXA measures the mass of bone mineral per projection area (g/cm^2), which is a size dependent measure [206]. Thus results must be corrected for height in short children to prevent underestimation of bone mineral density (BMD) in comparison with age matched controls. A North American cross-sectional study of 60 children with SSNS, who had received an average of 23 g of prednisone, demonstrated that whole body bone mineral content (BMC) was increased and lumbar spine BMC was normal in children with SSNS compared with age matched local controls when adjusted for bone area, height, age, sex, pubertal stage and race [207]. Nephrotic children had significantly lower z-scores for height and higher z-scores for weight and body mass index (BMI) compared with controls. The authors concluded that corticosteroid induced increases in BMI were associated with increased whole body BMC and maintenance of BMC of spine. In contrast in 100 non obese Indian children with SSNS, who had received 5.6–18 g of prednisone, 61% had low BMD levels compared to normal values from North American controls [201]. No children developed fractures. These data indicate that differences in growth and body composition in different study populations need to be considered when interpreting studies of bone mass in children with SSNS. In a prospective Canadian study, lumbar spine BMD was studied at baseline (median 18 days from prednisone initiation), 3, 6, 9 and 12 months after the onset of nephrotic syndrome [208]. Only 51% of children were receiving prednisone by 12 months. Mean lumbar spine BMD was significantly reduced at baseline and 3 months but subsequently mean values did not differ significantly from values in healthy children. Cross sectional and longitudinal studies using peripheral quantitative computed tomography (pQCT) in children with nephrotic syndrome have confirmed differential effects of corticosteroids on cortical and trabecular bone mineral densities with evidence of reduced bone formation [209, 210].

Corticosteroid associated fractures are rare in children with SSNS unlike children with chronic inflammatory disorders such as juvenile rheumatoid arthritis. In the Canadian study, only three children (6%) of 65 children had asymptomatic vertebral fractures by 12 months after commencing prednisone [208]. Children with SSNS appear to be at a low risk of osteoporosis [206]. Bone mineral density improves in children with SSNS, who are given vitamin D and calcium supplements during prednisone therapy [211–213]. The Committee on Nutrition of the French Society of Paediatrics recommend that children with nephrotic syndrome should receive daily vitamin D supplements particularly in winter months [214].

Long term corticosteroid therapy results in suppression of the hypothalamic-pituitary-adrenal (HPA) axis in 35–60% of children with nephrotic syndrome particularly in younger children and children with FRNS or SDNS [215–218]. In the most recent study, the mean duration of prednisone in 13 children with HPA suppression was 66 months compared with 30 months in 24 children without HPA suppression with mean daily doses of 22 mg/kg and 26 mg/kg in the two groups [217]. Families with children receiving long term prednisone therapy should be educated about the possible need for hydrocortisone

replacement at times of illness or stress including surgical procedures. To avoid HPA suppression, long periods of high dose prednisone therapy should be avoided by introducing non-corticosteroid sparing agents.

Corticosteroid Sparing Agents in Frequently Relapsing and Steroid Dependent SSNS

Corticosteroid sparing agents are indicated in children, who have frequent relapses despite low dose alternate day prednisone and/or who have significant adverse effects of prednisone therapy. Corticosteroid sparing agents used in children with FRNS or SDNS include alkylating agents (cyclophosphamide, chlorambucil), levamisole, calcineurin inhibitors (cyclosporin, tacrolimus), mycophenolate mofetil or mycophenolate sodium and rituximab. In addition mizoribine is used in Japan.

Alkylating Agents

Cyclophosphamide remains an important medication to treat FRNS in low and middle income countries where it is be cheaper than other non-corticosteroid immunosuppressive agents [219] while chlorambucil is less commonly used. In six RCTs combined in meta-analysis, oral cyclophosphamide 2–3 mg/kg/day for 8–12 weeks or chlorambucil 0.2 mg/kg/day administered for 8 weeks reduced the risk of relapse by 60% in frequently relapsing SSNS at 6–12 months after treatment compared with prednisone alone [220]. Two studies have shown that intravenous cyclophosphamide (500 mg/m^2/dose for 6 monthly doses) was more effective than oral cyclophosphamide (2 mg/kg/day for 12 weeks) in reducing the risk for relapse at 6 months (RR 0.56; 95% CI 0.33–0.92) but not at 2 years [220–222]; in both studies the cumulative dose of cyclophosphamide was lower in the intravenously treated groups. In a systematic review of 26 observational studies of cyclophosphamide and chlorambucil usage in SSNS. relapse-free survivals after 2–5 years were 72% and 36% in children with FRNS and 40% and 24% in children with SDNS. More recent single centre studies have found similar results [223–227].

Adverse effects with alkylating agents are frequent and may be severe. A third of children will experience leucopenia so white blood counts need to be checked regularly during treatment. If leucopenia occurs, the treatment dose should be reduced or ceased temporarily to reduce the risk of infection. Latta and co-workers identified adverse effects from 38 reports involving 866 children with FRNS or SDNS, who received 906 courses of cyclophosphamide and 638 children, who received 671 courses of chlorambucil (Table 13.3) [228]. They concluded that chlorambucil in the recommended dosage was potentially more toxic than cyclophosphamide based on a higher risk of infections, malignancies and seizures so chlorambucil is rarely used now. Cyclophosphamide may reduce male fertility and cause abnormal gonadal function in men and should not be used in peripubertal male children. In SSNS there is a dose dependent relationship

Table 13.3 Adverse effects of alkylating agents in children with steroid sensitive nephrotic syndrome

		Cyclophosphamide		Chlorambucil	
Adverse effect		Total assessed	N (%) with outcome	Total assessed	N (%) with outcome
Deaths	Patients	866	7 (0.8%)	625	7 (1.1%)
Malignancies	Patients	866	2 (0.2%)	534	3 (0.6%)
Seizures	Patients	866	0 (0%)	266	9 (3.4%)
Infections	Courses	609	9 (1.5%)	552	35 (6.3%)
Haemorrhagic cystitis	Courses	762	22 (2.2%)	552	0 (0%)
Leucopenia	Courses	619	210 (32.4%)	456	151 (33%)
Thrombocytopenia	Courses	214	5 (2.1%)	408	24 (5.9%)
Hair loss	Courses	736	131 (17.8%)	237	5 (2.1%)

Reproduced with permission from Latta K, von Schnakenburg C, Ehrich JH. A meta-analysis of cytotoxic treatment for frequently relapsing nephrotic syndrome in children. Pediatric Nephrology. 16(3):271–82, 2001 [228]

between the number of patients with sperm counts below 10^6/mL and the cumulative dose of cyclophosphamide [228]. The threshold cumulative dose for safe use of cyclophosphamide remains uncertain because of individual reports of oligospermia in boys receiving less than 200 mg/kg. These data suggest that single courses of cyclophosphamide at a dose of 2 mg/kg/day should not exceed 12 weeks (cumulative dose 168 mg/kg). There are few data on gonadal toxicity with chlorambucil in SSNS. In male patients treated for lymphoma, total doses of 10–17 mg/kg led to azoospermia [229]; similar total doses are used in SSNS. Gonadal toxicity is less severe in women with most reports observing little or no toxicity with alkylating agents in SSNS [228]. The 2012 KDIGO guidelines advise that second courses of alkylating agents should not be given because of their potential long term toxicity [125].

In many countries, cyclophosphamide has been largely replaced by other non-corticosteroid medications because of its adverse effects. The main advantage of cyclophosphamide over levamisole, MMF and CNIs is that it often results in a prolonged period of remission after the medication is ceased. Rituximab also leads to a prolonged period of remission off treatment. Two recent observational studies [230, 231], which compared outcomes of children treated with cyclophosphamide with those treated with rituximab, found that 1 and 2 year relapse-free survivals were similar between treatments but adverse effects were more common with cyclophosphamide. Importantly more children treated with rituximab were able to cease steroids completely [231]. However rituximab is expensive and may not be available in resource limited countries.

Calcineurin Inhibitors

Cyclosporin has been used to treat children with frequently relapsing or steroid dependent SSNS since 1985 [232]. Two small trials enrolling 95 children demonstrated no significant difference in the risk of relapse during treatment between alkylating agents given for 6 or 8 weeks and cyclosporin given for 6 or 9 months [233–235]. However most children treated with cyclosporin relapsed when therapy was ceased so the risk of relapse with alkylating agents was lower than with cyclosporin after cyclosporin had been ceased for 12–15 months. In a prospective 2 year follow up of 44 children, in whom cyclosporin was discontinued after completion of an RCT, 37 (84%) experienced a relapse [236]. Relapses occurred in 81% (26/32), without relapse during cyclosporin therapy, and in 92% (11/12) children, who had one or more relapses during cyclosporin therapy. The probability that children would re-develop FRNS or SDNS by 2 years was 75% and 53% respectively in children with or without relapse during their previous cyclosporin therapy. Adverse effects of cyclosporin reported in four trials were common with 13% of children developing hypertension, 10% reduced kidney function, 23% gum hypertrophy and 27% hirsutism [235]. A potentially serious adverse effect of CNI is posterior reversible encephalopathy syndrome (PRES) [237]. Nephrotic syndrome per se and hypertension also are predisposing factors for PRES.

Cyclosporin (microemulsified) is usually commenced at 4–5 mg/kg/day in two divided doses with subsequent dosing altered to achieve 12 h trough whole blood levels (C_0) of 80–150 ng/mL (67–125 nmol/L) initially. In an RCT the sustained remission rate at 2 years was significantly higher in children with C_0 levels maintained between 80–100 ng/mL (50–67 nmol/L) [mean dose 4.8 mg/kg/day] compared with children treated with a fixed dose of 2.5 mg/kg/day (sustained remission rates 50% versus 15%) [238]. Hypertension and mild arteriolar hyalinosis were less common in the fixed dose group. The cyclosporin dose, required to maintain trough levels, may be reduced by one third by administering ketoconazole as a cyclosporin sparing agent with reduction in drug costs [239]. Studies in children with SSNS have demonstrated better correlations between area under the curve concentrations of cyclosporin and 2 h post dose levels (C_2) than with trough levels [240]. A Japanese RCT compared the effect of two different C_2 levels on the relapse rate in children with FRNS or SDNS [241]. Children were randomised to receive cyclosporin to achieve whole blood C_2 levels of

600–700 ng/mL (499–582 nmol/L) for 6 months followed by 450–550 ng/mL (374–457 nmol/L) for 18 months or to achieve C_2 levels of 450–550 ng/mL (374–457 nmol/L) for 6 months and then 300–400 ng/mL (250–333 nmol/L). The sustained remission rate was slightly but not significantly higher in the high C_2 level group compared with the lower C_2 level group but the relapse rate was significantly lower in the high level group; adverse effects did not differ between groups.

Tacrolimus is now the preferred CNI agent for SSNS where available largely because of the cosmetic effects of cyclosporin though it is also nephrotoxic and may be associated with diabetes mellitus. No RCTs have compared tacrolimus with cyclosporin in children with SSNS. In a prospective uncontrolled study of 74 children (50 on tacrolimus; 24 on cyclosporin), relapse frequency during 2 years follow-up did not differ significantly between treatments [242]. Nephrotoxicity defined as an increase in serum creatinine >25% above baseline was less common in tacrolimus treated children and diabetes mellitus was not reported. The starting dose of tacrolimus was 0.5–1.5 mg/kg/day in two divided doses; subsequently the dose was adjusted to 12 h trough levels of 5–12 ng/mL (6–14 nmol/L). There are also some observational data in children with SDNS suggesting that tacrolimus may be associated with less CNI nephrotoxicity than cyclosporin [243]. In a retrospective cohort study of 340 children with FRNS or SDNS examining the relative efficacy and safety of tacrolimus, MMF and levamisole as the first non-corticosteroid agent, the 30-month relapse free survival was 62% with tacrolimus, 39% with MMF and 24% with levamisole [219]. Fewer adverse effects were seen with levamisole (three reports) compared with tacrolimus (33 reports). Serious adverse effects mainly related to infection were only seen with tacrolimus.

CNI toxicity is well documented in children receiving this therapy outside the transplant setting [244] though few studies have correlated clinical toxicity with morphologic features [245, 246]. The toxic effects are essentially the same in transplant and non-transplant settings. CNI toxicity may be characterized by reduction in glomerular filtration rate with no discernible histological abnormality or by acute and chronic tubular and/or vascular changes in the kidney. Acute changes of toxic tubulopathy are classically described as "isometric" vacuolation of proximal tubular epithelial cells. However this is often a focal phenomenon and may only be seen in a small number of tubules in a biopsy sample. The vacuoles are of similar size (hence "isometric") and occur on the basis of dilatation of the smooth endoplasmic reticulum of the cells. Non-specific changes of acute tubular necrosis may be seen in some cases, with intraluminal desquamation of epithelial cells, dilatation of the tubules and regenerative nuclear changes. Acute vascular changes may result in microvascular thrombosis, endothelial and myocyte necrosis. Chronic vascular changes include nodular hyaline arteriopathy, which arises on the basis of individual myocyte necrosis of arteriolar smooth muscle, and "striped" interstitial fibrosis and tubular atrophy that reflect focal ischaemic damage. Ultimately, chronic CNI nephrotoxicity can result in glomerular changes of chronic ischaemia and/or focal and segmental glomerulosclerosis. The morphological nephrotoxic effects of tacrolimus are essentially the same as those seen with cyclosporin and include acute tubular necrosis, acute and chronic vascular changes, and interstitial fibrosis.

Cyclosporin-induced tubulointerstitial lesions on kidney biopsy are reported in 30–40% of children who have received cyclosporin for 12 months or more [247–249]. Cyclosporin associated arteriopathy is uncommon. Risk factors for fibrosis are total duration of cyclosporin therapy, having heavy proteinuria for more than 30 days during therapy [247] and higher trough cyclosporin levels [250], higher 2 h peak cyclosporin levels and concurrent use of angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers [249]. Arteriopathy but not interstitial fibrosis improves after cyclosporin has been ceased for 12 months or more [251].

The duration of administration of CNIs is controversial with some authors suggesting that

duration should not exceed 2 years [247]. However other authors have suggested that longer periods of CNIs may be well tolerated [252]. There are few data on kidney histology in children with SSNS who have received tacrolimus but increases in interstitial fibrosis correlated with trough tacrolimus levels [253]. These few data suggest that as with cyclosporin, the lowest possible dose of tacrolimus should be used to maintain remission.

Levamisole

Levamisole is a synthetic antihelminthic agent with immunomodulatory properties [254]. Its use in childhood nephrotic syndrome was first described by Tanphaichitr and co-workers in 1980 [255] and since then many studies have described its benefits [254]. Levamisole is usually administered in a dose of 2.5 mg/kg on alternate days. Levamisole given for 4 months to 1 year reduced the risk of relapse by 50% in comparison with prednisone alone in 6 trials (474 patients; RR 0.41, 95% CI 0.27–0.61) [220, 256–261] but was ineffective in a seventh trial, in which a lower total dose of levamisole was given [220, 262]. However several of these RCTs were at high risk of bias because of methodological problems, which may lead to an overestimation of treatment effects [163]. Recently a multicentre, double-blind RCT evaluating levamisole compared with placebo in 99 children with FRNS or SDNS confirmed the efficacy of levamisole [263]. Between 100 days and 12 months from the start of the medications, the time to relapse was significantly increased in the levamisole group compared with the placebo group (HR 0.22; 95% CI 0.11–0.43). After 12 months, 26% of levamisole treated participants remained in remission compared with 6% of placebo-treated participants. Four of 50 children treated with levamisole suffered moderate leucopenia. Adverse effects of levamisole are uncommon but include leucopenia, gastrointestinal effects and occasionally vasculitis [264, 265]. Although levamisole is a valuable corticosteroid sparing agent, it is currently unavailable in many countries.

Mycophenolate Mofetil/ Mycophenolate Sodium

Mycophenolate mofetil (MMF) and mycophenolate sodium (MPS) are converted to mycophenolic acid (MPA), which is an inhibitor of the *de novo* purine pathway with inhibitory effects on T and B lymphocyte proliferation [266]. MMF has become an important corticosteroid sparing agent in children with FRNS or SDNS and is often used as the initial non-corticosteroid immunosuppressive agent [267]. MPS is used in adults with nephrotic syndrome and minimal change disease [268] but has rarely been used in children though its efficacy can be expected to be similar to MMF [269]. Numerous observational studies have demonstrated a reduction in relapse rate during MMF treatment compared with prednisone treatment [270] though no RCT has compared MMF with prednisone alone. Its efficacy in SSNS has been evaluated in three RCTs (144 children) comparing MMF (800–1200 mg/m^2/day in two divided doses) with cyclosporin (4–5 mg/kg/day) [271, 272] and in one RCT (149 children) comparing MMF (1200 mg/m^2/day) with levamisole (2.5 mg/kg on alternate days) [273]. When combined in a meta-analysis, the relapse rate was lower with cyclosporin compared with MMF (142 children. Mean difference 0.83; 95% CI 0.33–1.33) but adverse effects of hypertension, hypertrichosis and gum hypertrophy were lower and GFR higher in MMF treated children [220]. The main adverse effects of MMF are abdominal pain, diarrhoea, anaemia, leucopenia and thrombocytopenia although MMF has been well tolerated in children with SSNS with gastrointestinal disorders reported in only 4% of 130 children in a large observational study [219]. *In utero* exposure to MMF has been associated with prenatal defects so women should be counselled that they will need to cease MMF before becoming pregnant [270].

Higher MPA exposure is required in children with nephrotic syndrome compared with levels in kidney transplant recipients [270]. Studies of therapeutic drug monitoring show that the target MPA area under the curve (AUC) needs to be

above 45–50 μg h/mL to maintain remission though there is large between-patient variability [271, 274, 275]. *Post hoc* analysis of the Gellermann study revealed that the relapse rate in children with higher MPA exposure (mean MPA-AUC 74.0 mg h/mL) did not differ from that seen in cyclosporin treated children [271]. None of the RCTs reported to date have used therapeutic drug monitoring to determine the correct dose of MMF for individual patients so it remains possible that results of these RCTs would differ if drug monitoring had been included. Currently therapeutic drug monitoring of MMF in children with nephrotic syndrome is not widely available. No consistent single time point for measurement has been identified that correlates with AUC data and can be used to monitor children with FRNS or SDNS receiving MMF.

Rituximab

Rituximab is a mouse-human chimeric monoclonal antibody which binds to the CD20 antigen expressed on B cells. Treatment leads to a suppression of CD19 cells to below 1% and relapse generally occurs when levels of CD19 cells recover. Rituximab has now been evaluated in seven RCTs using one [276–278] (Fig. 13.4), two [279–281] or four doses [282] of rituximab (375 mg/m^2 per dose). In five studies including 296 children with difficult to treat SDNS, the risk for relapse was reduced by 63% at 6 months (4 studies, 239 children; RR 0.27; 95% CI 0.15–0.47) and 36% at 12 months (2 studies, 168 children; RR 0.74; 95% CI 0.58–0.94) [220]. In two studies (60 participants), which assessed RTX in children with SSNS treated with high (≥0.7 mg/kg/day) or low doses (≤0.4 mg/kg/day) of prednisone without other immunosuppressive agents, the risk of relapse was reduced by 94% and 74% at 6 and 12 months compared with prednisone alone [277, 278].

To gain further information about the optimum dosing regimen of rituximab, a study evaluated retrospectively the different dose regimens used in 11 tertiary centres in Asia, Europe and North America [283]. Among 511 children with complicated relapsing SSNS (defined as relapsing despite ongoing treatment with prednisone and at least one additional agent), 191 received low dose rituximab (375 mg/m^2), 208 received medium dose rituximab (750 mg/m^2) and 112 received high dose rituximab (1125–1500 mg/m^2). Fifty five percent [283] of children received concurrent immunosuppressive treatment (CNI, MMF, prednisone). Children who received low dose rituximab had shorter relapse-free periods (8.5 months) compared with those receiving medium dose (12.7 months) or high dose (14.3 months) rituximab. However when rituximab was combined with immunosuppressive therapy, relapse-free survival did not differ between different dosage groups. Two RCTs is underway to determine whether MMF compared with placebo maintains remission in children with SSNS after successful treatment with rituximab [284, 285].

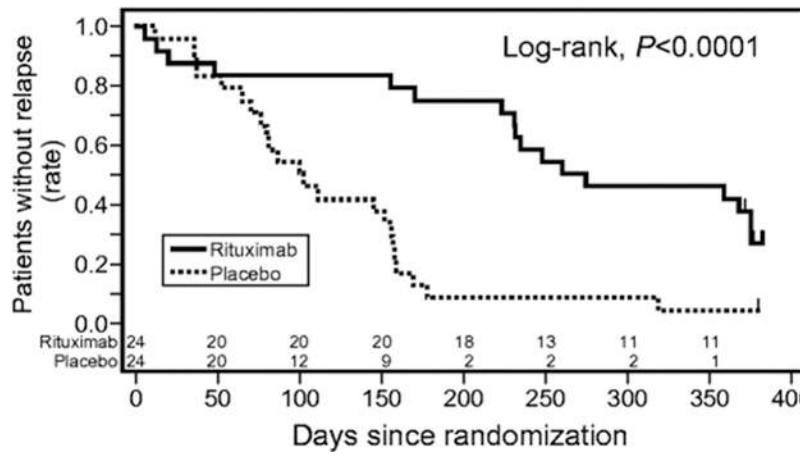

Fig. 13.4 Relapse-free survival probability in participants with relapsing nephrotic syndrome treated with rituximab. Reproduced with permission from Iijima K, Sako M, Kamei K, Nozu K. Rituximab in steroid-sensitive nephrotic syndrome: lessons from clinical trials. Pediatric Nephrology (2018) 33:1449–1455

The main adverse effects reported with rituximab have been acute episodes of bronchospasm, hypotension, fever and arthralgias occurring during or immediately after intravenous infusion. Premedication with anti-histamine and anti-pyretic agents is recommended. Adverse effects are generally mild with infusion reactions (13%) and infections (4%) being most common [283]. Mild to moderately serious infections are reported to be less commonly seen with rituximab compared with tacrolimus [280]. However rare but serious adverse effects reported in children with nephrotic syndrome treated with rituximab include fatal pulmonary fibrosis [286], *Pneumocystis jiroveci* pneumonia with respiratory failure [287, 288], bacterial pneumonia including *Pseuodomonas aeroginosa* pneumonia [289] and severe myocarditis requiring heart transplantation [290]. Though not yet reported in children with SSNS, a survey of patients with SLE treated with rituximab identified 57 patients with multifocal leucoencephalopathy caused by JC polyomavirus [291]. Fifty six children (14%) of 400 children in whom IgG levels were measured had persistent hypogammaglobulinaemia at 1 year following rituximab infusion [283]. Among 27 children who had received rituximab more than 2 years previously, most had a sustained reduction in total and switched memory B cells while 11 children had hypogammaglobulinaemia [292]. Younger patients appear to be at increased risk of hypogammaglobulinaemia [293]. More information is required to determine the longer term impact of these immunological abnormalities.

Because of the uncertainty about long term adverse effects and its cost, rituximab use was previously restricted to children with steroid and CNI dependent SSNS. However a study from the USA has demonstrated that the 1 year overall treatment costs of using rituximab compared with CNIs may not differ significantly [294]. Increasingly rituximab is being used in children with FRNS or SDNS because it can achieve long periods of remission off treatment [278] though the long term effects of prolonged B cell depression and hypogammaglobulinaema remain to be elucidated.

Choice of First Corticosteroid Sparing Agent for Children with FRNS or SDNS

RCTs to date have not provided sufficient data on the comparative efficacy and adverse effects of corticosteroid sparing agents to allow definitive recommendations on which medication should be the first agent used in FRNS or SDNS. Most international [125] and national guidelines [133, 165, 166] have not provided recommendations on which medication should be preferred as the first corticosteroid sparing agent. New guidelines from KDIGO and the International Pediatric Nephrology Association are awaited. Table 13.4 lists the advantages and disadvantages of each corticosteroid sparing medication. The choice of first agent will depend on clinician and family preferences based on an assessment of the benefits and harms as well as the cost and availability of medications.

Other Agents

In RCTs, no significant reduction in the risk of relapse has been demonstrated with azathioprine [235]. Mizoribine blocks purine biosynthesis pathways and is used in Japan for children with SSNS. In an RCT involving 197 children, who received 4 mg/kg/day of mizoribine or placebo, there was no significant difference in relapse rates and 16% of treated patients developed hyperuricaemia [295]. Recent studies suggest that some children with SSNS respond well to mizoribine and that differences in the pharmacokinetics in responders and non-reponders may explain differences in response. Mizoribine has a lower frequency of adverse effects compared with cyclophosphamide and cyclosporin so in Japan it may be used before other second line agents [296]. Two RCTs [297, 298] compared azithromycin (a macrolide antibiotic with immunomodulatory properties) and prednisone with prednisone alone and found a lower risk of relapse at 6 months in children treated with azithromycin. Adrenocorticotrophic hormone (ACTH) was widely used to try nephrotic syndrome in the 1950s but was subsequently replaced by prednisone. More recently there have been case reports of its efficacy in SSNS. However a

Table 13.4 Advantages and disadvantages of corticosteroid-sparing agents as first agent for use in frequently relapsing or steroid dependent steroid sensitive nephrotic syndrome

Medication	Advantages	Disadvantages
Cyclophosphamide	Prolonged remission off therapy Inexpensive	Less effective in SDNS Monitoring of blood count during therapy Potential serious short- and long-term adverse effects Only one course should be given
Chlorambucil	Prolonged remission off therapy Inexpensive	Less effective in SDNS Monitoring of blood count during therapy Potential serious short- and long-term adverse effects Only one course should be given
Levamisole	Prolonged remissions in some children with FRNS Generally inexpensive Few adverse effects	Continued treatment required to maintain remission Limited availability Not approved for SSNS in some countries
Mycophenolate mofetil/ Mycophenolate sodium	Prolonged remissions in some children with FRNS and SDNS Few adverse effects	Continued treatment required to maintain remission Less effective than CNIs Limited availability Expensive Risk of birth defects & pregnancy loss in first trimester of pregnancy Little data on use of mycophenolate sodium in children with SSNS
Cyclosporine	Prolonged remissions in some children with FRNS/SDNS	Continued treatment required to maintain remission Expensive Nephrotoxic Cosmetic side-effects
Tacrolimus	Prolonged remissions in some children with FRNS/SDNS	Continued treatment required to maintain remission Expensive Nephrotoxic Risk of diabetes mellitus Not approved for SSNS in some countries
Rituximab	Prolonged remissions off treatment in many children with FRNS/SDNS	Risk of prolonged B cell depression and hypogammaglobulinaemia Expensive Usually well tolerated but small risk of very serious adverse effects

FRNS frequently relapsing steroid-sensitive nephrotic syndrome; *SDNS* steroid dependent steroid-sensitive nephrotic syndrome

recent RCT found that ACTH at 80 U/1.73 m^2 administered twice weekly was ineffective at preventing disease relapses in children with steroid dependent SSNS [299].

Vaccinations in Children with SSNS

Physicians should encourage families to complete routine childhood vaccination programmes though the timing of administration of live vaccines (measles, mumps, rubella, varicella [MMRV]) may need to be altered if the child is receiving high dose corticosteroids or corticosteroid sparing agents. *Steptococcus pneumonia*e and *Haemophilis influenza* are important causes of invasive infections in children with SSNS. Vaccines against both organisms are included in the routine vaccination schedules of many countries. The safety and efficacy of the 13-valent pneumococcal conjugate vaccine (PCV13) and the 23-valent pneumococcal poly-

saccharide vaccine (PPSV23) have been demonstrated in children with SSNS [300–302]. In 42 children with nephrotic syndrome who received PCV13, serotype specific antibodies increased in all children and remained elevated for 12 months [302]. However serological responses were lower in children receiving both prednisone and non-corticosteroid immunosuppressive agents (tacrolimus, cyclosporin, MMF). Response to PPSV23 was similar in 30 nephrotic children who received PPSV23 while on prednisone 60 mg/m^2/day to that seen in 13 children on low dose alternate day prednisone [300]. Similar rises in anti-pneumococcal antibody levels were detected in both groups for up to 36 months. Response was not affected by non-corticosteroid immunosuppressive agents. Relapse rates did not increase following vaccination compared with the pre-vaccination period or compared with historical controls [300]. The Centre for Disease Control and Prevention's Advisory Committee on Immunization Practices recommends that the 13-valent pneumococcal conjugate vaccine (PCV13) be given routinely to all children aged below 60 months and to children with immunocompromising conditions including nephrotic syndrome to 18 years [303]. The Committee advises that PCV13 should be administered whether or not the child has previously received PCV7 and/or PPSV23. To broaden protection against serotypes not in PCV13, PPSV23 is also recommended for all children aged 2 years and over with immunocompromising conditions. Influenza vaccine should be given annually to children with nephrotic syndrome who are receiving corticosteroids and/or other immunosuppressive agents. Contacts of these children should also receive influenza vaccine. Hepatitis B vaccination should be administered to at risk children. Seroprotection rates were higher in SSNS than SRNS and higher in those who had received prednisone compared with those receiving prednisone with corticosteroid sparing agents [304].

Live vaccines are generally contraindicated in children on high dose prednisone or on other immunosuppressive agents. Most national recommendations on the administration of live vaccines in children do not specifically address children with SSNS. Based on a study of the South West Pediatric Nephrology Group [305], the 2012 KDIGO guidelines suggest that children not be given varicella vaccines until their prednisone dose is below 1 mg/kg/day (maximum 20 mg daily) or below 2 mg/kg on alternate days (maximum 40 mg on alternate days) and that live vaccines should not be given until children have been off cytotoxic agents for more than 3 months and off other immunosuppressive agents (CNIs, levamisole, MMF) for at least 1 month [125]. Varicella vaccination of household contacts is recommended [306]. A recent study from Japan has questioned the 2012 KDIGO guidelines [307]. The study reported no vaccine-related infections with satisfactory seroconversion rates to measles, rubella, varicella and mumps in 60 children with SSNS who were receiving immunosuppressive agent (CNI, MMF, mizoribine) with low dose prednisone when the vaccines were administered and had negative titres against one or more of these infections. Most children maintained seropositivity for these viruses at 1 year. No serious adverse reactions including vaccine-related infections were seen.

Of importance now to children with steroid sensitive nephrotic syndrome and their families is coronavirus disease 19 (COVID-19). In the United States, the Centers for Disease Control recommend COVID-19 vaccination for everyone aged 12 years and older for the prevention of COVID-19 disease [308] and their interim guidance states that immunocompromised individuals may receive COVID-19 vaccination if they have no contraindications to vaccination. There is limited information on the risk for and outcomes of COVID 19 in people with any immune related kidney disease though preliminary findings suggest that COVID 19 illnesses may be more severe than in people without such kidney disease particularly in people on high dose prednisolone [309]. The Immunonephrology Working Group of the ERA—EDTA (European Renal Association—European Dialysis and Transplant Association) recommend that all eligible people should receive vaccination against COVID-19 (except for those with known allergic reactions to any of the vaccine components) since the poten-

tial of COVID-19 vaccines to induce immunity protecting from severe COVID 19 should outweigh potential risks in most cases [310].

Conclusions

Though the long-term outlook in most children with SSNS is for resolution of nephrotic syndrome and continuing normal kidney function, approximately half of these children will suffer multiple relapses requiring corticosteroids and one or more corticosteroid sparing agents during the course of their disease and are at risk of multiple disease and treatment related complications. In summary:-

- SSNS is more common in Asian but less common in African and African-American children compared with Caucasian children.
- The aetiology and pathogenesis of SSNS remains largely uncertain.
- The outcome of SSNS is for resolution of disease and normal kidney function in the majority of patients.
- The prognosis for long-term kidney function in SSNS depends on complete remission of proteinuria rather than histology so that kidney biopsies are usually not required at presentation or before commencing corticosteroid sparing therapy in children with SSNS.
- Four large well-designed RCTs have demonstrated that there is no benefit of continuing prednisone therapy in the initial episode of SSNS beyond 2 or 3 months to reduce the risk of relapse or FRNS.
- Small studies suggest that relapses of SSNS can be treated successfully with smaller doses of prednisone (1 mg/kg/day) compared with 2 mg/kg/day but adequately powered RCTs are required to confirm this.
- RCTs and large observational studies have evaluated the relative efficacies of alkylating agents, levamisole, mycophenolate mofetil and CNIs. However these data remain insufficient to provide definitive recommendations on which corticosteroid sparing agent should be preferred as the first agent in a child with FRNS or SDNS so the choice of agent depends on patient and physician preferences based on adverse effects, availability and costs.
- In RCTs, rituximab reduces the risk of relapse compared with other therapies. However many children relapse within 6–12 months and uncertainties remain about the risk of serious adverse effects and the significance of persistent hypogammaglobulinaemia.

Further information on the underlying cause of SSNS is needed to guide therapy. New randomised controlled trials are required both to compare new therapies with existing therapies and to determine the optimal regimens for using corticosteroid therapy in SSNS. In particular further studies are required to determine the optimum dose and duration of corticosteroid therapy required in the initial episode of SSNS and in relapsing disease. Further studies on the safety of rituximab are required to determine whether it should be used ahead of other non-corticosteroid agents in children with FRNS and SDNS.

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Steroid Resistant Nephrotic Syndrome

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Rasheed Gbadegesin, Keisha Gibson, and Kimberly Reidy

Introduction

Idiopathic nephrotic syndrome is characterized by severe proteinuria, hypoalbuminemia, and/or presence of edema. Whereas approximately 85% of affected children achieve complete remission of proteinuria upon corticosteroid treatment, those who do not achieve remission are labeled as having "steroid resistant nephrotic syndrome" (SRNS). While details related to steroid sensitive forms of nephrotic syndrome are discussed in Chap. 14 of this book, our chapter will focus on the epidemiology, diagnosis, treatment and clinical outcomes of those children who fail to enter clinical remission after treatment with glucocorticoids.

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Definitions

The most important implication for a child given the label of SRNS is that he or she is at increased risk for both the development of disease complications as well as progression to chronic kidney disease (CKD) and eventually end stage kidney disease (ESKD). A major challenge of discussing the multiple issues related to children with SRNS is that its very definition has been standardized within the pediatric nephrology community only recently. In 2020, an IPNA expert committee launched a set of clinical practice recommendations for SRNS in children and in 2021, KDIGO published a clinical practice guideline for the management of glomerular diseases including a pediatric section [1, 2]. The two guidance documents provide the same uniform definitions of SRNS and its subsets:

Steroid Resistant Nephrotic Syndrome (SRNS): Children who fail to enter complete clinical remission within 4 weeks of treatment with prednisone or prednisolone at standard dose [3, 4].

It should be noted that several alternative definitions of SRNS have been used in the past, such as failure to enter remission after 6 weeks of daily oral prednisone, 4 weeks of daily followed by 4 weeks of alternate day oral prednisone, or 4 weeks of daily oral prednisone followed by three intravenous pulses of methylprednisolone [5, 6]. It is hoped that the new definition will be

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followed both in research settings and in routine clinical practice. This will be a prerequisite to compare the efficacy of established and novel treatments for nephrotic syndrome.

Calcineurin inhibitor responsive SRNS: Partial remission after 6 months of treatment and/or complete remission after 12 months of treatment with a CNI at adequate doses and/or levels.

Calcineurin inhibitor resistant SRNS: Absence of at least partial remission after 6 months of treatment with a CNI at adequate doses and/or levels.

Multidrug resistant SRNS: Absence of complete remission after 12 months of treatment with two mechanistically distinct steroid-sparing agents at standard doses.

Secondary SRNS: A steroid sensitive nephrotic syndrome patient at disease onset who at subsequent relapse fails to achieve remission after 4 weeks of therapy with daily prednisone or prednisolone at standard dose. Emerging data in the literature has drawn attention to this subgroup of patients with NS [7]. A unique characteristic of this group is that up to 80% of patients in this subgroup who progress to ESKD will develop recurrence of disease following kidney transplantation [8, 9].

Epidemiology

The annual incidence of nephrotic syndrome in most countries studied to date is ~1.2–17.0 new cases per 100,000 children [4, 10–14], and the prevalence is ~16 cases per 100,000 children [4]. The incidence varies widely between countries and different ethnicities [14]. In young children there is a male preponderance, with a male to female ratio of 2:1, although this gender disparity completely disappears by adolescence [11, 15–18]. Steroid resistant nephrotic syndrome (SRNS) is seen in about 15–20% of all cases of childhood nephrotic syndrome. Monogenic SRNS is responsible for 10–30% of all SRNS. The higher percentage is seen in the regions of the World here in breeding is very high and also in population where there are founder mutations. The most common causes of monogenic autosomal SRNS are mutations in nephrin (*NPHS1*), podocin (*NPHS2*), phospholipase c epsilon 1 (*PLCE1*) and *nucleoporin* genes. Majority of all cases of autosomal dominant monogenic SRNS are due to mutations in inverted formin2 (*INF2*), transient receptor potential cation channel, subfamily C, member 6 (*TRPC6*) actinin4 alpha (*ACTN4*), and wilms tumor type 1 (*WT1*) genes.

The incidence of nephrotic syndrome has been largely unchanged over the last 35 years, but the histopathologic lesions associated with nephrotic syndrome appear to be evolving. Some reports from various countries suggest that the incidence of focal segmental glomerulosclerosis (FSGS) is increasing, even after correction for variations in renal biopsy practices, and also assuming that children who did not undergo a renal biopsy had minimal change nephrotic syndrome (MCNS) [9, 15–18].

The histologic patterns and incidence of nephrotic syndrome are also affected by **ethnicity and geographic location**. For instance, idiopathic nephrotic syndrome in the United Kingdom was found to be more common among Asian children living in the UK and Canada compared to European children [19, 20]. In contrast, in Sub-Saharan Africa, idiopathic nephrotic syndrome occurs less commonly and disease is more commonly due to infection-associated glomerular lesions [21–23]. In the US, nephrotic syndrome has a relatively higher incidence among children of various ethnic backgrounds. A review of children with nephrotic syndrome in Texas reported that the distribution of children closely resembled the ethnic composition of the surrounding community [15]. These data in conjunction with the data from African countries suggests that the interaction of environmental and genetic factors plays an important role in the pathogenesis of nephrotic syndrome. Despite this, race alone seems to have a clear correlation with the histologic lesion associated with nephrotic syndrome. Indeed, 47% of African American children with nephrotic syndrome in the above study were found to have FSGS, while only 11% of Hispanic and 18% of Caucasian children had this unfavorable pattern of injury [15]. The genetic basis for the high prevalence of

FSGS in people of African ancestry was established in 2010 when homozygous or compound heterozygous G1 and G2 genotype in the gene encoding apolipoprotein 1 (*APOL1*) were shown to confer ten times the odds of developing FSGS in African Americans [24].

The age at presentation with nephrotic syndrome also has strong correlations with the frequency of presentation, as well as the associated renal histology. The most common age for presentation with nephrotic syndrome is 2 years, and 70–80% of all cases of nephrotic syndrome develop in children <6 years of age [4, 10]. In addition, children diagnosed prior to 6 years of age comprised 80% of those with MCNS, compared to 50% of those with FSGS, and only 2.6% of those with MPGN [25]. When analyzed based on renal histology, the median age at presentation was 3 years for MCNS, 6 years for FSGS, and 10 years for MPGN [25]. Therefore, excluding presentation in the first 12 months of life, these data suggest that the likelihood of having MCNS as a cause for nephrotic syndrome decreases with increasing age, while the likelihood for having the less favorable diagnoses of FSGS or MPGN increases [25, 26].

The most common renal histologies seen in children with SRNS are FSGS, MCNS, MPGN and membranous nephropathy.

Additional variables associated with clinical steroid responsiveness include ethnicity and geographic location. While 20% of children in Western countries have steroid resistant nephrotic syndrome, studies from Africa reported steroid resistance in 50–90% of children with nephrotic syndrome, with higher proportions of children with steroid responsive disease in more affluent and diverse urban centers [23, 27–29].

Histopathological Findings

SRNS is a heterogeneous clinical condition with multiple etiologies. The histopathologic entities that may cause SRNS vary in different series depending on the age group and the population being studied. However, in different series focusing on children presenting after the first year of life, the common pathologic variants associated with SRNS include focal segmental glomerulosclerosis (FSGS), membranous glomerulopathy, membranoproliferative glomerulopathy (MPGN), and minimal change disease (MCD) [11, 15, 31–34]. The majority of cases are due to disease on the continuum between MCD and FSGS (Table 14.1). Since the MCD/FSGS spectrum represents the most common pathologic variants of SRNS, and since other chapters are devoted to each of the other histologic variants, the rest of this chapter will focus mainly on FSGS.

Table 14.1 Pathologic findings in steroid resistant nephrotic syndrome

Histology	South-Asia[a] [25, 26] n = 326	South-Africa [28] n = 183	Poland [27] n = 34	USA[b] [9, 12] n = 253
MCD	38.4	36.1	5.9	45.4
FSGS	41.5	36.1	32.4	26.5
MESGN	14.1	8.1	55.8	10.3
MEMB	4.0	–	–	1.2
MPGN	1.0	–	5.9	7.5
Others	1.0	19.7	–	9.1

[a] Two studies one each from Pakistan and India [25, 26]
[b] Summary of two studies, some of the patients were diagnosed with frequent relapsing and steroid dependent NS [9, 12]

Focal Segmental Glomerulosclerosis (FSGS)

FSGS is a pathologic finding that is characterized by focal glomerulosclerosis or tuft collapse, segmental hyalinosis, IgM deposits on immunofluorescence staining, and podocyte foot process effacement on electron microscopy [35]. In the majority of children, it is characterized by SRNS and progression to end-stage kidney disease (ESKD) within 5–10 years of diagnosis [30]. It was first described in kidney biopsies of adults with nephrotic syndrome by Fahr in 1925, although it was Rich who later made the observation that the lesion of FSGS in children with nephrotic syndrome classically starts from the corticomedullary junction before involving other parts of the renal cortex [36, 37]. The observation of Rich is probably the explanation for why many cases of FSGS

are initially misdiagnosed as MCD since early disease may be confined to the corticomedullary junction. The incidence of FSGS is estimated at seven per million people, and the incidence is higher in blacks than whites and the rate of decline in kidney function is also worse in blacks [38]. The incidence of FSGS is increasing in all populations. In a predominantly adult cohort, Kitiyakara et al. reported an 11-fold increase among dialysis patients over a 21 year period, and a similar pattern was reported in a population-based study in the USA [39, 40]. The most compelling pediatric data to date is a metanalysis that examined over 1100 nephrotic patients over two time points. This study demonstrated a twofold increase in the incidence of FSGS in children [41]. The reason for the increasing incidence is unknown, but possible explanations include changing criteria in the selection of patients for kidney biopsy, better diagnostic instruments, or changing environmental factors such as infection-driven disease. For example, patients with severe acute respiratory syndrome due to coronavirus 2 (SARS-CoV-2) can develop nephrotic syndrome with renal histology findings of FSGS due to direct podocyte infection and or cytokine production [42].

Table 14.2 Etiology of FSGS

Primary/idiopathic FSGS (80% of all cases)
Familial FSGS
Infections
HIV infection
Hepatitis B and C
Cytomegalovirus
Epstein-Barr virus
Parvovirus B19
SARS-CoV-2 (COVID-19)
Drugs/toxic agents
Gold
Interferon-α
Lithium
Pamidronate
Mercury
Heroin
Hyperfiltration
Obesity
Bilateral or unilateral renal dysplasia
Reflux nephropathy
Other causes of glomerulonephritis associated with nephron loss
Aging
Ischemia
Renal artery stenosis
Hypertensive kidney disease
Calcineurin inhibitor nephrotoxicity
Acute and chronic renal allograft rejection
Cholesterol crystal embolism
Cyanotic congenital heart disease

Clinical and Pathologic Classification of FSGS

Until recently, FSGS was classified based on presumed causes. The etiology was unknown in more than 80% of cases (primary or idiopathic FSGS) and the remainder secondary to other disease processes such as infectious agents like hepatitis, HIV, toxic agents, ischemia, obesity and other glomerulonephritides. A list of causes of FSGS is shown in Table 14.2.

With the recent advances in genomic science, hereditary causes of FSGS are increasingly being recognized. Although this group is estimated to be responsible for not more than 30% of all cases, detailed studies of hereditary FSGS have shed more light on the molecular pathogenesis of the disease [43].

The morphological changes in kidney biopsies of patients with FSGS are heterogeneous. In order to standardize the pathological diagnosis of FSGS and relate histologic findings to clinical course, the Columbia classification of FSGS was proposed [44]. In this classification schema, five patterns of FSGS have been proposed including: (1) FSGS not otherwise specified (NOS), (2) Perihilar variant, (3) Cellular variant, (4) Tip variant, and (5) Collapsing variant (Table 14.3 and Fig. 14.1). The clinical significance of the

Table 14.3 Outcome of FSGS histologic subtypes in the NIH-sponsored FSGS trial [21]

FSGS subtypes	Frequency (%) n = 138	% with ESKD at 3 years
NOS	68	20
Collapsing	12	47
Tip	10	7
Perihilar	7	Number too small
Cellular	3	Number too small

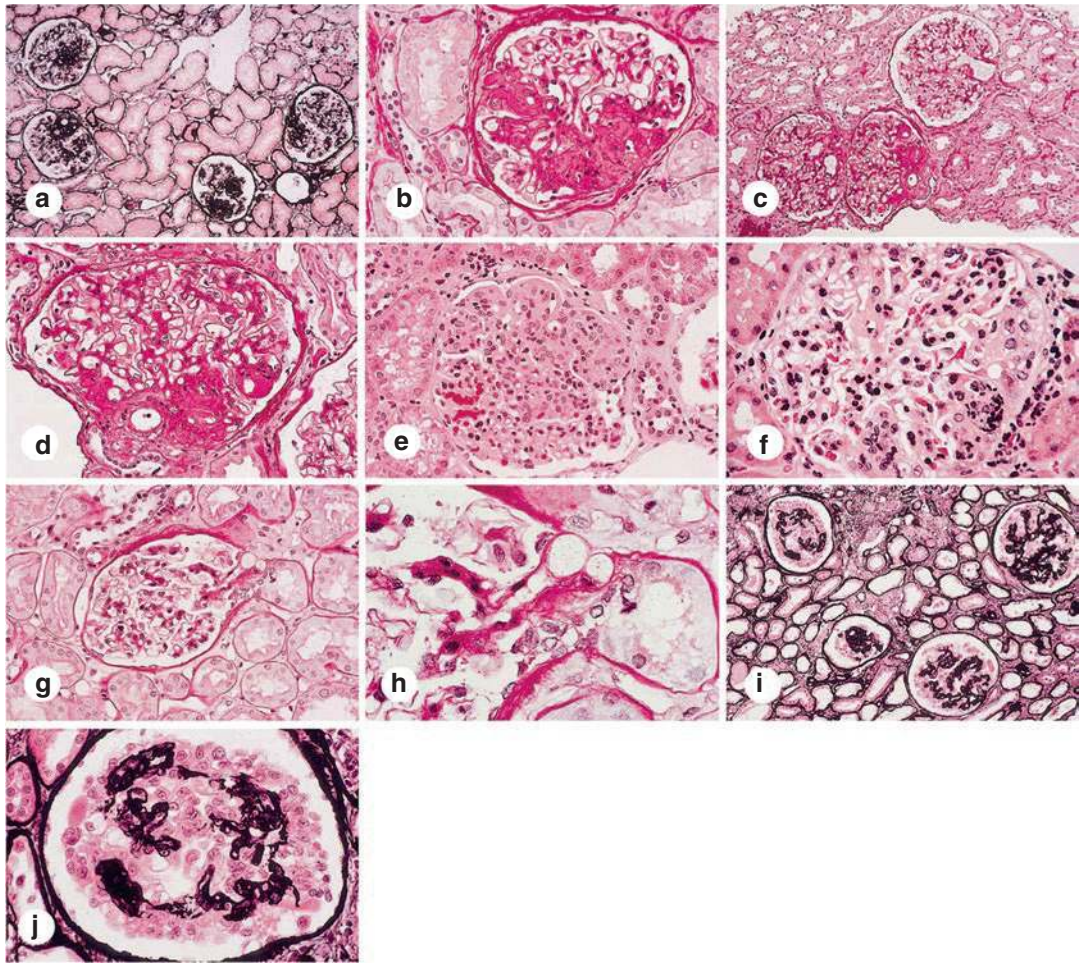

Fig. 14.1 Columbia classification of FSGS: *FSGS NOS:* (**a**) Low power magnification showing segmental sclerosis in two glomeruli. Lesions are characterized by increased matrix and obliteration of the capillary lumen. Distribution of lesions within the tuft is variable. (**b**) PAS staining at higher magnification showing obliteration of glomerular tuft by increased matrix and hyaline deposit. Sclerosed segments form adhesions to Bowman's capsule, note that there is no podocyte hypertrophy or hyperplasia. *FSGS perihilar variant:* (**c**) Low power examination showing segmental sclerosis affecting the vascular pole in one of three glomeruli. The lesion shows increased sclerosis and hyalinosis and there is adhesion of the sclerotic segment to the Bowman's capsule in the vascular pole region. (**d**) Higher magnification of C showing increased sclerosis and glassy hyalinosis deposited in the vascular pole segment of the tuft. *FSGS cellular variant:* (**e**) The glomerulus in this image shows endocapillary hypercellularity. The involved segments are engorged with endocapillary cells including mononuclear leukocytes. (**f**) Further demonstration of numerous endocapillary leukocytes mimicking endocapillary glomerulonephritis. In addition there is hypertrophy and hyperplasia of overlying podocytes. *FSGS tip variant:* (**g**) Low power view shows a segmental lesion involving the tip domain at the origin of the tubular pole. (**h**) Higher magnification of the lesion in G showing endocapillary foam cells and adhesion of the sclerotic segment to Bowman's capsule at the mouth of the proximal tubule. *FSGS collapsing variant*: (**i**) Low power magnification shows four glomeruli with global collapse of the tuft and podocyte hypertrophy and hyperplasia with tubular degenerative changes. (**j**) High power magnification shows global occlusion of capillary lumina by implosive collapse of the glomerular basement membranes. There is no significant increase in intracapillary cells or matrix. Overlying podocytes form a cellular corona over the collapsed tuft. Some of the enlarged podocytes appear binucleated and have lost their cohesion to the tuft. (Adapted with permission from reference [44])

variants is still being studied. In a cohort of adults with FSGS, it was reported that collapsing FSGS had the highest rate of renal insufficiency at presentation and worst long term outcome [45]. The most comprehensive prospective report of the clinical significance of the classification in children comes from the analysis of the kidney biopsies from the patient cohort in the NIH sponsored FSGS trial [46]. In this study FSGS NOS was the most common variant, being responsible for 68% of all cases, with collapsing, tip, perihilar and cellular variants responsible for 12%, 10%, 7% and 3%, respectively. Patients with collapsing FSGS were more likely to be black and to have nephrotic syndrome with renal impairment at presentation, compared to patients with NOS and tip variants [46]. Furthermore, globally sclerotic changes were found more commonly in the NOS variant while segmental sclerosis, tubular atrophy and interstitial fibrosis were found more commonly in collapsing FSGS [46]. At the end of 3 years follow up, 47% of patients with collapsing FSGS were in ESKD compared with 20% and 7% for the NOS and tip variants, respectively [46] (Table 14.3). These findings were confirmed in a study of 201 Japanese FSGS patients [47].

Integrated molecular and morphologic classification: Emerging data is recognizing the fact that FSGS and related morphologic descriptions such as diffuse mesangial sclerosis and minimal change disease are non-specific diagnoses but morphologic changes resulting from multiple injuries to the podocyte [48]. It is now proposed that these morphologic entities should be called **podocytopathies** [49]. The advantages of looking at FSGS and the other morphologic patterns as podocytopathies are (1) focusing on a cell that is central to pathogenesis and therefore a target for biochemical analysis and cellular therapy, (2) facilitating identification of other cellular lineage that may be working in concert with the podocyte to preserve the function and the integrity of the GFB, and (3) enabling clinical work-up focusing on identifying causes or risk factors for podocyte injury and therefore more informed prognosis and personalized therapy [48].

Pathogenesis

The hallmark of nephrotic syndrome is glomerular proteinuria [48]. While there are other causes of proteinuria, proteinuria in nephrotic syndrome results from leakage of protein through the glomerular filtration barrier (GFB). The GFB is composed of three layers: podocyte (glomerular epithelial cell), glomerular basement membrane, and fenestrated endothelium (Fig. 14.2) [24, 48–108]. Defects in any of the three layers can result in proteinuria [107, 108].

Hereditary and Monogenic Forms of SRNS

Over the past 20 years, investigations of inherited forms of nephrotic syndrome led to recognition of the importance of the podocyte in the pathogenesis of SRNS [24, 48–110]. The majority of

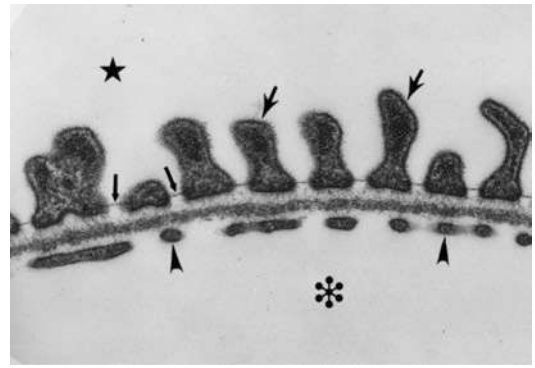

Fig. 14.2 Electron micrograph of the components of the glomerular filtration barrier. During normal glomerular filtration, plasma water is filtered from the glomerular capillary lumen (asterisk) through the fenestrated endothelial cell layer (arrowheads), then across the glomerular basement membrane (GBM) and through the slit diaphragms (small arrows) which bridge the filtration slits between adjacent podocyte foot processes (large arrows) and finally into the urinary space (star) where it enters the lumen of the proximal tubule. These podocyte foot processes are normally tall and evenly-spaced along the GBM, but during nephrotic syndrome they become spread out along the GBM, with apical displacement of the slit diaphragms. The layer of negatively-charged glycocalyx can be seen in this image as a blurry coating on the apical surfaces of the podocyte foot processes. (Adapted with permission from reference [50])

monogenic causes of SRNS affect the structure and function of the podocyte [24, 48–110]. The podocyte is a terminally differentiated epithelial cell with limited ability to regenerate [111]. The prominence of the podocyte in the pathophysiology of SRNS is highlighted by the fact that most common causes of monogenic NS are genes with preferential or selective expression in the podocyte.

In a large cohort of patients with SRNS, the top six monogenic causes of SRNS were *NPHS2* (encodes podocin), *NPHS1* (encodes nephrin), *PLCE1* (encodes phospholipase C epsilon 1), *WT-1* (encodes Wilms tumor 1), *LAMB2* (encodes laminin beta 2) and *SMARCAL* (encodes SW/SNF2 related, matrix associated, actin dependent regulator of chromatin, subfamily a-like 1) (Fig. 14.4 and Table 14.4) [112, 113]. Beyond

Table 14.4 Genetic causes of FSGS and SRNS

Gene	Protein	Mode of Inheritance
Slit diaphragm genes		
NPHS1	Nephrin	AR
NPHS2	Podocin	AR
PLCE1	Phospholipase C epsilon 1	AR
CD2AP	CD2-associated protein	AD, AR
TRPC6	Transient receptor potential channel C6	AD
CRB2	Crumbs family member 2	AR
FAT1	FAT atypical cadherin	AR
KIRREL1	kirre like nephrin family adhesion molecule 1	AR
Transcription factors and nuclear genes		
WT1	Wilm's tumor protein 1	AD
LMX1B	LIM homeobox transcription factor 1-beta	AD
SMARCL1	SMARCA-like protein	AR
NUP93	Nuclear pore complex protein 93	AR
NUP107	Nuclear pore complex protein 107	AR
NUP205	Nuclear pore complex protein 205	AR
NUP160	Nuclear pore complex protein 160	AR
NUP85	Nuclear pore complex protein 85	AR
NUP133	Nuclear pore complex protein 133	AR
XPO5	Exportin 5	AR
E2F3	E2F transcription factor	AD
NXF5	Nuclear RNA export Factor 5	X-linked recessive
PAX2	Paired box protein 2	AD
LMNA	Lamin A and C	AD
WDR73	WD repeat domain 73	AR
Cytoskeletal and membrane genes		
ACTN4	Alpha-actinin 4	AD
INF2	Inverted formin 2	AD
MYO1E	Myosin 1E	AR
MAGI2	Membrane Associated Guanylate kinase, inverted 2	AR
ANLN	Anillin actin binding protein	AD
PTPRO	Protein-tyrosine phosphatase-R O	AR
EMP2	Epithelial membrane protein 2	AR
CUBN	Cubilin	AR
PODXL	Podocalyxin	AR, AD
ARHGAP24	Rho GTPase-activating protein 24	AD
ARHGDIA	Rho GDP dissociation inhibitor alpha	AR

(continued)

Table 14.4 (continued)

Gene	Protein	Mode of Inheritance
DAAM2	Dishevelled associated activator of morphogenesis 2	AR
SYNPO	Synaptopodin	AD
SYNPO2 (Also localized to mesangial cells)	Synaptopodin 2	AR
DLC1	Deleted in liver cancer 1	AR
KANK 1/2/4	Kidney ankyrin repeat-containing protein	AR
ITSN1/2	Intersectin protein	AR
CDK20	Cyclin-dependent kinase 20	AR
NOS1AP	Nitric oxide synthase 1 adaptor protein	AR
Mitochondrial, lysosomal, metabolic, and cytosolic genes		
COQ2	Coenzyme Q2 4-hyroxybenzoate polyprenyl transferase	AR
COQ6	Coenzyme Q6 monooxygenase	AR
PDSS2	Prenyl-diphosphate synthase subunit 2	AR
ADCK4	AarF domain containing kinase 4	AR
SCARB2	Scavenger receptor class B, member 2	AR
PMM2	Phosphomannomutase 2	AR
ALG1	Asparagine-linked glycosylation 1	AR
TTC21B	Tetratricopeptide repeat protein 21B	AR
CDK20	Cyclin-dependent kinase 20	AR
CFH	Complement factor H	AR
DGKE	Diacylglycerol kinase epsilon	AR
Glomerular basement membrane genes		
LAMB2	Laminin subunit beta-2	AR
ITGB4	Integrin beta 4	AR
ITGA3	Integrin alpha 3	AR
COL4A 3/4/5	Type IV collagen alpha 3,4,5	AR, AD, X-linked
Endosomal regulator genes		
GAPVD1	GTPase Activating Protein And VPS9 Domains 1	AR
ANKFY1	Ankyrin Repeat And FYVE Domain Containing 1	AR

these top six monogenic causes of SRNS, pathogenic variants have been identified in over 60 genes in patients with SRNS (Table 14.4). Recent large cohort studies revealed that altogether 20–30% of sporadic childhood onset SRNS may be due to single gene defects [112–115]. In animal models, including transgenic mice and zebrafish, most identified single gene causes of SRNS result in podocyte dysfunction. Mechanisms of podocyte dysfunction include: (1) slit diaphragm abnormalities (*CD2AP, NPHS1, NPHS2*, and *FAT1*) (2) impaired podocyte actin cytoskeleton regulation and/or adhesion to the glomerular basement membrane (*ACTN4, ANLN, ARHGAP24, INF2, SMARCAL and TRPC6*); (3) defective podocyte differentiation (*PLCE1* and *WT1*), (4) mitochondrial dysfunction (*ADCK4*, *COQ2*, *COQ6, COQ8B* and *tRNA (Leu))* and (5) nuclear pore dysfunction (*NUP94, NUP107, NUP160*), Table 14.4 [48, 116–118].

Beyond the podocyte, pathogenic variants in genes encoding for key molecular components of the glomerular basement membrane are increasingly being recognized as monogenic causes of SRNS. These include *COL4A3* and *COL4A4*, which encode for type 4 collagen of the GBM, and *LAMA5* and *LAMB2*, forming laminin LM-521; α5β2γ1 that is a key component of the glomerular basement membrane. While *COL4A3* and *COL4A4* mutations typically present with the more classic phenotype of Alport syndrome (see Chap. 16), they may also phenocopy FSGS and present with SRNS [119, 120]. Further discussion of monogenic SRNS can be found in Chap. 15.

Common Gene Variants Associated with SRNS/FSGS

In addition to monogenic causes of SRNS, common variants in the gene encoding for apolipoprotein L1 (*APOL1*) are associated with FSGS [24, 122]. *APOL1* contributes to innate immunity and protection against *Trypanosoma*, the cause of African sleeping sickness. The APOL1 protein forms a channel that contributes to *Trypansomal* lysis. Two common variants (known as G1 [2 single nucleotide polymorphisms S342G and I384M] and G2 [a 6 base pair deletion (p.NYK388K] are common in people of West African descent and are associated with protection against resistant *Trypanosoma brucei rhodesiense* and *gambiense*. However, carriage of any combination of *APOL1* high risk genotype defined as homozygous or compound heterozygous G1/G2 genotypes: (G1/G1; G1/G2; or G2/G2) are associated with increased risk for kidney disease [123]. In children of African descent with SRNS, prevalence of *APOL1* high risk genotype may be as high as 40% [124, 125]. The mechanisms of *APOL1* related kidney disease continue to be a focus of ongoing investigations. The prevalence of high risk genotype is about 14% in African Americans, however less than 25% of individuals with these high risk genotypes will develop kidney disease suggesting that genetic and environmental second hits may be needed for phenotypic manifestations. Increased podocyte APOL1 expression with enhanced inflammatory signaling may be one such second hit [126, 127]. Other pathways implicated in APOL1 related kidney disease include podocyte lipid and mitochondrial dysfunction and alterations in ion channel functions [126, 128, 129]. Interestingly *APOL1* high risk genotype is also associated with increased susceptibility to infection related nephropathy, including HIV and COVID-19 nephropathy [130, 131].

Circulating Factors

Beyond genetic factors, a major mechanism of idiopathic SRNS is the presence of a circulating pathogenic factor or absence of factors that prevent proteinuria [132]. Evidence supporting the role of circulating factors includes the recurrence of FSGS post-transplant that is amenable to treatment with plasmapheresis and immunosuppression in some patients [133]. In addition, administration of plasma from FSGS patients alters glomerular and podocyte morphology *in vitro* [134]. Despite extensive efforts, a single circulating factor has not been identified to date. Several candidate factors have been proposed; one of these is sUPAR, the soluble urokinase receptor, which was shown to be elevated post FSGS recurrence and induced proteinuria in a mouse model of FSGS [135]. However, additional studies failed to confirm sUPAR as the circulating factor, although its role in disease progression remains the subject of ongoing investigations [133]. Other circulating factors that have been implicated include cardiotrophin-like cytokine factor-1 (CLCF-1), CD40 antibodies, and apolipoprotein A-Ib (ApoA-Ib) [133]. CLCF-1 is a cytokine that functions in B-cell stimulation. CD40 is a costimulatory protein expressed on immune cells. Elevated CLCF1 levels and anti-CD40 antibodies were identified in sera from patients with recurrent FSGS [136]. Application of CLCF1 or anti-CD40 antibodies to cultured podocytes induced actin cytoskeleton alterations [136]. ApoA-1 is a circulating component of the HDL complex; The ApolA-1b variant was identified by urine proteomics studies as increased in patients with recurrent FSGS [137].

Podocyte Endowment, Loss, Regeneration and Glomerulosclerosis

Glomerulosclerosis is the most common pathologic finding underlying SRNS. Regardless of the initial factor, podocyte damage and loss is key to development of the lesions of glomerulosclerosis [138]. The mechanisms by which podocyte damage evolves into the pathological appearance seen in FSGS have been studied extensively in murine models of FSGS [73]. The initial defect appears to be a reduction in podocyte number and the inability of podocytes to completely cover the glomerular tufts. The reduction in podocyte density causes the loss of separation between the glomerular tuft and Bowman's capsule, leading to the formation

of synechiae or adhesions between the tuft and the Bowman's capsule [73]. The perfused capillaries lacking podocytes at the site of tuft adhesion then deliver their filtrate into the interstitium instead of Bowman's space (Fig. 14.3) [73]. This misdirected filtration through capillaries lacking podocytes leads to progression of segmental injury, tubular degeneration and interstitial fibrosis [73]. Further evidence for the role of podocytopenia in the pathogenesis of FSGS was shown using a rat model of diphtheria toxin-induced podocyte depletion in which the extent of podocyte loss is regulated [74]. In this model, mild podocyte loss resulted in hypertrophy of the remaining podocytes to cover the glomerular basement membrane. However, with moderate to severe depletion FSGS and global sclerosis developed; 30–40% of podocyte loss appear to be sufficient to drive progressive glomerulosclerosis [74].

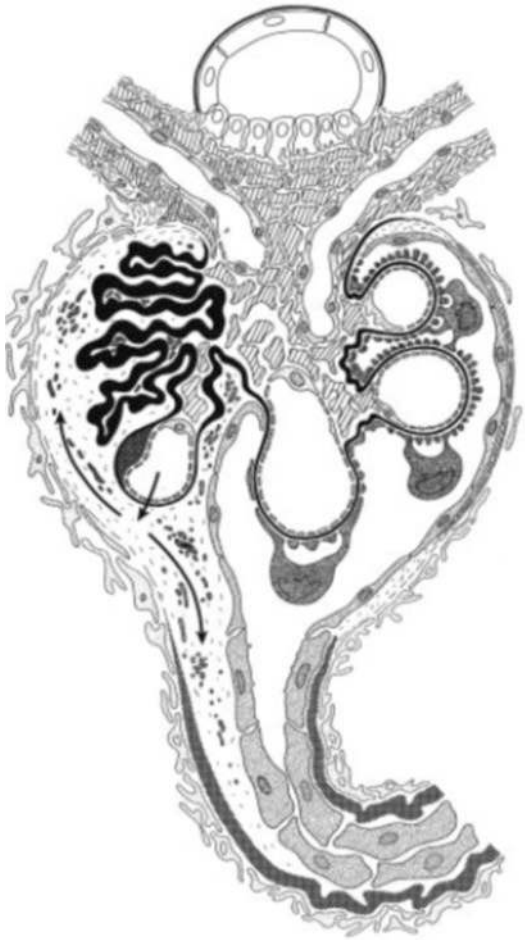

Fig. 14.3 Kriz's misdirected filtration hypothesis of evolving FSGS lesion: The glomerular basement membrane (GBM) is shown in black, podocytes are densely stippled, parietal epithelial cells are less densely stippled and interstitial as well as endothelial cells are loosely stippled, mesangial cells are hatched. The tuft adhesion contains several collapsed capillary loops. It also contains a perfused loop, which is partially hyalinized. The filtrate of this loop is delivered into a paraglomerular space that is separated from the interstitium by a layer of fibroblasts. This newly created space extends onto the outer aspect of the tubule by expanding and/or separating the tubular basement membrane from its epithelium. (Reproduced with permission from reference [73])

Diagnostic Evaluation

Patient and Family History

In patients diagnosed with SRNS, the medical history should be explored for potential secondary causes of the disease such as sickle cell disease, HIV, SLE, as well as recent hepatitis B, malaria or parvovirus B19 infections. Family history should be assessed for other family members affected by nephrotic syndrome and/or chronic kidney disease, and parental consanguinity should be checked.

Clinical Assessment

The clinical evaluation of children with SRNS should include an assessment of fluid status, as well as careful exploration of extrarenal disease features such as dysmorphic features, ambiguous genitalia, skeletal, skin, ocular, hearing and neurological abnormalities. Any abnormalities should prompt further diagnostic evaluation.

Laboratory Workup

Proteinuria should be quantitated by measuring the urinary protein:creatinine ratio (uPCR) in spot urine or 24-h protein excretion. Urine dipstick is not considered sufficient to make the diagnosis or monitor treatment responsiveness in

SRNS. Basic chemistries including serum creatinine, serum albumin, a complete blood count, a lipid profile and coagulation testing are important for estimating renal function, confirming the presence or absence of overt nephrosis, and evaluating the risk for disease complications.

SRNS patients require a diligent effort to rule out secondary disease processes. Tests for systemic autoimmune disorders, including antinuclear antibody (ANA), anti–double stranded DNA (anti-dsDNA) antibodies, ANCA, and complement C3 levels should be performed and testing for hepatitis B and C, HIV, malaria, parvovirus B19 and depending on geographic area and ethnicity, sickle cell disease, tuberculosis, and even syphilis may be indicated.

Genetic Testing

Genetic screening is emerging as a critically important clinical tool in the management of children with SRNS. Identification or exclusion of pathogenic gene variants potentially allows for (1) a rational approach to the use of immunosuppressive agents and avoidance of side effects; (2) selection of targeted therapies that may induce remission and/or delay progression to ESKD (e.g., COQ10 supplementation in patients with hereditary COQ10 deficiency; (3) prediction of clinical course and risk of post-transplant disease recurrence; (4) avoidance of kidney biopsy; and (5) genetic counseling and possible prenatal screening [139]. In view of these considerations, the IPNA SRNS guideline recommends genetic testing for all children as soon as the diagnosis of primary SRNS is established [1]. When considered later in the course of the disease, genetic screening is not indicated in patients who have responded to intensified immunosuppressive therapy and in patients with secondary SRNS.

Kidney Biopsy

Kidney biopsy allows the confirmation of a primary podocytopathy (MCD, FSGS, or DMS) and the exclusion of other differential diagnoses such as membranous nephropathy or MPGN. Biopsy is therefore indicated in all children with SRNS except in those with an established monogenic cause of SRNS, potentially in familial and/or syndromic cases, and in patients with known secondary SRNS due to infection or malignancy. Even in suspected or confirmed hereditary forms of SRNS, kidney biopsy may sometimes be indicated, particularly in patients with CKD stage 2 and higher, to grade the amount of tubular atrophy, interstitial fibrosis and glomerulosclerosis as prognostic markers [32, 33].

Management

The IPNA SRNS Clinical Practice Recommendation contains a refined algorithm for the management of SRNS in children (Fig. 14.4). We will describe and explain the rationale for the preferred therapeutic approaches along the lines of this recommendation.

Confirmation Period

When the diagnosis of SRNS is established after 4 weeks of standardized oral corticosteroid therapy, it is suggested to utilize a 2- to 3-week period to further confirm and elaborate the diagnosis before starting new immunosuppressive therapies other than steroids. During this period, genetic testing should be initiated and a kidney biopsy performed, oral prednisone therapy continued and/or three intravenous steroid pulses may be optionally performed. Importantly, renin-angiotensin system (RAS) blockade should be implemented by up-titrating an angiotensin converting enzyme (ACE) inhibitor or angiotensin receptor blocker (ARB) to full antiproteinuric efficacy. It is essential to measure proteinuria at the end of this period to avoid confounding the antiproteinuric effect of RAS blockade with that of any subsequent immunomodulatory therapies. If genetic screening reveals a monogenic form of SRNS, RAS blockade should be continued at the maximally effective dose, steroid therapy discontinued and no alternative immunosuppressive

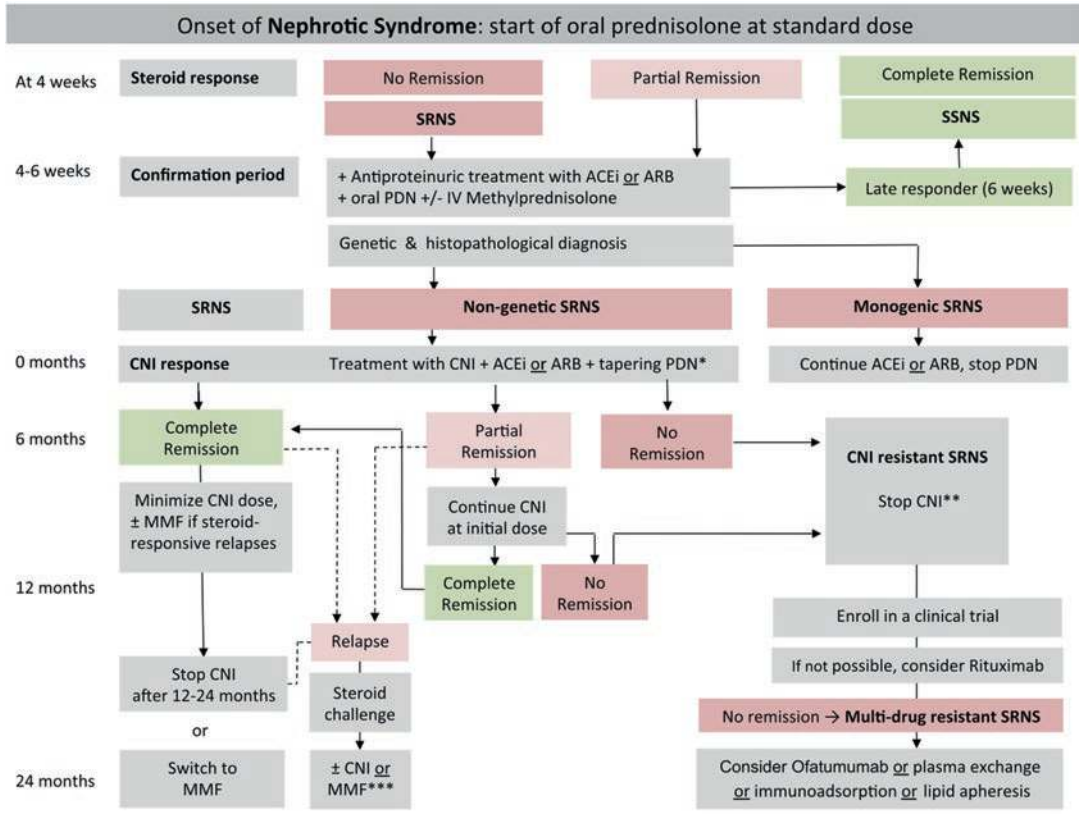

Fig. 14.4 IPNA clinical practice recommendation for management of SRNS in children (Reproduced from [1] with permission)

therapies should be started (or stopped if already started) in order to avoid a futile, potentially toxic treatment.

Therapeutic Pathway

If no genetic disorder is identified, a calcineurin inhibitor (CNI, tacrolimus or cyclosporin A) should be started, the RAS blocker continued at unchanged dose, and oral prednisone weaned over 4–5 months. The response to calcineurin inhibition should be evaluated after 6 months of treatment. If complete remission has been achieved, the patient can be classified as **CNI-responsive SRNS**. In this case, the CNI dose should be minimized and optionally supplemented by MMF. In case of persistent remission, the CNI should be stopped after 12–24 months, optionally continuing or switching to MMF monotherapy. If partial remission is achieved at 6 months, treatment should be continued for a total of 12 months. If complete remission does not occur, the CNI should be discontinued and the patient should receive the diagnosis **CNI-resistant SRNS**. In these patients, a B-cell depleting monoclonal antibody (e.g. Rituximab) can be tried. If this treatment does not yield full remission, the patient should receive the diagnosis **multidrug-resistant SRNS**. Children with CNI-resistant or multidrug-resistant SRNS are candidates for clinical trials and experimental extracorporeal rescue therapies, such as plasma exchange, immunoadsorption and lipid apheresis.

Pharmacotherapies

In the following, we describe the evidence base supporting the use of antiproteinuric and immunosuppressive pharmacotherapies in the therapeutic pathway above. It should be emphasized that due to the rarity of the disease, randomized trial evidence for any of the drugs is scarce or absent. Generally, the apparent efficacy of all of the immunosuppressive agents is lower in SRNS compared to those with frequently relapsing or steroid sensitive nephrotic syndrome [140, 141]. However, most previous treatment studies did not identify and exclude patients with genetic forms of SRNS who are highly unlikely to respond to immunosuppressive treatment. Since these cases comprise up to 30% of SRNS cases, most trial results must be considered substantially confounded. Finally, spontaneous remission of SRNS can occur and may explain some occasional responses of largely ineffective therapies.

RAS Blockade

Several controlled and non-controlled clinical studies have demonstrated the antiproteinuric efficacy of ACE inhibitors and angiotensin-receptor blockers (ARBs) in adults and children with glomerular diseases [142–148]. The antiproteinuric effects of ACEIs and ARBs are due to their ability to reduce glomerular capillary plasma flow rate, decrease transcapillary hydraulic pressure, and alter the permselectivity of the glomerular filtration barrier. On average, RAS blockers reduce proteinuria by approximately 30% in children with SRNS [149], although even complete remissions have been reported [150]. ACEIs and ARBS should be administered at the maximum approved and tolerated dosages since proteinuria reduction is dose dependent. However, the use of RAS blockers may increase the risk for AKI in patients with intravascular volume depletion [151]. Combined treatment with ACEi and ARBs is not recommended as it increases the risk for adverse events [152]. ACE inhibitor therapy may lead to a phenomenon known as 'aldosterone escape' with a long-term increase in plasma aldosterone levels. The addition of aldosterone blockade with ACE inhibition reduces urine protein excretion by 30–58% in patients with both diabetic and non-diabetic proteinuria [153]. The long-term safety of this form of combined RAS blockade remains to be elucidated.

Calcineurin Inhibition

Several randomized trials have suggested improved complete or partial remission rates in patients with SRNS when treated with cyclosporine compared with placebo, no treatment, or intravenous methylprednisolone pulses (~75% vs. 22%) [154–158].

Out of 433 children with primary SRNS in the PodoNet registry treated with CsA or Tacrolimus in the year following diagnosis, 30% achieved complete and 19% partial remission [159]. CsA and tacrolimus show similar efficacy in inducing remission [160].

In addition to immunomodulation, antiproteinuric effects of the calcineurin inhibitors may in part be mediated by hemodynamic effects that reduce renal blood flow [161]. In addition, calcineurin inhibitors may reduce proteinuria by inhibition of calcineurin-mediated degradation of synaptopodin and stabilization of the podocyte actin cytoskeleton [162, 163].

While CNIs are generally not recommended in patients with reduced eGFR due to their nephrotoxic effects, their use may be justified in SRNS patients with CKD and no other option for disease control [162]. CsA and tacrolimus have similar nephrotoxicity, but gingival hyperplasia and hypertrichosis are more prevalent with CSA and glucose intolerance occurs more frequently with tacrolimus (Table 14.5).

Mycophenolate-Mofetil (MMF)

The efficacy of MMF in inducing or maintaining remission in children with SRNS has not been demonstrated against placebo or no treatment in randomized clinical trials.

Table 14.5 Treatment options for steroid resistant nephrotic syndrome

Drug	Efficacy Evidence	Toxicity	Benefit
ACE Inhibitors/ ARBs	Good	May lower eGFR Teratogenicity	Slowed progression of CKD
IV Corticosteroids	Good	Weight gain Hypertension Glucose intolerance Hyperlipidemia Striae	May ensure achieving therapeutic drug levels.
Cyclosporine	Good	Nephrotoxicity Hypertension Gingival hyperplasia	May only require low dose
Tacrolimus	Good	Nephrotoxicity Hypertension Glucose Intolerance	May only require low dose
Mycophenolate Mofetil	Mixed	GI intolerance Teratogenicity	No nephrotoxicity
Rituximab	Mixed	Infections Hypogammaglobulinemia Long-term effects unknown	May enable discontinuation of daily immunosuppressive medications

In a multicenter randomized trial of 192 children and young adults with steroid resistant FSGS, MMF in combination with dexamethasone was similarly effective as CsA (33% vs. 46%] and the rates of adverse events were similar [163]. MMF was less effective than tacrolimus in maintaining remission (45% vs. 90%) [164]. In the PodoNet registry, MMF therapy was associated with complete or partial remission in only 4 of 24 cases (17%) [159]. CNI/MMF co-treatment yielded full remission in four and partial remission in 10 of 34 patients, i.e. not different from CNI monotherapy [159].

B-Cell Depleting Agents

Rituximab is a chimeric monoclonal antibody directed against CD20. Rituximab-induced B-lymphocyte depletion may act on proteinuria in nephrotic syndrome by inducing regulatory T lymphocytes, as has been observed in patients with lupus nephritis [164]. Experimental findings suggest that Rituximab may also directly protect podocytes by stabilizing the podocyte cytoskeleton and preventing apoptosis through an interaction with the sphingomyelin phosphodiesterase acid-like 3b protein that is expressed in podocytes [165].

In a retrospective review of 33 patients with SRNS treated with two to four doses of intravenous rituximab, and followed for ≥12 months, 9 (27%) patients with SRNS showed complete remission, 7 (21%) had partial remission, and 17 (52%) had no response after 6 months of observation [166]. A similar response pattern was reported from a randomized trial in Korean children and in 66 children followed in the PodoNet registry [159, 167]. However, in an open-label, controlled trial that randomized 31 children with SRNS to either receive rituximab or continue prednisone and calcineurin inhibitors, no subjects in either arm achieved significant reduction of proteinuria. Hence, the efficacy of rituximab in the treatment of SRNS is unclear and there is a need for a randomized control trial [168].

More recently, case reports suggested that Ofatumumab (OFA), a fully humanized anti-CD20 monoclonal antibody, may be useful in inducing remission in patients with hypersensitivity reaction to rituximab or in children who are resistant to multiple immunomodulators including rituximab [169, 170]. However, a recent low-dose ofatumumab randomized placebo-controlled trial was prematurely terminated because the first 13 patients (25% of targeted

enrollment) did not respond to the treatment [171]. Meanwhile, Ofatumumab has been withdrawn from the market.

Newer Therapies and Ongoing Clinical Trials

For children with CNI-resistant SRNS, consideration for entry into clinical trials evaluating novel therapies on the horizon should be strongly considered.

The FONT2 study (Novel Therapies in the Treatment of Resistant FSGS) aimed to compare novel therapies in patients with FSGS that have failed standard immunosuppressive therapies with conservative management [172]. In vitro studies have documented decreases in glomerular permeability when isolated glomeruli were incubated with galactose-containing sera [173]. The proposed mechanism suggests galactose may bind a glomerular permeability factor thus rendering it ineffective. Another proposed mechanism for proteinuria in patients with SRNS implicate Tumor Necrosis Factor-alpha (TNF-α), a pro-inflammatory cytokine that is important in the recruitment of leukocytes to the site of glomerular injury, induction of cytokines and growth factors, generation of oxygen radicals with increased glomerular endothelial cell permeability, cytotoxicity, and induction of apoptosis. In the FONT2 trial, 21 patients were randomized to one of the three study arms to receive the TNF-α antibody **adalimumab, galactose,** or standard medical therapy for 26 weeks. None of the adalimumab-treated subjects achieved the primary outcome of ≥50% reduction in proteinuria, whereas two subjects in the galactose and two in the standard medical therapy arm had a 50% reduction in proteinuria without a decline in eGFR, suggesting that some patients may benefit from treatment with oral galactose [173].

ACTH (Adrenocorticotropic Hormone) was the therapy of choice for children with nephrotic syndrome in the 1950s before corticosteroids became widely available [174, 175]. The development of an ACTH analog has made this therapy available once again as a second line agent in the treatment of SRNS. The largest published series to date by Hogan et.al reports a cumulative remission rate of 29% in 24 patients with SRNS and SDNS treated with subcutaneous ACTH [176]. In a recent systematic review that included 98 patients with FSGS, 42% achieved remission following treatment with ACTH. However, it should be noted that the population comprised frequently relapsing, steroid dependent, and steroid resistant patients [177].

Sparsentan, a dual endothelin and ARB was found to decrease proteinuria by 45% vs. 19% in a phase 2 randomized double-blind trial of those treated only with irbesartan with no differences in serious adverse events between the groups [178]. A phase-3 multicenter trial is in progress.

A small post-approval study for **low-density lipoprotein (LDL) apheresis** for children with CNI-resistant SRNS has shown increased treatment responsiveness and improved or stable eGFR over the follow-up period, it should be noted that this was not a randomized control trial [179].

Common variants in APOL1 gene termed G1 and G2 account for a significant proportion of the excess risk of progressive kidney disease in individuals of recent African ancestry with an estimated lifetime risk of kidney disease in 15% in those with a high-risk genotype [180, 181]. Novel **APOL1 inhibitors** are currently in clinical development. A Phase II trial investigating the APOL1 inhibitor VX-147 has started recruitment [182]. Antisense oligonucleotides are short, synthetic, modified chains of nucleotides that bind to the target mRNA, inducing its degradation and preventing the mRNA from being translated into a detrimental protein product. Preclinical studies with antisense oligonucleotides are showing promise as a novel therapeutic approach for APOL1 associated nephropathy [183, 184].

Treatment of Monogenic SRNS

Increased availability of genetic testing for children with SRNS has enabled the development of more personalized treatment decisions. The clinical value of genetic testing in SRNS is illustrated by our ability to make decisions on the intensity and duration of immunosuppression, as well as pre- and post-transplant management based on genomic findings [8, 9, 185, 186]. Generally, >95% of patients with monogenic SRNS will not respond to treatment with immunomodulatory agents [121, 159], and hence it is recommended to withdraw immunosuppressive therapy. RAS inhibitors should be administered at maximally effective and tolerated doses. There are anecdotal reports of individuals with mutations in *WT1, PLCE1, and MYO1E* who achieved partial or complete remission when exposed to immunosuppressive treatments, in particular calcineurin inhibitors [64, 69, 121, 187]. It is unclear if these responses, which are usually transient, are due to immunomodulatory effects or rather to their effects on stabilizing the podocyte cytoskeleton.

One of the promises of the genomic revolution is that identification of genetic causes of SRNS will lead to identification of specific and non-toxic therapeutic agents. Along this line, some monogenic causes of SRNS have given clue to novel therapeutic agents. An intriguing example is Coenzyme Q10 (CoQ10) supplementation in patients with mutations in genes encoding for components of the CoQ10 synthase complex (COQ6, COQ2, COQ8B) [67, 139, 188]. Other examples are Vitamin B12 supplementation in patients with mutations in the cubilin (CUBN) gene, and targeting of *TRPC6*, *TRPC5*, and RhoGTPases for potential treatment of some form of monogenic SRNS [62, 189, 190].

Long-Term Prognosis of SRNS

Most studies examining the long-term prognostic factors for kidney survival in patients with SRNS were from small cohorts, frequently single-center studies, with short term follow up, and often incomplete datasets [191, 192]. A multi-center study of 75 children with FSGS reported that within 5 years from the diagnosis of FSGS, 21% of children had developed ESKD, 23% had developed CKD, and 37% had developed persistent proteinuria, while only 11% remained in remission [30]. The most comprehensive study to date has been performed by the PodoNet consortium [159]. In this study, clinical, treatment-related, genetic, and laboratory data including kidney biopsy findings were collected from >1300 patients with SRNS with an average follow up time of about 4 years but extending up to 15 years. The overall proportion of SRNS patients with preserved kidney function was 74%, 58%, and 48% at 5, 10, and 15 years respectively. Risk factors for disease progression included lacking responsiveness to intensified immunosuppression (IIS) protocols, genetic disease, and FSGS on biopsy. Ten-year ESKD-free survival rates were 94%, 72%, and 43% in patients with complete remission, partial remission, and IIS resistance respectively (Fig. 14.5). After 15 years, kidney function was preserved in 96% of IIS-responsive, 53% of multidrug resistant and 17% of genetic SRNS patients. The histopathological findings at time of diagnosis were also predictive of outcome but less so than IIS responsiveness and genetic disease status, with 37% 15-year ESKD-free survival in patients with FSGS as compared to 79% in those with minimal change disease. The predictive value of IIS responsiveness and genetic status was independent of the histopathological diagnosis.

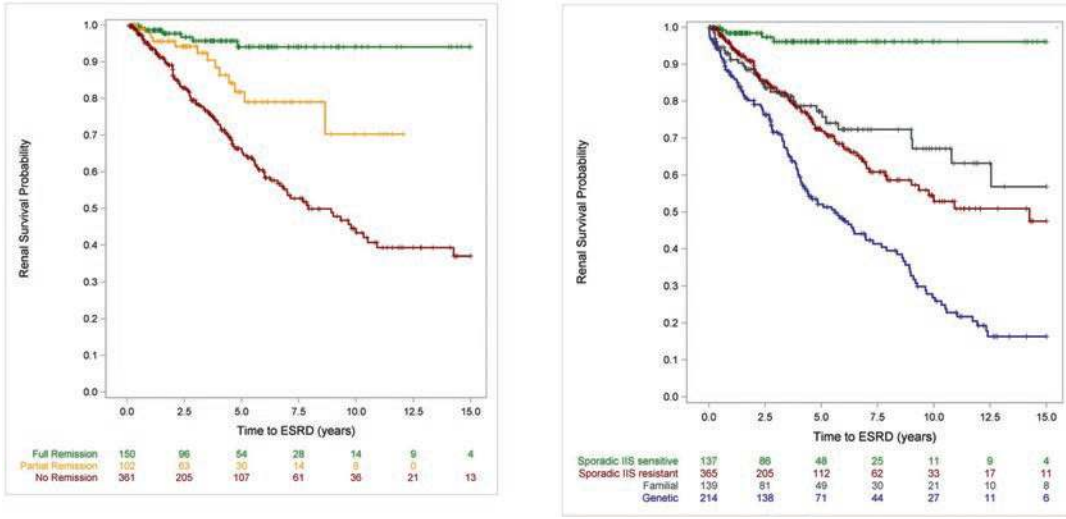

Fig. 14.5 ESKD-free survival in children with SRNS followed in PodoNet Registry. *Left panel:* Survival according to responsiveness to calcineurin inhibitor therapy (green; full remission, yellow; partial remission, red; no remission). *Right panel*: ESKD-free survival according to responsiveness to intensified immunosuppression (IIS) and genetic familial disease status (green: IIS responsive sporadic SRNS, red: multidrug resistant SRNS; grey: familial SRNS without identified genetic cause; blue: genetic SRNS). Reproduced from [159] with permission

Complications of Nephrotic Syndrome

Hyperlipidemia

Hyperlipidemia is a common clinical finding in children with nephrotic syndrome. The characteristic lipid profile includes elevations in total plasma cholesterol, very low-density lipoprotein (VLDL), and low-density lipoprotein (LDL) cholesterol, triglyceride, lipoprotein A, as well as variable alterations (more typically decreased) in high-density lipoprotein (HDL) cholesterol [193, 194]. While the hyperlipidemia in children with SSNS is often transient and usually returns to normal after remission, children with SRNS refractory to therapy often have sustained hyperlipidemia. Such chronic hyperlipidemia has been associated with an increased risk for cardiovascular complications and progressive glomerular damage in adults [195–199]. Based on this, pharmacologic treatment of hyperlipidemia in children with refractory nephrotic syndrome may both reduce the risk for cardiovascular complications later in life and reduce the risk of disease progression.

The potential usefulness of hydroxymethylglutaryl CoA (HMG CoA) reductase inhibitors (statins) in children with SRNS has been reported in a few uncontrolled trials. One study reported a 41% reduction in cholesterol and 44% reduction in triglyceride levels within 6 months of treatment [200]. A second study found significant reductions within 2–4 months in total cholesterol (40%), LDL cholesterol (44%), and triglyceride (33%) levels, but no significant changes in HDL cholesterol levels [201]. Treatment was found to be very safe in these studies, with no associated adverse clinical or laboratory events. Although the long-term safety of statins in children has not yet been established, these medications appear to be generally well tolerated in adults with nephrotic syndrome, with only minor side effects such as asymptomatic increases in liver enzymes, creatine kinase, and rarely diarrhea [202].

Thrombosis

The risk of thromboembolic events in children with nephrotic syndrome is estimated to be 1.8–5% with a higher risk reported in children with SRNS compared with those with SSNS [203, 204]. Factors contributing to an increased risk of thrombosis during nephrotic syndrome include abnormalities of the coagulation cascade, such as increased clotting factor synthesis in the liver (factors I, II, V, VII, VIII, X, and XIII) and loss of coagulation inhibitors such as anti-thrombin III in the urine. Other prothrombotic risks present in these children include increased platelet aggregability (and sometimes thrombocytosis), hyperviscosity resulting from increased fibrinogen levels, hyperlipidemia, prolonged immobilization, and the use of diuretics. In one series, the use of diuretics was the major iatrogenic risk factor for thrombosis [204].

The majority of episodes of thrombosis are venous in origin. The most common sites for thrombosis are the deep leg veins, ileofemoral veins, and the inferior vena cava. In addition, use of central venous catheters can further increase the risk of thrombosis. Renal vein thrombosis (RVT) can also occur and may manifest as gross hematuria with or without acute renal failure. Development of these features should prompt either renal Doppler ultrasonography or magnetic resonance angiography to rule out RVT. Pulmonary embolism is another important complication that may be fatal if not recognized early. Rarely, cerebral venous thrombosis, most commonly in the sagittal sinus, has also been reported [205]. In addition to imaging studies, development of thrombosis should prompt an evaluation for possible inherited hypercoagulable states.

The typical acute management of thrombosis in children with nephrotic syndrome includes initial heparin infusion or low molecular weight heparin, followed by transition to warfarin for 6 months. Children with a history of prior thrombosis and patients with severe proteinuria should also receive prophylactic anticoagulation therapy during future relapses.

Nutrition

Several recommendations supported by observational data exist regarding nutrition in pediatric patients with nephrotic syndrome. Specifically, children with nephrotic syndrome and edema should be evaluated for malabsorption and subsequent malnutrition due to bowel wall edema. In edematous patients, long-term sodium restriction is appropriate with a maximum goal of approximately 2500 mg/day. In patients with persistent hyperlipidemia due to inability to control nephrosis, a low saturated fat diet should be instituted with their HMG CoA-reductase inhibitor. Protein intake should only be supplemented at the Recommended Daily Allowance (RDA) [206]. Although it would appear intuitive that states of excess urinary protein loss should warrant increase dietary protein intake, several studies have successfully challenged this notion. In nephrotic rats, augmentation of dietary protein was found to stimulate albumin synthesis by increasing albumin mRNA content in the liver, but there was also a notable increase in glomerular permeability and subsequently increased albuminuria [207]. No change in albumin synthesis was noted with dietary protein restriction in this model or in nephrotic patients.

Immunization

Children with nephrotic syndrome are at increased risk for infections, including but not limited to streptococcus and staphylococcal species due to urinary losses of IgG, loss of factors crucial for regulation of the alternative complement pathway, and large fluid collections prone to breeding bacteria. Live-viral vaccines (rotavirus vaccine, varicella vaccine, measles, mumps, and rubella vaccine, and the live-attenuated influenza vaccine) are generally recommended to be avoided in CKD patients who are immunosuppressed and therefore should be avoided in patients that are frankly nephrotic and/or currently receiving immunosuppressive therapy. Anti-pneumococcal vacci-

nation using the 23-valent polysaccharide vaccine (PPSV23) is recommended for children with nephrotic syndrome [208]. Due to the low immunogenicity of this vaccine in children less than 2 years of age, the 13-valent polysaccharide pneumococcal vaccine (PCV13) is recommended in this age group, followed by supplemental immunization with PPSV23 over the age of 2 years at least 8 weeks after the final dose of PCV13. A second dose of PPSV23 should be repeated in 5 years.

Kidney Transplantation

Recurrence of nephrotic syndrome may occur in up to 30% in the first kidney allograft of patients with ESKD due to FSGS and approach 100% in those who have a history of prior allograft loss due to FSGS. The risk post-transplant recurrence appears to be mainly determined by the underlying disease etiology, i.e. immune-mediated vs. genetic. Whereas patients with secondary steroid resistance have about 80% risk of recurrence, the risk appears to be close to zero in patients with genetic forms of SRNS [7–9, 139]. Hence, genetic testing should be considered mandatory in all children with SRNS considered for kidney transplantation.

In addition, young age, mesangial proliferation in the native kidneys, rapid progression to ESKD, pre-transplant bilateral nephrectomy, and white ethnicity have been associated with increased risk of recurrence post-transplant [209, 210]. The histologic variant type of FSGS does not appear to be predictive of disease recurrence. There is a higher risk of recurrence in living donor transplant pediatric recipients; however, the reduced risk of rejection and a lower immunosuppression in living-related transplants may overcome the deleterious effect of recurrent glomerulonephritis [211].

The management of recurrent FSGS disease remains controversial and results from observational reports vary. The implementation of plasma exchange is supported in part by the idea of a circulating permeability factor. Up to 70% of children with recurrent FSGS treated with repeated plasma exchanges and/or rituximab may achieve at least a partial remission.

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Hereditary Nephrotic Syndrome

15

Stefanie Weber

Introduction

During the past two decades defects in various genes have been associated with the development of steroid resistant nephrotic syndrome (SRNS) in children and adults. These genes encode for proteins that participate in the development and structural architecture of glomerular visceral epithelial cells (podocytes). These insights moved the podocyte with its interdigitating foot processes and slit diaphragm (SD) into the center of interest regarding the pathophysiology of proteinuria.

While light microscopy shows variable aspects ranging from minimal change nephropathy to diffuse mesangial sclerosis or focal-segmental glomerulosclerosis (Fig. 15.1a, b), all hereditary proteinuria syndromes share a common phenotype when evaluated by electron microscopy, which uniformly demonstrates the typical flattening of the foot processes and loss of the SD. With respect to the clinical course, different entities can be distinguished, especially referring to the onset of the disease and modi of inheritance. Disorders of early glomerular development most often manifest prenatally, directly after birth or in early infancy. Disorders with late-onset nephrotic syndrome typically manifest as FSGS in adolescence or adulthood, frequently following an autosomal-dominant mode of inheritance with incomplete penetrance and variable expression. In rare cases, extrarenal symptoms are associated with hereditary nephrotic syndrome, e.g., in Denys-Drash, Frasier, Schimke and Pierson syndrome as well as in mitochondrial disorders. In the following, important genes involved in hereditary nephrotic syndrome will be discussed. Given the rapid development in the field, this list is comprehensive, though may not be complete.

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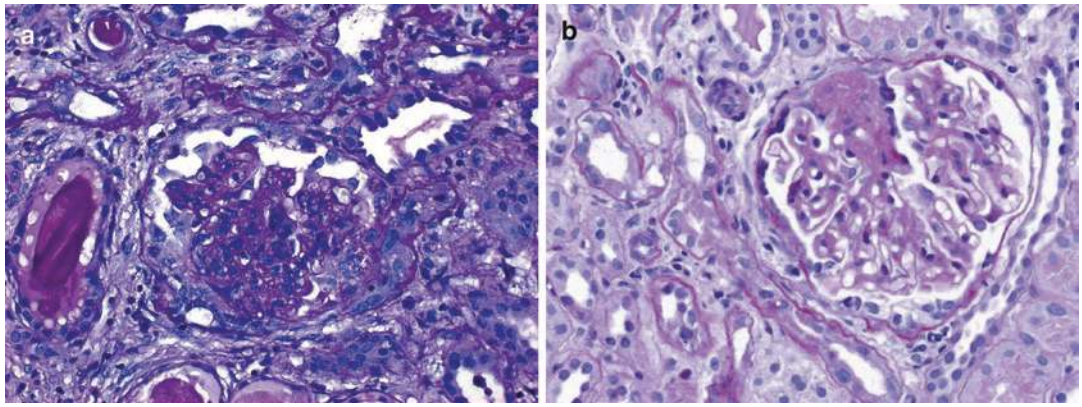

Fig. 15.1 (**a**, **b**) Kidney histology of a patient with diffuse mesangial sclerosis (**a**) and focal-segmental glomerulosclerosis (**b**), respectively. PAS staining; magnification 40×. (Courtesy of Kerstin Amann, Institute of Pathology, Department of Nephropathology, University Hospital Erlangen, Germany)

Hereditary Disorders of Early Glomerular Development

Podocytes develop from the nephrogenic blastema in a chain of events in conjunction with the development of the renal glomeruli. First, local condensation of the mesenchyme leads to the formation of the nephron anlage, i.e., the comma-shaped and the S-shaped bodies and eventually the formation of the mature glomerulus. Podocytes are the first cells that can clearly be distinguished in this process, forming a disk-like layer of epithelial cells. The subsequent differentiation to mature podocytes with interdigitating primary and secondary foot processes is associated with a general loss of the ability for further proliferation. At this stage, early cell-cell contacts (adherens junctions) have developed into a specialized structure, the SD, spanning the intercellular space. The final glomerular filtration barrier is constituted by the fenestrated endothelium, the glomerular basement membrane (GBM) and interdigitating podocytes.

A number of genes are involved in these processes (Table 15.1 and Fig. 15.2), and *WT1* is one of the major mediators of podocyte differentation. *NPHS1* and *NPHS2* code for nephrin and podocin, respectively, two proteins that have important roles for the organization of the SD. *LAMB2* encodes laminin ß2, one component of the heterotrimeric laminins that link the podocyte to the GBM. *LMX1b* encodes the transcription factor Lmx1b that in the kidney is exclusively expressed in podocytes. It is one of the crucial genes regulating gene expression during early steps of podocyte development. Another group of genes involved in early-onset nephrotic syndrome, e.g. *PLCE1*, encoding for phospholipase C epsilon-1, are involved in podocyte signaling processes.

WT1 Gene Mutations

The *Wilms tumor* is one of the most common solid tumors of childhood, occurring in 1 of 10,000 children and accounting for 8% of childhood cancers. The Wilms' tumor suppressor gene (*WT1*) was first identified in 1990 [1]. *WT1* locates on chromosome 11p13 and encodes a zinc finger transcription factor that regulates the expression of many genes during kidney and urogenital development. Mutations in *WT1* were first identified in pediatric patients affected by Wilms'

Table 15.1 Overview on important disorders causing hereditary nephrotic syndromes

	Inheritance	Locus	Gene	Protein	OMIM accession no.
Early-onset nephrotic syndrome					
Isolated DMS	AR	11p13	*WT1*	WT1	256370
Denys-Drash syndrome (typically DMS)	AD	11p13	*WT1*	WT1	194080
Congenital nephrotic syndrome/Finnish type	AR	19q13	*NPHS1*	Nephrin	602716
Recessive familial SRNS (MC/FSGS)	AR	1q25	*NPHS2*	Podocin	600995
Pierson syndrome	AR	3p21	*LAMB2*	Laminin β2	609049
Nail-patella syndrome	AD	9q34.1	*LMX1B*	LMX1B	161200
Recessive nephrotic syndrome (DMS/FSGS)	AR	10q23-q24	*NPHS3/PLCE1*	PLCE1	608414
Recessive nephrotic syndrome (FSGS)	AR	12p12.3	*PTPRO*	PTPRO	614196
Recessive congenital nephrotic syndrome (DMS)	AR	17q25.3	*ARHGDIA*	ARHGDIA	615244
Recessive childhood-onset nephrotic syndrome	AR	16p13.13	*EMP2*	EMP2	602334
NUP nephropathy	AR	multiple loci	*NUP93, NUP205, XPO5, NUP107, NUP85, NUP133, NUP160*	NUP93, NUP205, XPO5, NUP107, NUP85, NUP133, NUP160	multiple accession nos.
Late-onset nephrotic syndrome/FSGS					
Frasier syndrome (typically FSGS)	AD	11p13	*WT1*	WT1	136680
FSGS1	AD	19q13	*ACTN4*	α-Actinin 4	603278
FSGS2	AD	11q21-22	*TRPC6*	TRPC6	603965
FSGS3 (*CD2AP*-associated disease susceptibility)	AR/AD	6	*CD2AP*	CD2AP	607832
FSGS4 (*MYH9*-associated disease susceptibility)	AD	22q12.3	*MYH9/APOE1*	MYH9/APO1	612551
FSGS5	AD	14q32.33	*INF2*	INF2	613237
FSGS6	AR	15q22.2	*MYO1E*	MYO1E	614131
Mitochondrial disease					
Early-onset SRNS with variable extrarenal symptoms	AR	4q21-q22	*COQ2*	COQ2	609825
Early-onset SRNS with sensorineural deafness	AR	14q24.3	*COQ6*	Q10 mono-oxigenase 6	614647
SRNS/FSGS	AR	19q13.2	*ADCK4*	ADCK4	615573
Syndromal disease					
Schimke immuno-osseous dysplasia	AR	2q34-q36	*SMARCAL1*	SMARCAL1	242900
Galloway-Mowat Syndrome (GAMOS)	AR	15q25.2	*WDR73*	WDR73	616144
	AR	14q11.2, 20q13.12 2p13.1	*OSGEP, TP53RK, TPRKB*	OSGEP, TP53RK, TPRKB	617729 617730 617731
	X-linked	Xq28	*LAGE3*	LAGE3	301006

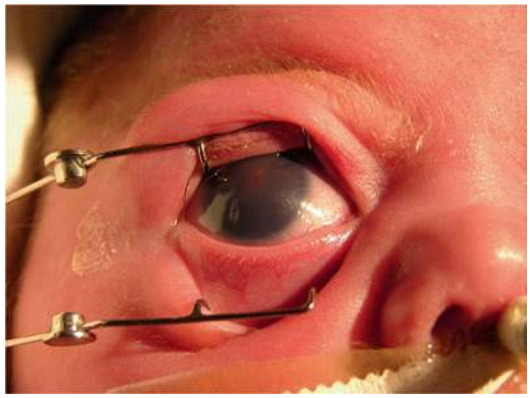

Fig. 15.2 Typical aspect of a patient with Pierson syndrome and microcoria. (Courtesy of Kveta Blahova, Pediatric Clinic, Charles University, Prague, Czech Republic)

tumor, aniridia, genitourinary malformations and mental retardation (WAGR syndrome), a contiguous gene deletion syndrome including *WT1* and *PAX6* [2]. *WT1* mutations were also identified in patients with isolated Wilms' tumor [3]. These are classically truncating mutations, associated with a complete loss-of-function of WT1. In tumor material of isolated cases both germline and somatic mutations have been detected. Familial Wilms tumor forms seem to follow a dominant pattern of inheritance, with dominant germline mutations. However, in a number of these cases the classical two-hit inactivation model, with loss of heterozygosity due to a second somatic event, has been described as the underlying cause of tumor development [4]. Alternative genes involved in familiar Wilms tumors are *CTR9*, *REST* and multiple others.

Subsequently, *WT1* mutations were also associated with Denys-Drash syndrome (DDS) [5], Frasier syndrome (FS) [6], and diffuse mesangial sclerosis (DMS) with isolated nephrotic syndrome (NS) [7]. The full picture of autosomal dominant *Denys-Drash syndrome* is characterized by early onset NS, male pseudohermaphroditism, gonadal dysgenesis and the development of Wilms tumor (in more than 90% of patients). The Wilms tumor may precede or develop after the manifestation of NS.

Of note, besides the Wilms tumor risk, individuals with 46,XY and associated disorders of sexual development (DSD) or complete gonadal dysgenesis (CGD) are at an increased risk for germ cell tumors, particularly gonadoblastoma with an observed incidence of one per 30 years at risk.

Age at onset of NS is generally within the first months of life [8]. In rare cases enlarged and hyperechogenic kidneys were already demonstrated by prenatal ultrasound [9]. Renal histology typically presents with DMS [10] and electron microscopy reveals foot process effacement. In rare cases, a histology reminiscent of MPGN may be observed. The NS is resistant to steroid treatment and renal function is deteriorating rapidly to end-stage renal disease (ESRD) already during infancy. Bilateral nephrectomy is generally advised in ESRD in order to prevent the development of Wilms tumor [11]. Recurrence of NS after kidney transplantation has not been observed so far [12].

Dominant *WT1* mutations are identified in the vast majority of DDS patients. These mutations predominantly affect exons 8 and 9 of the *WT1* gene and most of them are de novo mutations not observed in the parents. Most *WT1* mutations associated with DDS are missense mutations affecting conserved amino acids of the zinc finger domains, with p.R394W being the most frequent mutation observed. These alterations of the zinc finger structure reduce the DNA binding capacity of the *WT1* protein [13]. A heterozygous knock-in mouse model has been created for the p.R394W missense mutation, presenting with DMS and male genital anomalies [14] supporting the dominant nature of the disease.

Of note, some of the patients affected by *WT1* mutations in exons 8 and 9 do not present with the full picture of DDS but with isolated DMS or isolated (steroid-resistant) proteinuria. *WT1* analysis should therefore be performed in all children with isolated DMS and early-onset NS because of the risk of Wilms tumor development in case of a positive mutation analysis result. Close monitoring by renal ultrasound (e.g., every 6 months) is important in all children with *WT1* mutation and early-onset NS. In addition, karyotype analysis is recommended in all girls with isolated DMS to detect a possible male pseudohermaphrodit-

ism. Some patients with isolated DMS present with recessive mutations in *WT1* with both the maternal and paternal allele being affected [7].

Frasier syndrome is also characterized by a progressive glomerulopathy and male pseudohermaphroditism [15]; however, there are specific differences to DDS: the onset of proteinuria occurs later in childhood and the deterioration of renal function is slower. ESRD develops only in the second or third decade of life. As in DDS, proteinuria and NS are steroidresistant. Renal histology in FS patients typically shows focal and segmental glomerulosclerosis (FSGS) [16]; in a minority of patients only minimal change lesions are observed. In female patients, the genitourinary tract is normally developed, whereas a complete sex reversal with gonadal dysgenesis is observed in 46,XY patients. Primary amenorrhoea in conjunction with NS is a typical feature of these 46,XY patients and should prompt molecular analysis of *WT1*. While the risk to develop a Wilms tumor is low in patients with FS, gonadoblastomata, developing from gonadal dysgenesis, are frequently observed (in up to 40% of FS patients). After the diagnosis of FS, gonadectomy is highly recommended in 46,XY patients.

In 1997, it was first demonstrated that mutations in the *WT1* gene also underly the pathogenesis of FS [6]. Notably, the class of mutations in FS classically differs from DDS: whereas mutations affecting the coding sequence of exons 8 and 9 cause typical DDS, mutations associated with FS represent donor splice-site mutations located in intron 9. Similar to DDS, these mutations occur in a heterozygous state and, frequently, they are de novo mutations not observed in the parents. The donor splice-site of intron 9 plays an important role for the generation of the KTS isoform of the WT1 protein. This isoform contains three additional amino acids (lysine-threonine-serine; KTS). It has been demonstrated that the (+) KTS/(−) KTS protein dose ratio is of high relevance for WT1 action during genitourinary and kidney development. In FS patients, this ratio is markedly reduced due to the splice-site mutations [6].

Large genotype-phenotype studies confirm these associations based on the nature of the underlying mutation [17]. Still, there is some overlap and phenotypic heterogeneity in selected cases: splice-site mutations typical for FS may in some cases be found in patients with DDS [5] or isolated DMS [18], and patients with typical DDS mutations may display with isolated FSGS [19] or Wilms' tumor without NS [20]. Since the histologic findings often do not correlate with clinical findings and remarkable histopathologic heterogeneity is observed even among individuals with the same *WT1* pathogenic variant, kidney biopsy is no longer considered a first-tier diagnostic measure for patients of any age in the view of some authors. Instead, analysis of *WT1* should be included in routine genetic screening in all patients with SRNS or unexplained proteinuria. Owing to the experience of phenotypic heterogeneity, the group of clinical pictures is now entitled **WT1 disorder**s.

NPHS1 Gene Mutations Associated with Autosomal Recessive CNS of the Finnish Type (CNF)

CNS of the Finnish type is characterized by autosomal recessive inheritance and the development of proteinuria in utero [21]. The responsible gene was mapped in 1994 to chromosome 19q13 [22] and mutations in *NPHS1* have been subsequently identified in affected children [23]. *NPHS1* encodes for nephrin, a zipper-like protein of the glomerular SD. Typically, severe NS manifests before 3 months of age and renal biopsy specimens show immature glomeruli, mesangial cell hypercellularity, glomerular foot process effacement and microcystic dilatations of the proximal tubules. NS is steroid-resistant in these patients and treatment options comprise albumin infusions, pharmacological interventions with ACE inhibitors and indomethacin and ultimately uni- or bilateral nephrectomy [24–27].

Nephrin is exclusively expressed in podocytes, at the level of the SD once full differentiation has occurred [28]. Nephrin belongs to the immunoglobulin superfamily with a single putative transmembrane domain, a short intracellular N-terminus and long extracellular C-terminus

[23]. The extracellular C-terminus is predicted to bridge the intercellular space between the interdigitating foot processes, rendering nephrin a key component of the SD. Nephrin strands contribute to the porous structure of the SD, forming pores of approximately 40 nm in size [29]. These pores are currently believed to be in part responsible for the size selectivity of the SD and the glomerular filtration barrier.

Apart from its role as a structural protein, nephrin also appears to participate in intracellular signaling pathways maintaining the functional integrity of the podocyte [30–32]. The SD is discussed to constitute a highly dynamic protein complex that recruits signal transduction components and initiates signaling to regulate complex biologic programs in the podocyte. A number of proteins within this signaling platform were identified to interact with nephrin, among these podocin, CD2AP and TRPC6, all of which are also associated with the development of NS when altered by gene mutations (see below). It is suggested that the plasma membrane of the filtration slit has a special lipid composition comparable to lipid rafts [33]. Lipid rafts are specialized microdomains of the plasma membrane with a unique lipid content and a concentrated assembly of signal transduction molecules [34]. It was shown that nephrin is a lipid raft-associated protein at the SD and that podocin serves to recruit nephrin into these microdomains. Disease-causing podocin mutations fail to target nephrin into rafts, altering nephrin-induced signal transduction [32]. In summary, these studies confirm the extraordinary role of SD proteins for the maintenance of the glomerular filtration barrier.

Mutations in *NPHS1* were first identified in the Finnish population, leading to the classification of "Finnish type" CNS. Two truncating mutations were found with high frequency in affected Finnish children suggesting an underlying founder effect in the Finnish population: p.L41fsX90 (Fin major, truncating the majority of the protein) and p.R1109X (Fin minor, truncating only a short C-terminal part). In subsequent studies, *NPHS1* mutations were also identified in non-Finnish patients throughout the world. The Fin major and Fin minor mutations are only rarely observed in non-Finnish patients. Several mutational hot spots were identified affecting the immunoglobulin domains of the nephrin protein [35]. The immunoglobulin domains 2, 4 and 7 appear particularly important for gene function. In addition to the high prevalence in Finland, *NPHS1* mutations are also common among Mennonites in Pennsylvania, 8% of this population is carrier of a heterozygous mutant allele [36].

Recent studies pointed out that congenital nephrotic syndrome can also be caused by recessive mutations in *NPHS2* (see below), particularly involving nonsense-, frameshift or the homozygous missense mutation p.R138Q [37–39]. In rare cases a triallelic digenic mode of inheritance was observed in patients with CNS/SRNS: in these patients, sequence variations in both *NPHS1* and *NPHS2* were identified with a total of three affected alleles (two *NPHS1* mutations and one *NPHS2* sequence variation or vice versa) [35]. It is speculated that the additional sequence variation of the second gene plays a role as a genetic modifier, possibly aggravating the clinical phenotype.

NPHS2 Gene Mutations Associated with Autosomal Recessive Steroid-Resistant Nephrotic Syndrome

The *NPHS2* gene was mapped by linkage analysis in eight families with autosomal recessive SRNS to chromosome 1q25-q31 [40] and recessive mutations in *NPHS2* were identified subsequently [41]. NS in these families was characterized by steroid-resistance, age at onset between 3 and 5 years and no recurrence of proteinuria after renal transplantation. *NPHS2* mutations have never been reported in patients with SSNS. Renal histology typically shows FSGS; however, some patients present with only minimal change lesions. In some cases, progression from minimal change lesions to FSGS has been demonstrated in repeat biopsies.

NPHS2 encodes for podocin, a 42 kD integral membrane protein expressed in both fetal and mature glomeruli [41]. By electron microscopy

and immunogold labeling it was demonstrated that the site of expression is the SD of the podocytes. As both protein termini are located in the cytosol and podocin is predicted to have only one membrane domain, a hair-pin like structure of the protein was proposed. Interacting with both nephrin and CD2AP, podocin appears to link nephrin to the podocyte cytoskeleton. In patients affected by recessive mutations in *NPHS2*, SD formation is impaired and the typical foot process effacement is visible. These observations suggest that podocin has an important function for maintaining the glomerular filtration barrier. The knock-out of *Nphs2* in mice is associated with a phenotype highly reminiscent of the human disease with podocyte foot process effacement, nephrotic range proteinuria and chronic renal insufficiency [42]. As nephrin, podocin is localized in lipid rafts [33] and is important for recruiting nephrin to these microdomains of the plasma membrane [32]. Some mutations in *NPHS2* impair the ability of podocin to target nephrin to the rafts, especially the most frequent mutation identified in European patients (p.R138Q) [32].

Up to now, more than 125 pathogenic mutations have been described in *NPHS2* [43]; most mutations affect the stomatin domain located in the C-terminal part of the protein [37, 44]. Mutations in *NPHS2* were first identified in infants with SRNS and rapid progression to ESRD [41]. Subsequently, however, it became evident that defects in podocin can be responsible for SRNS manifesting at any age from birth to adulthood [45–47]. A partial genotype-phenotype correlation is apparent: while frameshift-, nonsense- and the p.R138Q mutation in homozygosity are typically associated with early-onset NS, other missense mutations (e.g., p.V180M, p.R238S) are predominantly found in patients with a later onset of SRNS [37]. A single nucleotide polymorphism in *NPHS2* (p.R229Q) has been identified to be a common cause of adulthood-onset of hereditary nephrotic syndrome when present in compound heterozygosity with specific pathogenic *NPHS2* mutations [48–50]. In the study of Machuca et al., among 119 patients diagnosed with NS presenting after 18 years of age, 18 patients were found to have one pathogenic mutation and p.R229Q, but none with two pathogenic mutations in *NPHS2* [50]. Screening for the p.R229Q variant seems therefore recommended in adolescent or adult patients. Recent data demonstrated that the pathogenicity of p.R229Q depends on the *trans*-associated mutation. It was shown that the association of *NPHS2*-p.R229Q with specific exon 7 or exon 8 mutations in *NPHS2* altered heterodimerization and mislocalisation of the encoded p.Arg229Gln podocin protein [51]. Following this study, homozygosity of *NPHS2*-p.R229Q alone is not disease causing. This observation is important as the p.R229Q variant is prevalent in heterozygous state in approximately 3% of the normal population (range 0.5–7%, depending on the genetic background) [51], resulting in a frequency of homozygous carriers of up to 1%.

Still, the p.R229Q variant is discussed to be a non-neutral PM with an enhanced frequency in FSGS patient cohorts of different ethnical origins [52]. In vitro studies have demonstrated that p.R229Q podocin shows decreased binding to its interacting protein partner nephrin [48] and in a large study of more than 1500 individuals of the general population p.R229Q was significantly associated with the prevalence of microalbuminuria, a risk factor for developing chronic renal insufficiency and cardiovascular events [53].

LAMB2 Gene Mutations Associated with Pierson Syndrome

Pierson syndrome is characterized by CNS and peculiar eye abnormalities including a typical nonreactive narrowing of the pupils (microcoria, Fig. 15.3) but also additional lens and corneal abnormalities [54]. Recessive mutations in *LAMB2* on chromosome 3p21 were identified as underlying genetic defect [55]. *LAMB2* encodes for the protein laminin β2, one component of the trimeric laminins in the kidney that crosslink the basolateral membrane of the podocyte to the GBM. Most disease-associated alleles identified in Pierson patients were truncating mutations leading to loss of laminin β2 expression in the

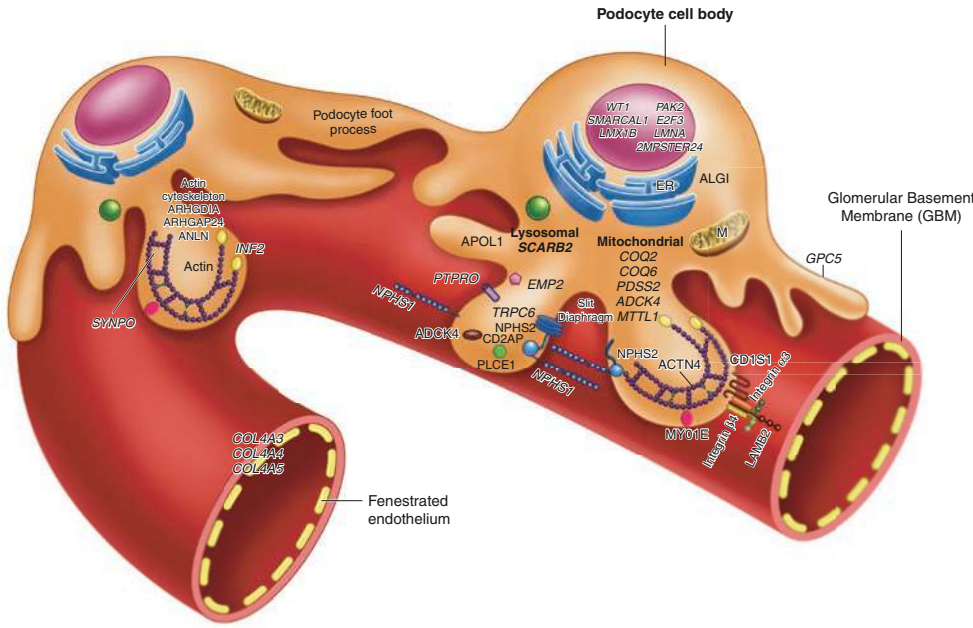

Fig. 15.3 Schema of a podocyte foot process cross-section, depicting important components involved in hereditary nephrotic syndrome

kidney [55]. Ocular laminin β2 expression in unaffected controls was strongest in the intraocular muscles, corresponding well to the characteristic hypoplasia of ciliary and pupillary muscles observed in affected patients. Subsequent genotype-phenotype studies revealed that some mutations in *LAMB2*, especially hypomorphic missense mutations, can be associated with a phenotypic spectrum that is much broader than previously anticipated including isolated CNS or CNS with minor ocular changes different from those observed in Pierson syndrome [56]. Fetal ultrasound in four consecutive fetuses of a family with Pierson syndrome and positive *LAMB2* mutation analysis consistently revealed marked hyperechogenicity of the kidneys and variable degrees of pyelectasia by 15 weeks of gestation [57]. Placentas were significantly enlarged. Hydrops fetalis due to severe hypalbuminemia demonstrated by chordocentesis occurred in one fetus and anencephaly was detected in another fetus. Development of oligohydramnios indicated a prenatal decline of renal excretory function. From these studies it can be concluded that mutational analysis in *LAMB2* should also be considered in isolated CNS if no mutations were found in *NPHS1*, *NPHS2*, or *WT1*, and in cases with prenatal onset of nephrotic disease with typical sonomorphologic findings of the kidneys and the development of oligohydramnios.

The *LAMB2* missense mutation p.C321R, for example, has been identified in congenital nephrotic syndrome with only mild extrarenal symptoms. Functional studies in cell culture and mice suggested defective intracellular trafficking of the mutant protein, associated with endoplasmatic reticulum stress [58]. Another missense mutation (p.S80R) has been identified in homozygous state a teenage girl with severe myopia since early infancy and nephrotic range proteinuria first detected at the age of 6 but normal renal function. Renal biopsy revealed mild DMS and a residual expression of laminin β2 [59].

Summarizing these reports, it becomes obvious that the phenotype associated with pathogenic *LAMB2* mutations can be very variable and that genetic testing for *LAMB2* mutations should be considered in all patients with either early-onset proteinuria or glomerular proteinuria with an abnormal ocular phenotype.

LMX1b Gene Mutations Associated with Autosomal Dominant Nail-Patella Syndrome

Nail-patella syndrome (NPS) or onychoosteodysplasia is caused by dominant mutations in the *LMX1b* gene, located on chromosome 9q34.1 and encoding the LIM-homeodomain protein Lmx1b. Lmx1b plays a central role in dorsal/ventral patterning of the vertebrate limb and targeted disruption of Lmx1b results in skeletal defects, including hypoplastic nails, absent patellae, and a unique form of renal dysplasia [60]. Prominent features of affected children are dysplasia of nails and absent or hypodysplastic patellae. In many patients, also iliac horns, dysplasia of the elbows, glaucoma and/or hearing impairment are detected. *LMX1b* is highly expressed in podocytes and patients can also present with an involvement of the kidney comprising proteinuria, nephrotic syndrome or renal insufficiency. Overall, nephropathy is reported in approximately 40% of affected patients (microalbuminuria or overt proteinuria) [61] but ESRD in less than 10% [62]. Interestingly, renal involvement appears significantly more frequent in females and in patients with a positive family history of NPS nephropathy [61]. In NPS patients with renal involvement, electron microscopy shows collagen fibril-like deposition in the GBM with typical lucent areas [63]. These characteristic ultrastructural changes can even be present in patients without apparent nephropathy [64]. Large genotype-phenotype studies demonstrated that individuals with an *LMX1B* mutation located in the homeodomain showed a significantly higher frequency of renal protein loss and higher values of proteinuria than subjects with mutations in the LIM domains [61]. Recent studies identified *LMX1B* missense mutations affecting residue p.R246 also in a subset of patients with isolated FSGS without extrarenal symptoms, expending the spectrum of FGSG-related genes to *LMX1B* [65, 66]. Insight into Lmx1b function was further obtained by the generation of *Lmx1b* knockout animals [67]. In *Lmx1b*(−/−) mice the expression of GBM collagens is reduced and podocytes have a reduced number of foot processes, are dysplastic, and lack typical SD structures. Interestingly, mRNA and protein levels for CD2AP and podocin are greatly reduced in these kidneys and several *LMX1B* binding sites were identified in the putative regulatory regions of both *CD2AP* and *NPHS2* (encoding podocin) [67]. These observations support a cooperative role for Lmx1b, CD2AP and podocin in foot process and slit diaphragm formation.

PLCE1 Gene Mutations Associated with Autosomal Recessive Nephrotic Syndrome

Following positional cloning, a gene locus for nephrotic syndrome (*NPHS3*) was mapped to chromosome 10q23-q24 and homozygous truncating mutations were identified in the gene *PLCE1*, encoding the enzyme phospholipase C-ε1 which is involved in intracellular signal transduction [68]. Onset of nephrotic syndrome was generally early in affected children and renal histology revealed DMS in most cases. In subsequent studies, mutations in *PLCE1* were identified in a relevant percentage of patients with DMS (28–33%) [69, 70] and with a lower frequency in hereditary FSGS (8%) [70]. Interestingly, two of the individuals with truncating *PLCE1* mutations entered sustained remis-

sion following steroid or cyclosporin A treatment [69]. The observation of a possible responsiveness to immunosuppression in *PLCE1* mutation carriers awaits confirmation in a larger number of affected patients.

PLCE1 is widely expressed in many tissues including also the podocytes. The knock-down of *pcle1* in zebrafish is associated with the development of podocyte foot process effacement and edematous outer appearance of the fish [68] confirming a specific role of phospholipase C epsilon-1 for the maintenance of the glomerular filtration barrier. Still, the pathogenesis of isolated podocyte damage and the development of proteinuria in patients lacking phospholipase C epsilon-1 remains to be elucidated.

PTPRO Gene Mutations Associated with Autosomal Recessive Nephrotic Syndrome

Homozygous *PTPRO* splice-site mutations were identified in two families of Turkish origin with childhood-onset nephrotic syndrome and minimal change nephropathy or FSGS on renal biopsy [71]. Nephrotic syndrome was resistant to oral prednisone therapy but a partial response to an intensified immunosuppressive regimen including pulse methylprednisolone and cyclosporin A was observed in some cases. PTPRO, identical to glomerular epithelial protein-1 (GLEPP1), is a receptor-like membrane protein tyrosine phosphatase expressed at the apical membrane of the podocyte foot processes in the kidney. Disruption of *Ptpro* in mice results in alterations of the podocyte structure and a reduction of the glomerular filtration rate indicating a role of PTPRO for proper podocyte function.

ARHGDIA Gene Mutations in Autosomal Recessive DMS

A recessive mutation in *ARHGDIA*, encoding a regulator of Rho-GTPases, was detected in two female siblings born to consanguineous parents. Both girls presented with congenital nephrotic syndrome and DMS on renal histology [72]. The homozygous 3-bp in-frame deletion mutation in *ARHGDIA* seems to be implicated in the hyperactivation of Rho-GTPases, causing a derangement of the podocyte actin cytoskeleton. *Arghdia* knock-out mice develop podocyte damage, severe proteinuria and progressive renal failure, supporting a role of human *ARHGDIA* in the pathogenesis of proteinuric disease.

EMP2 Gene Mutations Associated with Autosomal Recessive Childhood-Onset Nephrotic Syndrome

Mutations in podocyte genes have only exceptionally been identified in children with steroid-sensitive nephrotic syndrome (SSNS) [44, 73]. However, by homozygosity mapping and whole exome sequencing in 67 families biallelic mutations in *EMP2* were identified in one pair of siblings of Turkish origin, affected by frequently relapsing nephrotic syndrome with remission after cyclophosphamide treatment. In both, onset of the disease was below 3 years of age. Subsequently, more than 1600 individuals with nephrotic syndrome were screened for recessive mutations in *EMP2* and two more patients with SRNS (of Turkish and of African origin) were identified [74]. *EMP2* encodes epithelial membrane protein 2, discussed to be involved in cell proliferation and cell-cell interactions. The knock-down of *emp2* in the zebrafish resulted in pericardial effusions, consistent with a role of epithelial membrane protein 2 in keeping-up glomerular filtration [74].

NUP Nephropathy

A novel large group of genes has recently been identified to be involved in autosomal recessive steroid-resistant nephrotic syndrome leading to CKD and ESRD. These genes encode proteins of the nuclear pore complex (NPC) [75, 76]. These proteins belong to the group of nucleoporins (NUP), assembling to form NPCs, one of the

largest protein complexes found in eukaryotic cells. NPCs are channels that span the nuclear envelope and allow the transport of proteins, RNAs, and ribonucleoprotein particles between the cytoplasm and nuclear interior [77]. Hitherto, mutations were reported in multiple genes encoding proteins of the NPC, including *NUP93, NUP205,* and *XPO5* (inner ring of the NPC) and *NUP107*, *NUP85*, *NUP133*, and *NUP160* (outer ring of the NPC) in patients with childhood-onset SRNS, accounting for approximatively 3% of cases. These human mutations seem to disturb important mechanisms of glomerulogenesis. Kidney biopsy in selected cases showed FSGS or DMS, and extrarenal symptoms identified in patients with mutations in *NUP95*, *NUP107,* and *XPO5* comprise short stature, intellectual disability, microcephaly, or dilated cardiomyopathy. However, more studies focusing on deep phenotyping will be needed to establish more reliable genotype-phenotype correlations.

Hereditary Disorders with Late-Onset Nephrotic Syndrome

Hereditary late-onset FSGS is a heterogeneous condition generally transmitted in an autosomal dominant fashion (with the exception of autosomal recessive FSGS6). Different disease loci have been mapped in affected families (FGSG1-FSGS6) and responsible genetic defects have been identified in many podocyte-associated genes, frequently involving actin cytoskeleton architecture and dynamics.

ACTN4 Gene Mutations Associated with Adulthood FSGS (FSGS1)

In 1998, a locus for autosomal dominant late-onset FSGS was mapped to chromosome 19q13 (FSGS1) [78] and mutations in *ACTN4* were identified as the underlying pathogenic cause [79]. *ACTN4* encodes for α-actinin-4, an actin-bundling protein of the cytoskeleton highly expressed in podocytes. Both a knock-down and an overexpression transgenic mouse model have been established for *Actn4*, demonstrating proteinuria and podocyte alterations. It was therefore discussed that α-actinin-4 plays an important role for the cytoskeletal function of the podocyte. Young knock-out mice present with focal areas of foot process effacement and older animals with diffuse effacement and globally disrupted podocyte morphology [80]. Moreover, *Actn4* was shown to be upregulated in the kidneys of different animal models of proteinuria. Human *ACTN4* mutations were identified in three different families with FSGS [79]. The clinical course in affected family members was characterized by progressively increasing proteinuria starting in adolescence and developing into FSGS and chronic renal insufficiency later in adult life. ESRD was observed in a number of affected individuals. All *ACTN4* mutations identified so far represent non-conservative amino acid substitutions affecting the actin-binding domain of α-actinin-4. In vitro studies demonstrated that mutant α-actinin-4 binds filamentous actin more strongly than wild-type protein. Based on this observation it was proposed that dominant mutations in *ACTN4* interfere with the maintenance of podocyte architecture: a proper organisation of the cytoskeleton seems to be important for normal functioning of podocyte foot processes. Interestingly, however, not all mutation carriers of the families reported by Kaplan et al. presented with a renal phenotype. The observed incomplete penetrance suggests that additional (genetic or non-genetic) factors are involved in the pathogenesis that in conjunction with a mutation in *ACTN4* lead to the manifestation of FSGS. *ACTN4* mutations may confer *disease susceptibility*, as also discussed for mutations in *CD2AP* and *TRPC6*. However, mutations in *ACTN4* represent a rare cause of hereditary FSGS, accounting for approximately 0–4% of familial FSGS, depending on the study cohort [45, 81, 82].

TRPC6 Gene Mutations Associated with Late-Onset FSGS (FSGS2)

In 1999, a second gene locus for autosomal dominant FSGS was mapped to chromosome 11q21-q22 using a 399-member Caucasian kindred of British heritage dating back seven generations

[83]. Fourteen deceased family members had suffered from ESRD, 14 living family members were on dialysis or had undergone renal transplantation, and 3 individuals were proteinuric. Six years later, the responsible gene *TRPC6* was identified [84, 85]. *TRPC6* encodes the transient receptor potential cation channel TRPC6 that is thought to mediate capacitative calcium entry into cells. Expression analysis revealed that TRPC6 is highly expressed in the kidney and also in podocytes at the site of the SD. A dominant missense mutation was identified in the original family of Winn et al., and five additional families with mutations in *TRPC6* were characterized by Reiser et al. Two of the missense mutations in the latter study were shown to increase the current amplitudes of TRPC6, consistent with a gain-of-function effect of the mutations. Interestingly, however, both studies describe carrier individuals with a normal renal phenotype, pointing to an incomplete penetrance of the mutations. *TRPC6* mutations have been identified in very few children; early disease onset seems to be exceptional. So far, it is unknown how the dysfunction of a cation channel is related to the development of podocyte damage and loss of the glomerular filtration barrier. One hypothesis is related to the observation that MEC-2, a *C. elegans* homologue of podocin, participates in the mechanosensation of the worm. MEC-2 is physically and functionally linked to ion channels, transducing the signals of mechanosensation. Since TRPC6 interacts with podocin and nephrin at the SD, it was proposed that podocin takes part in mechanosensation processes at the glomerular filtration barrier, transducing signals to TRPC6 which in turn modulates intracellular calcium concentrations in the podocyte. Nephrin, on the other hand, is thought to stimulate different pathways of the intracellular signaling machinery. Therefore, a complex protein network involving nephrin, podocin, CD2AP and the cation channel TRPC6 is established to maintain the SD structure of the foot process. Mutations in TRPC6 likely affect this functional network by altering the intracellular calcium concentration of the podocyte.

CD2AP Gene Mutations Associated with Adulthood FSGS (FSGS3)

In 1999, FSGS3 was shown to map to chromosome 6 and reported to be caused by haploinsufficiency for *CD2AP* [86]. *CD2AP* encodes for the CD2-associated protein CD2AP, an actin-binding protein that was originally identified as a cytoplasmic ligand of the CD2 receptor on T and natural killer cells. *CD2AP* knock-out mice presented not only with impaired immune functions but also with severe NS and FSGS, accompanied by mesangial hypercellularity and extracellular matrix deposition [87]. Electron microscopy showed the typical loss of podocyte foot process integrity with process effacement and loss of the SD structure. Screening in FSGS patients led to the identification of a dominant *CD2AP* mutation (a 2-bp substitution altering the exon 7 splice acceptor site) in two adult patients with late-onset FSGS [86]. Enhanced disease susceptibility for FSGS conferred by the change in CD2AP expression was postulated as the underlying pathogenic mechanism. CD2AP interacts with nephrin and both proteins localize to lipid rafts in the plasma membrane [33], suggesting that CD2AP is required to connect nephrin (and thus the SD) to the cytoskeleton of the podocyte. An impairment of CD2AP function might be associated with enhanced cytoskeletal fragility, predisposing to podocyte damage.

Susceptibility to Genetic Locus 22q12.3 (FSGS4), Including the Genes MYH9 and APOE1

Multiple single nucleotide polymorphisms (SNP) in the gene *MYH9* were recessively associated with idiopathic and HIV-associated FSGS and hypertensive end-stage renal disease (ESRD) in African American adult patients [88]. However, subsequent genomewide analyses in large patient cohorts suggested that a positively selected risk variant could be in a larger interval containing the *APOL* genes rather than be confined to *MYH9*. Two *APOL1* risks variants for FSGS were identi-

fied to be common in African but absent in European chromosomes. As APOL1 is a serum factor that lyses trypanosomes and in vitro assays revealed that only the kidney disease-associated APOL1 variants lysed Trypanosoma brucei rhodesiense it was speculated that evolution of a critical survival factor in Africa may have contributed to the high rates of renal disease in African Americans [89].

INF2 Gene Mutations Associated with Autosomal Dominant FSGS (FSGS5) and Charcot-Marie-Tooth Disease

Mutations in *INF2*, encoding inverted formin-2, were first identified in 11 FSGS families with onset of proteinuria in adolescence or adulthood [90]. Proteinuria was typically moderate, accompanied by microscopic hematuria and hypertension in some cases. Proteinuria was progressive, often leading to ESRD. Renal biopsies showed the presence of FSGS and unusually prominent actin bundles within the foot processes, on electron microscopy. Being part of the actin cytoskeleton Inf2/inverted formin-2 interacts with actin and is involved in both polymerization and depolymerization of actin filaments. Interestingly, mutations in *INF2* were also identified in patients with dominant intermediate Charcot-Marie-Tooth (CMT) disease and FSGS-associated proteinuria [91], localizing to a distinct area of the *INF2* gene. Mutant INF2 protein was abnormally distributed in the cytoplasm and the actin cytoskeleton and microtubule network were disorganized, not only in podocytes but also in peripheral Schwann cells, leading to disturbed myelin formation and CMT.

Mutations in MYO1E in Autosomal Recessive FSGS (FSGS6)

Recessive homozygous mutations in *MYOE1*, encoding a non-muscle membrane-associated class I myosin, were reported in children and adolescent patients of consanguineous union affected by nephrotic-range proteinuria, microhematuria, hypoalbuminemia, and edema [92]. Renal biopsy demonstrated FSGS, tubular atrophy and interstitial fibrosis. *Myoe1*-knockout mice show a similar phenotype with proteinuria, hematuria and progressive renal failure, indicating a defect in the glomerular filtration barrier. Impaired intracellular trafficking of the mutant protein seems to be implicated in the pathogenesis of *MYO1E*-associated disease.

Mitochondrial Disorders Associated with Nephrotic Syndrome

Several gene mutations in different components of the coenzyme Q_{10} (CoQ_{10}) biosynthesis pathway have recently been identified to be involved in hereditary nephrotic syndrome, frequently associated with extrarenal symptoms. Recessive mutations in *COQ2* were first identified in 2006 in a pair of siblings with early-onset glomerular lesions, steroid resistant nephrotic syndrome and CoQ_{10} deficiency [93]. The gene *COQ2* encodes for the para-hydroxybenzoate-polyprenyl transferase enzyme of the CoQ_{10} synthesis pathway. An increased number of abnormal mitochondria in podocytes and other glomerular cells was demonstrated by electron microscopy. Following this initial study, more patients were identified with a similar clinical course and loss-of-function mutations in *COQ2* [94, 95]. Extrarenal symptoms of mitochondrial disease are not obligatory but developed in a subset of affected patients to a varying degree, including encephalopathy, lactacidosis, myoclonic epilepsy and hypertrophic cardiomyopathy.

Early-onset SRNS associated with sensorineural deafness has subsequently been attributed to recessive mutations in *COQ6* encoding for CoQ_{10} biosynthesis monooxygenase 6 [96]. Renal histology revealed FSGS lesions in most cases with COQ6 mutation but DMS has also been observed.

Subsequently, mutations in *ADCK4 (=COQ8B)* were associated with disturbed CoQ_{10} biosynthesis and identified by homozygosity mapping and whole exome sequencing in children with SRNS

and school age onset of nephrotic syndrome [97]. *ADCK4/COQ8B* encodes the aarF domain containing kinase 4, a protein partially expressed in podocyte foot processes but also in podocyte mitochondria. ADCK4 colocalizes with COQ6 and COQ7. Serum CoQ_{10} levels are reduced in affected patients.

Of note, *COQ2*, *COQ6* and *COQ8B* are all nuclear genes encoding mitochondrial proteins. Therefore, the inheritance of associated disorders follows the Mendelian rules of autosomal recessive disease.

These findings indicate that the podocyte reacts very sensitively to disturbances of energy supply and it can be expected that mutations in other genes encoding mitochondrial proteins will be discovered in patients with SRNS in the future.

Most importantly and in contrast to all other hereditary disorders of the podocyte, these mitochondriopathies offer for a first time a potential causal treatment option by supplementation of $CoQ_{10.}$ In several patients affected by mutations in *COQ2*, *COQ6* and *COQ8B/ADCK4*, remarkable reductions of proteinuria were induced by oral medication with CoQ_{10}, opening a new therapeutic avenue for these patients with otherwise steroid resistant NS [96–98]. The recommended oral CoQ10 supplementation dose is 30 mg/kg/day.

Syndromal Disorders Associated with Nephrotic Syndrome

A large number of syndromes have been described on clinical grounds in patients presenting with (steroid-resistant) proteinuria in addition to various extrarenal manifestations. A genetic basis has been identified only in a minority of these syndromes. Here, we discuss two important syndromes that invariably present with SRNS, Schimke syndrome and Galloway-Mowat syndrome.

Schimke Syndrome

Schimke immuno-osseous dysplasia (SIOD) maps to chromosome 2q34–36 and is caused by recessive mutations in the *SMARCAL1* gene [99]. *SMARCAL1* encodes the SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin subfamily a-like protein 1, a protein involved in the remodeling of chromatin to change nucleosome compaction for gene regulation, replication, recombination, and DNA repair. The clinical phenotype of Schimke immuno-osseous dysplasia is characterized by growth retardation due to spondyloepiphyseal dysplasia, a slowly progressive immune defect, cerebral infarcts, skin pigmentation, and SRNS beginning in childhood. FSGS lesions are frequently observed in kidney biopsy specimen and the majority of patients progress early to ESRD. However, disease severity and age at onset follow a continuum from early onset and severe symptoms with death early in life to later onset and mild symptoms with survival into adulthood. A considerable fraction of patients with SRNS due to SIOD is oligosymptomatic, presenting only with proteinuria and short stature [100], underpinning the role of next generation sequencing (NGS) panel sequencing including *SMARCAL1* in non-syndromic unsolved SRNS cases. Proteinuria associated with very short stature may be a reliable clue to SIOD. Genotype-phenotype studies suggest that recessive loss-of-function mutations (frameshift, stop and splice-site mutations) are generally associated with a more severe course of the disease while some missense mutations allow a retention of partial *SMARCAL1* function and thus cause milder disease [99]. This genotype-phenotype correlation is typically observed for onset of extra-renal symptoms while the renal course (median age at ESKD 8 years) seems to be independent of the mutation type [100].

Galloway-Mowat Syndrome

The Galloway-Mowat syndrome (GAMOS) is characterized by microcephaly and other brain anomalies, severe mental retardation and early-onset NS (CNS) [101]. Both FSGS and DMS were observed in kidney biopsies of affected individuals [101, 102]. An important number of

patients also presents with hiatus hernia. Both males and females are affected and an occurrence in siblings of the same family has been reported. Different associated gene loci have been identified. In 2014, loss-of-function mutations in *WDR73* were described in two unrelated families [103]. In 2017, a large international consortium identified multiple mutations in KEOPS-complex genes in patients affected by autosomal recessive (*OSGEP*, *TP53RK*, *TPRKB*) or X-chromosomal linked (*LAGE3*) GAMOS [104].

Clinical Aspects

Clinical aspects of NS are discussed in all detail in former chapters. Here, we want to focus on some issues specific for genetic forms of SRNS.

Long-Term Outcome and Renal Prognosis

Large multicenter studies have provided significant data and insight into the long-term renal prognosis of hereditary forms of nephrotic syndrome. In an analysis of more than 1000 SRNS patients followed in the PodoNet Registry study the diagnosis of a genetic disease markedly impacted ESKD risk. ESKD-free survival rates were 27% and 17% in patients with a genetic diagnosis, contrasting with a favorable prognosis in children with sporadic non-genetic SRNS responsive to intensified immunosuppression, who exhibited a 15-year renal survival rate of 96% [105, 106]. Within the same study, the histopathologic diagnosis was also clearly predictive of ESKD. When adjusting for genetic status, age, proteinuria level, CKD and responsiveness to intensified immunosuppression, DMS or FSGS on biopsy implied an increased risk of progressing to ESKD [105].

Therapeutic Implications

The therapy of SRNS in general is demanding. Numerous immunosuppressive agents have shown some efficacy in a fraction of the SRNS population, including cyclosporine, mycophenolate mofetil or the anti-CD20-antibody rituximab mostly in combination with glucocorticoids. However, genetically determined forms of SRNS have mostly proven insensitive to immunosuppressive interventions, which is pathophysiologically explained by the presence of intrinsic defects in podocyte architecture and function [105, 107, 108]. A detailed review of the pertinent literature is available in the recent IPNA expert recommendations [109]. Generally, it is suggested to spare children with hereditary SRNS from immunosuppressive treatment.

Conversely, specific gene mutations have not been reported in patients with steroid sensitive NS [44], so genetic screening seems generally not indicated even in patients with reduced steroid sensitivity (such as frequent relapsers or steroid dependency). However, in former studies addressing a possible genetic basis of SSNS, associations to various gene loci and candidate genes (e.g. *CLVS1* encoding clavesin-1) have been identified [110–113].

Therefore, novel insights into the genetics and pathophysiology of SSNS can be expected.

Antiproteinuric and renoprotective pharmacological treatment with ACE inhibitors or AT1 receptor blockers (RAASi) is advised in all children with SRNS once the diagnosis is made in the maximally approved and tolerated dose in order to slow down the progression of renal insufficiency also in genetic SRNS. ACEi or ARBs should be used with caution in patients with CKD stage 4, and they should not be started or should be stopped in case of intravascular volume depletion, acute kidney injury (AKI), hyperkalemia, or frequent vomiting/diarrhea. In female adolescents, contraception should be ensured in order to avoid the teratogenic effects of RAASi [109].

Living Related Donor Transplantation

Living-related kidney transplantation is generally considered the therapy of first choice in pediatric patients with ESRD. However, in patients affected by SRNS due to germline mutations in

podocyte genes several aspects need to be considered. First, it is unknown as yet how kidneys of a heterozygous donor behave and develop in a recipient with recessive SRNS: The parents of affected children with recessive SRNS carry one mutant allele each that is also present in the transplanted kidney. It could be speculated that these kidneys are more easily prone to develop proteinuria if other pro-proteinuric factors (e.g., arterial hypertension, salt-rich diet) are superposed. While animal models of SRNS do not support this hypothesis so far, comprehensive human data addressing this question are mostly lacking. Consequences for the donor should also be considered. It is as yet unknown whether the prognosis of the remaining single kidney in the heterozygous parental donor is impaired by the gene mutation. Again, the remaining heterozygous kidney might be more susceptible to proteinuric disease than single kidneys of individuals without mutations. Up to now the experience with living-related donor transplantation in hereditary SRNS is very limited and does not support a restriction in affected children. Still, careful surveillance of both donor and recipient seems advisable.

In families of patients affected by autosomal dominant late-onset SRNS, only one of the parents is carrier of the pathogenic sequence variation. Genetic testing of family members will be helpful to delineate mutation carriers in the family. If the mutation occurred as a de novo mutation in the patient, both parents are equally suitable for living donor transplantation from a genetic point of view.

Recurrence of Nephrotic Syndrome After Renal Transplantation

Many investigators have studied the pathogenesis of increased glomerular permeability and recurrence of proteinuria after transplantation in FSGS. In general, recurrence of proteinuria after renal transplantation is observed in approximately 30% of FSGS patients [108, 114]. This risk appears higher in children than in adult patients [115]. Affected patients present with proteinuria, which is often in the nephrotic range. Frequently proteinuria recurs within few days after renal transplantation. In children, the mean time to recurrent proteinuria is 14 days post-transplant [116]. Recurrence of proteinuria/FSGS following renal transplantation negatively impacts graft survival in both children and adult patients. Risk factors are an age less than 15 years, rapid progression of renal insufficiency and diffuse mesangial proliferation in the initial biopsy of the native kidney [117]. In non-hereditary FSGS/SRNS, the recurrence of proteinuria is discussed to follow a T cell dysfunction and production of proteinuric circulating factors, including soluble urokinase receptor, hemopexin, and cardiotrophin-like cytokine-1 [118, 119].

In *NPHS2*-associated SRNS/FSGS recurrence of post-transplant proteinuria is a rare phenomenon, observed in less than 10% of transplanted patients [37, 44, 120]. The identification of a homozygous truncating *NPHS2* mutation in one patient with post-transplant NS prompted the search for anti-podocin antibodies but all results were negative excluding a de novo glomerulonephritis as underlying cause [37]. Anti-podocin antibodies were also not identifiable in a study including patients with *NPHS2* missense mutations and post-transplant NS [121].

In CNF, the risk of a recurrence of proteinuria after transplantation seems to be important: it was demonstrated that especially patients affected by the Fin major mutation have a risk of approximately 25% of post-transplant NS. Subsequent studies revealed that the pathogenesis of this recurrence is related to the development of anti-nephrin antibodies directed against the wildtype nephrin protein residing in the transplanted kidney [122], analogous to the anti-GBM antibodies against type IV collagen causing post-transplant de novo glomerulonephritis in patients with Alport syndrome. Treatment options of post-transplant NS in these patients are controversially discussed due to a relative paucity of data; a subset of patients seems to respond to rituximab or cyclophosphamide [108].

Genetic Testing

Following the rapid technological development in human genetics and genome research, different approaches can be applied in order to identify gene mutations in patients with SRNS and/or FSGS. In countries where next generation sequencing (NGS) techniques are not available there may still be a rationale for conventional Sanger sequencing in patients with specific phenotypes, e.g., sequencing of *WT1* in Denys-Drash or *LAMB2* in Pierson syndrome. Sanger sequencing can also be useful in patients with congenital NS (*NPHS1*) or school-aged patients with SRNS (*NPHS2*), where the likelihood of a positive result is relatively high. In adolescents and young adults, a different screening rationale should be applied. The *NPHS2*-p.R229Q sequence variation in compound heterozygosity with specific pathogenic *NPHS2* mutations is frequently found in late-onset SRNS [45, 50, 51]. In addition, autosomal dominant disease due to mutations in *WT1*, *TRPC6*, *INF2* and *ACTN4* can be identified in adolescents and young adults, particularly in case of dominant transmission but to a minor extent also in sporadic cases [45, 123, 124].

However, panels of podocytopathy- and even more broadly kidney-disease-associated genes have been developed by many commercial and non-commercial laboratories, which now allow standardized and simultaneous sequencing of more than 50 genes in one experimental run by next NGS techniques. In addition, whole exome and whole genome sequencing (WES/WGS) are available at low costs and has been developed for many clinical diagnostic applications. Advantages of the WES approach include the identification of gene mutations in novel genes, non-coding regions (WGS), microdeletions/microduplications, copy number variations and the unraveling of gene mutations in phenotypically complex cases. Still, the interpretation of huge data sets can be challenging and necessitates biostatistical expertise. Large databases have been established offering an annotation of sequence variations in different populations. Numerous ethical issues still need to be addressed, especially with respect to the possible incidental identification of mutations in SRNS-unrelated genes with high phenotypic penetrance associated with severe disorders, e.g. different types of cancer. In these cases, genetic counseling is demanding and of high importance.

Genetic Counseling

Positive results of mutational analysis in pediatric patients with SRNS should be followed by adequate genetic counseling. This demands close collaboration between pediatric nephrologists and human geneticists. Parents of children affected by recessive disease will have a chance of 25% to give birth to another affected child. In parents of children with dominant disease, this risk amounts to 50% (with the exception of patients with de novo mutations, in these families, the risk of recurrence is very low). Parents of affected children need to be informed that treatment options are limited in hereditary SRNS and that renal function may deteriorate rapidly. Close monitoring of renal function and early treatment of complications of chronic renal insufficiency are advised. In autosomal dominant FSGS, genetic counseling might be difficult due to the fact of incomplete penetrance and variable expressivity. It seems that individual mutation carriers can be affected to a differing degree with an obvious mild phenotype in some family members and ESRD in others. Genetic counseling is not only important for the parents but also for the affected child. Children with recessive disease will transmit a heterozygous mutation to their own children in the future. As long as the other parent is not mutation carrier, all offspring will be healthy. Patients affected by dominant FSGS will transmit the pathogenic mutation in 50% of cases and offspring carrying the mutation might be affected by FSGS.

In some cases, established genotype-phenotype correlations might be helpful to estimate the risk of a more severe clinical course. In *NPHS2*-associated SRNS, for example, some mutations have been associated with early-onset and aggravated clinical course while other muta-

tions were shown to be less pathogenic [40]. For other disease entities, the analysis of clinical symptoms of other affected family members can be of help to predict the severity of the disease: in NPS, the risk of having a child with NPS nephropathy is about 1:4 and the risk of having a child in whom renal failure will develop is about 1:10 if NPS nephropathy occurs in other family members [53]. Genetic counseling is especially important in families affected by NS with serious prognosis. In children affected by CNS with female outer appearance, mutation analysis in *WT1* is mandatory in order to rule out a risk for Wilms tumor development.

Due to the implementation of NGS techniques in the clinical routine setting, many patients are identified to carry a significant number of sequence variations of unknown relevance in different podocyte genes, not following simple Mendelian inheritance. Whether these gene variants act as modifiers, accumulate to confer a susceptibility for the development of NS or are just polymorphisms without biological function remains difficult to be designated in many cases. Genetic counseling will has to make allowance for this.

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Alport Syndrome and Other Type IV Collagen Disorders

16

Michelle N. Rheault and Rachel Lennon

Introduction

Several forms of familial glomerular hematuria syndromes result from genetic variants that affect type IV collagen, the major collagenous constituent of glomerular basement membranes (GBM): Alport syndrome (AS) and hereditary angiopathy with nephropathy, aneurysms and cramps (HANAC) syndrome. Persistent hematuria is a cardinal feature of each of these disorders. Variants in any of three type IV collagen genes, *COL4A3, COL4A4* or *COL4A5* can cause AS, which is characterized by progressive deterioration of kidney function with associated hearing and ocular involvement in many affected individuals. A majority of affected individuals demonstrate X-linked inheritance; however, autosomal recessive and autosomal dominant transmission is also observed. Heterozygous variants in these genes are also significant and link to a wider spectrum of kidney disease [1–3]. Variants in *COL4A3, COL4A4 or COL4A5* [4] account for about 30–50% of children with isolated glomerular hematuria seen in pediatric nephrology clinics [5–8]. HANAC syndrome arises from variants in *COL4A1* [9].

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Alport Syndrome

Introduction

The first description of a family with inherited hematuria appeared in 1902 in a report by Guthrie [10]. Subsequent monographs about this family by Hurst in 1923 [11] and Alport in 1927 [12] established that affected individuals in this family, particularly males, developed deafness and uremia. The advent of electron microscopy led to the discovery of unique GBM abnormalities in patients with AS [13–15], setting the stage for the histochemical [16–18] and genetic [19, 20] studies that resulted in the identification of disease causing variants in *COL4A5* [20] followed by *COL4A3* and *COL4A4* [21, 22]. AS occurs in approximately 1:50,000 live births and accounts for 1.3% and 0.4% of pediatric and adult end-stage kidney disease (ESKD) patients in the United States, respectively [23].

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Etiology and Pathogenesis

Type IV Collagen Proteins, Tissue Distribution and Genes

Six chains of type IV collagen, α1(IV)-α6(IV), are encoded by six genes, *COL4A1-COL4A6*. The type IV collagen genes are arranged in pairs on three chromosomes: *COL4A1-COL4A2* on chromosome 13, *COL4A3-COL4A4* on chromosome 2, and *COL4A5-COL4A6* on the X chromosome. The paired genes are arranged in a 5′-5′ head-to-head fashion, separated by sequences of varying length containing regulatory elements [24, 25]. All type IV collagen chains share several basic structural features: a major collagenous domain of approximately 1400 residues containing the repetitive triplet sequence glycine (Gly)-X-Y, in which X and Y represent a variety of other amino acids; a *C*-terminal noncollagenous (NC1) domain of approximately 230 residues; and a noncollagenous *N*-terminal sequence of 15–20 residues. The collagenous domains each contain approximately 20 interruptions of the collagenous triplet sequence, while each NC1 domain contains 12 conserved cysteine residues. Type IV collagen chains self-associate to form triple helical structures or "trimers". The specificity of chain association is determined by amino acid sequences within the NC1 domains and results in only three trimeric species that are found in nature: $\alpha1_2\alpha2$(IV), α3α4α5(IV) and $\alpha5_2\alpha6$(IV) [26]. Unlike interstitial collagens, which lose their NC1 domains and form fibrillar networks, type IV collagen trimers form open, nonfibrillar networks through NC1-NC1 and N-terminal interactions [27].

$\alpha1_2\alpha2$(IV) trimers are found in all basement membranes, whereas α3α4α5(IV) and $\alpha5_2\alpha6$(IV) trimers have a more restricted distribution. In normal human kidneys, α3α4α5(IV) trimers are found in GBM, Bowman's capsules, and the basement membranes of distal tubules, while $\alpha5_2\alpha6$(IV) trimers are detectable in Bowman's capsules, basement membranes of distal tubules and collecting ducts, but not GBM [28, 29]. $\alpha5_2\alpha6$(IV) trimers are also present in normal epidermal basement membranes as well as some alimentary canal, ocular, and vascular basement membranes. α3α4α5(IV) trimers also occur in several basement membranes of the eye and of the cochlea [30–32].

Pathogenic variants in any of the *COL4A3*, *COL4A4*, or *COL4A5* genes will affect the formation and composition of affected basement membranes. If any of the α3(IV), α4(IV), or α5(IV) chains are absent due to loss of function variants (deletions, frame shift variants, premature stop codons), then the other collagen chains are degraded and no α3α4α5(IV) trimers are deposited in basement membranes [33]. In this case, the embryonic $\alpha1_2\alpha2$(IV) network persists. Missense variants, particularly those that affect the glycine residues involved in triple helix formation, may lead to the formation of abnormally folded trimers that are either degraded or deposited into the basement membrane with formation of an abnormal type IV collagen network. Due to a greater number of disulfide bonds, the α3α4α5(IV) network is more highly cross-linked and is considered more resistant to proteases and therefore mechanical strain than the $\alpha1_2\alpha2$(IV) network [33, 34]. In support of this network being mechanically stronger, absence of the α3α4α5(IV) network leads to increased distensibility in the lens capsule when tested in experimental models of AS [35]. Indeed, the glomerular capillary walls of AS patients may also be mechanically weak and provoke pathologic stretch-related responses in glomerular cells [36].

Genetics

AS is described in three genetic forms: X-linked (XLAS), autosomal recessive (ARAS) and autosomal dominant (ADAS), although opinions vary as to how a single gene can cause both recessive and dominant disease (Table 16.1). XLAS, caused by variants in *COL4A5,* was classically thought to account for approximately 80% of AS patients while ARAS, caused by variants in both alleles of *COL4A3* or *COL4A4,* accounted for about 15% of the AS population. Affected males with XLAS are hemizygous and carry a single abnormal *COL4A5* allele, while affected females are heterozygous with normal and abnormal alleles. Individuals with ARAS may be either homozygous, with identical variants in both

Table 16.1 Familial glomerular hematuria due to type IV collagen variants

	Genetic locus	Protein product	Kidney manifestations	Kidney failure	GBM ultrastructure	Extrakidney manifestations
Alport syndrome						
X-linked	*COL4A5*	α5(IV)	Hematuria Proteinuria Hypertension	All males, some females	Thinning (early) Lamellation (late)	Deafness Lenticonus Perimacular flecks
Autosomal recessive	*COL4A3* *COL4A4* (biallelic or digenic)	α3(IV) α4(IV)	Hematuria Proteinuria Hypertension	All males and females	Thinning (early) Lamellation (late)	Deafness Lenticonus Perimacular flecks
Autosomal dominant	*COL4A3* *COL4A4* (heterozygous)	α3(IV) α4(IV)	Hematuria Proteinuria Hypertension	Males and females (late)	Thinning (early) Lamellation (late)	Deafness
HANAC syndrome						
Autosomal dominant	*COL4A1*	α1(IV)	Hematuria Cysts CKD	?	Normal	Arterial aneurysms Muscle cramps

Abbreviations: *GBM* glomerular basement membrane; *CKD* chronic kidney disease

alleles of the affected gene or they may be compound heterozygotes, with different variants in the two alleles or even demonstrate digenic inheritance with one variant in *COL4A3* and the other in *COL4A4* [37, 38]. With the advent of next generation sequencing, studies are suggesting a higher percentage of patients with ADAS, up to 31% in one report [39]. ADAS is used by some clinicians to describe heterozygous variants in *COL4A3* or *COL4A4* with a progressive clinical course [40]. It is not clear why some individuals develop a progressive nephropathy while others have a slower or unremarkable clinical course; this may relate to the presence of co-segregating genetic modifiers [41].

Over 2500 pathogenic variants have been identified in the *COL4A5* gene in patients with XLAS [42]. Variants can be found along the entire 51 exons of the gene without identified hot spots. About 10–15% of *COL4A5* variants occur as spontaneous events; therefore, a family history of kidney disease is not required for a diagnosis of XLAS. A range of variants have been described: large rearrangements (~20%), small deletions and insertions (~20%), missense variants altering a glycine residue in the collagenous domain of α5(IV) (30%), other missense variants (~8%), nonsense variants (~5%) and splice-site variants (~15%) [43]. The type of *COL4A5* variant has a significant impact on the course of XLAS in affected males [43–45]. In males with a large deletion, nonsense variant or an indel causing a reading frame shift, the risk of developing kidney failure before age 30 is 90%. In contrast, progression to kidney failure before age 30 occurs in 70% and 50% of patients with splice-site and missense variants, respectively [43]. In addition, XLAS patients with 5′ glycine missense variants demonstrate a more severe phenotype than those with 3′ glycine variants [44]. In contrast to males with XLAS, a statistical relationship between *COL4A5* genotype and kidney phenotype has not been demonstrated in females with XLAS [46].

Clinical Manifestations

Males with XLAS and ARAS inevitably develop kidney failure at a rate that is influenced by genotype [37, 43, 45]. While most females with XLAS have non-progressive or slowly progressive kidney disease, a significant minority demonstrates progression to kidney failure [47]. The course of ARAS is similar in females and males [37]. In general, patients

with ADAS progress less rapidly than patients with XLAS or ARAS and are less likely to have extra-kidney manifestations [48].

Kidney Phenotype

Persistent microscopic hematuria (MH) occurs in all males with AS, regardless of genetic type, and is probably present from early infancy. Approximately 95% of heterozygous females with XLAS have persistent or intermittent MH [46], and 100% of females with ARAS have persistent MH. Gross hematuria is not unusual in boys and girls with Alport syndrome, occurring at least once in approximately 60% of affected males [43, 49].

In males with XLAS, and in males and females with ARAS, proteinuria typically becomes detectable in late childhood or early adolescence and progresses from microalbuminuria to overt proteinuria [50]. In one large cohort of females with XLAS, 75% had proteinuria, although the timing of onset was not investigated [46].

Blood pressure is typically normal in childhood but, like proteinuria, hypertension is common in adolescent males with XLAS or ARAS, and in females with ARAS. Most females with XLAS have normal blood pressure, but hypertension may develop, particularly in those with proteinuria.

All males with XLAS eventually require kidney replacement therapy, with 50% of untreated males reaching kidney failure by age 25, 80% by age 40 and 100% by age 60 [43]. The timing of kidney failure in patients with ARAS is similar to XLAS males, although ARAS patients with normal kidney function in their 30's and 40's have been reported [37]. In patients with ADAS, the age at which 50% of patients have progressed to kidney failure is approximately 50 years, or twice as long as XLAS males [48].

Females who are heterozygous for *COL4A5* variants demonstrate widely variable disease outcomes, with some women demonstrating only lifelong asymptomatic hematuria while others develop chronic progressive kidney disease including kidney failure [51]. About 12% of XLAS females reach kidney failure by age 45, 30% by age 60 and 40% by age 80 [46]. The explanation for the wide variability in outcomes for XLAS females is uncertain, but likely multifactorial. Risk factors for kidney failure in XLAS females include proteinuria and sensorineural deafness [46, 52]. X-inactivation, the process by which one X chromosome in females is silenced to adjust for gene dosage differences between males and females, may play a role in kidney disease progression in XLAS females [53, 54]. In a mouse model of female XLAS, modest skewing of X-inactivation to favor expression of the wild type α5(IV) was associated with a survival advantage [55].

AS nephropathy progresses predictably through a series of clinical phases. Phase I typically lasts from birth until late childhood or early adolescence, and is characterized by isolated hematuria, with normal protein excretion and kidney function. In Phase II, microalbuminuria followed by overt proteinuria is superimposed on hematuria, but the glomerular filtration rate (GFR) remains normal. Patients in Phase III exhibit declining GFR in addition to hematuria and proteinuria, and those in Phase IV have kidney failure. These phases have histological correlates, as described in the next section. The rate of passage through these phases is primarily a function of the causative genetic variant, at least in males with XLAS. Patients with *COL4A5* variants that prevent production of any functional protein (deletions, nonsense variants) proceed through these phases more rapidly than those whose variants allow synthesis of a functional, albeit abnormal, protein (some missense variants). Females with XLAS can be viewed as passing through the same phases as males, although the rate of progression is typically slower, and the journey to kidney failure may not be completed during the individual's lifetime.

Hearing

Newborn hearing screening is normal in males with XLAS, and in males and females with ARAS, but bilateral impairment of perception of high frequency sounds frequently becomes detectable in late childhood. The hearing deficit

is progressive, and extends into the range of conversational speech with advancing age. Affected individuals benefit from hearing aids since the deficit usually does not exceed 60–70 dB and speech discrimination is preserved. Sensorineural hearing loss (SNHL) is present in 50% of males with XLAS by approximately age 15, 75% by age 25, and 90% by age 40 [43]. Like the effect on kidney disease progression, missense variants in *COL4A5* are associated with an attenuated risk of hearing loss. The risk of SNHL before age 30 is 60% in patients with missense variants, while the risk of SNHL before age 30 is 90% in those with other types of variants [43]. SNHL is less frequent in females with XLAS. About 10% of XLAS females have SNHL by 40 years of age, and about 20% by age 60 [46]. SNHL is also common in ARAS, with approximately 66% of individuals affected [37].

The SNHL in AS has been localized to the cochlea [56]. In control cochleae, the α3(IV), α4(IV) and α5(IV) chains are expressed in the spiral limbus, the spiral ligament, stria vascularis and in the basement membrane situated between the Organ of Corti and the basilar membrane [57–59]. However, these chains have not been detected in the cochleae of ARAS mice [58], XLAS dogs [59] or men with XLAS [32]. Examination of well-preserved cochleae from men with XLAS revealed a unique zone of separation between the organ of Corti and the underlying basilar membrane, as well as cellular infiltration of the tunnel of Corti and the spaces of Nuel [60]. These changes may be associated with abnormal tuning of basilar membrane motion and hair cell stimulation, resulting in defective hearing. An alternative hypothesis is that hearing is impaired by changes in potassium concentration in the scala media induced by abnormalities of type IV collagen in the stria vascularis [61].

Ocular Anomalies

Abnormalities of the lens and the retina are common in individuals with AS, typically becoming apparent in the second to third decade of life in XLAS males and in males and females with ARAS. The α3(IV), α4(IV) and α5(IV) chains are normal components of the anterior lens capsule and other ocular basement membranes, and variants that interfere with the formation or deposition of α3α4α5(IV) trimers prevent expression of these chains in the eye [30, 57]. Anterior lenticonus, which is considered virtually pathognomonic for AS [62], is absent at birth and manifests during the second and third decades of life in ~13–25% of affected individuals [43, 63]. In this disorder, the anterior lens capsule is markedly attenuated, especially over the central region of the lens, and exhibits focal areas of dehiscence, leading to refractive errors and, in some cases, cataracts [64, 65]. Anterior lenticonus has been described only rarely in heterozygous females with *COL4A5* variants [47]. Dot-fleck retinopathy, a characteristic alteration of retinal pigmentation concentrated in the perimacular region [66], is also common in AS patients and does not appear to be associated with any abnormality in vision [43]. Recurrent corneal erosions [67, 68] and posterior polymorphous dystrophy, manifested by clear vesicles on the posterior surface of the cornea [69], have also been described in AS.

Leiomyomatosis

Several dozen families in which AS is transmitted in association with leiomyomas of the esophagus and tracheobronchial tree have been described [70]. Affected individuals carry X-chromosomal deletions that involve the *COL4A5* gene and terminate within the second intron of the adjacent *COL4A6* gene [71–73]. The genotype-phenotype relationship in this disorder is uncertain because deletions in this region may occur without associated leiomyomas, and conversely some families with XLAS and leiomyomas do not have deletions involving *COL4A6* [74]. Those affected tend to become symptomatic in late childhood, and may exhibit dysphagia, postprandial vomiting, epigastric or retrosternal pain, recurrent bronchitis, dyspnea, cough or stridor. Females with the AS-leiomyomatosis complex may develop genital leiomyomas, with clitoral hypertrophy and variable involvement of the labia majora and uterus.

Other Findings

AS associated with mental retardation, mid-face hypoplasia and elliptocytosis has been described in association with large *COL4A5* deletions that extend beyond the 5′ terminus of the gene [75]. Early development of aortic root dilatation and aneurysms of the thoracic and abdominal aorta, as well as other arterial vessels, have been described in AS males, perhaps due to abnormalities in the $\alpha5_2\alpha6$(IV) network in arterial smooth muscle basement membranes [76].

Kidney Pathology

Children with AS typically show limited kidney parenchymal changes by light microscopy before 5 years of age. Older patients may have mesangial hypercellularity and matrix expansion. As the disease progresses, focal segmental glomerulosclerosis, tubular atrophy and interstitial fibrosis become the predominant light microscopic abnormalities. Although some patients exhibit increased numbers of immature glomeruli or interstitial foam cells, these changes are not specific for AS.

Electron microscopy is frequently diagnostic, although the expression of the pathognomonic lesion is age-dependent and, for those with XLAS, gender-dependent. In early childhood, the predominant ultrastructural lesion in males is diffuse attenuation of the GBM. The classic ultrastructural appearance is diffuse thickening of the glomerular capillary wall, accompanied by "basket-weave" transformation; intramembranous cellular components, which have been described as podocyte protrusions; scalloping of the epithelial surface of the GBM; and effacement of podocyte foot-processes (Fig. 16.1) [77]. These changes are more prevalent in affected males, typically becoming prominent in late childhood and adolescence. Affected females can display a spectrum of lesions, demonstrating predominantly normal-appearing GBM, focal GBM attenuation, diffuse GBM attenuation, focal thickening/basket-weaving, or diffuse basket-

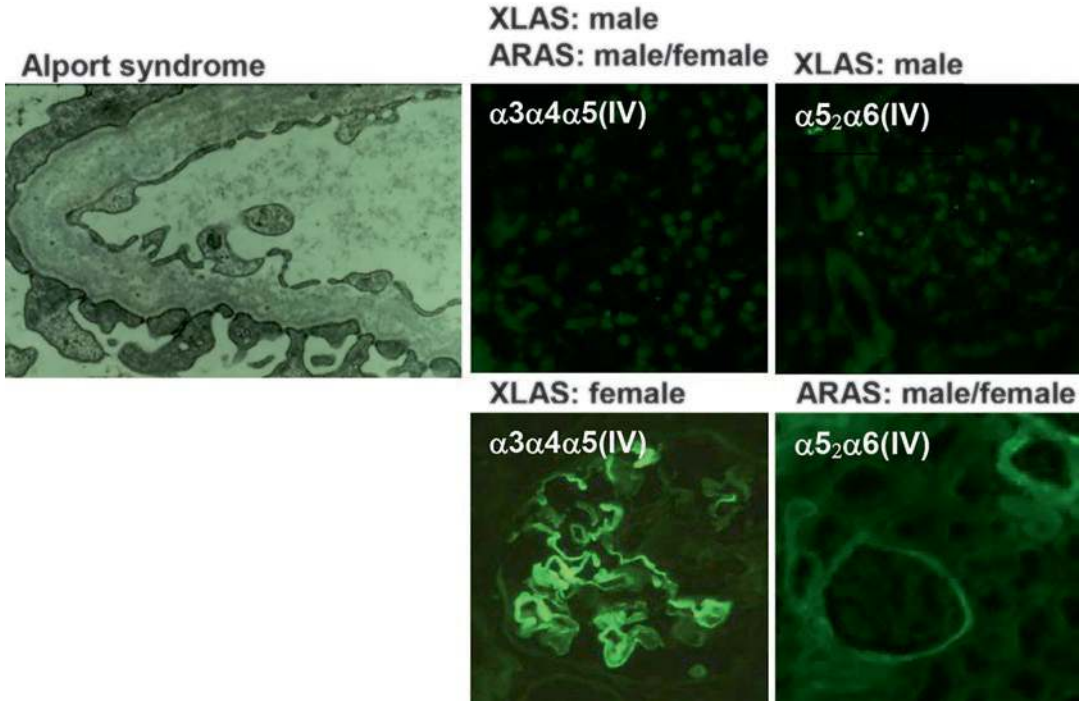

Fig. 16.1 Typical findings on electron microscopy and type IV collagen immunostaining for α5(IV) in Alport syndrome. Abbreviations: *XLAS* X-linked Alport syndrome; *ARAS* autosomal recessive Alport syndrome

weaving. The extent of the GBM lesion progresses inexorably in males, although the rate of progression may be influenced by *COL4A5* genotype. Females may have static or progressive GBM lesions. X-chromosome inactivation pattern, age and *COL4A5* genotype could all contribute to the GBM changes in affected females.

The classic GBM lesion is not found in all kindreds with AS. Adult patients who demonstrate only GBM thinning, yet have *COL4A5* variants, have been described. Although these represent a minority of Alport patients and families, they are also seen in individuals with heterozygous variants and in such patients there is an association with focal segmental glomerulosclerosis (FSGS) [1, 2]. Indeed, patients with a diagnosis of FSGS should have careful evaluation of GBM ultrastructure and, if defects are identified, genetic testing for Alport gene variants is warranted since a diagnosis of AS will enable further phenotypic evaluation in the individual as well as testing in other family members.

Routine immunofluorescence microscopy in patients with AS is normal or shows nonspecific deposition of immunoproteins. In contrast, specific immunostaining for type IV collagen α chains is frequently diagnostic, and can distinguish between XLAS and ARAS (Fig. 16.1). The utility of this approach derives from the fact that most disease-causing variants in AS alter the expression of the α3α4α5(IV) and $\alpha5_2\alpha6$(IV) trimers in kidney basement membranes. Most *COL4A5* variants prevent expression of both trimer forms in the kidney, so that in about 80% of XLAS males immunostaining of kidney biopsy specimens for α3(IV), α4(IV) and α5(IV) chains is completely negative [78]. About 60–70% of XLAS females exhibit mosaic expression of these chains, while in the remainder immunostaining for these chains is normal. The biallelic variants in *COL4A3* and *COL4A4* that cause ARAS often prevent expression of α3α4α5(IV) trimers, but have no effect on expression of $\alpha5_2\alpha6$(IV) trimers. In kidney biopsy specimens from patients with ARAS, immunostaining for α3(IV) and α4(IV) chains is negative in the GBM. However, while immunostaining of GBM for the α5(IV) chain is negative due to the absence of α3α4α5(IV) trimers, Bowman's capsules, distal tubular basement membranes and collecting duct basement membranes are positive for α5(IV) due to the unimpaired expression of $\alpha5_2\alpha6$(IV) trimers. Heterozygous carriers of a single *COL4A3* or *COL4A4* mutation have normal kidney basement membrane immunostaining for α3(IV), α4(IV) and α5(IV) chains.

The $\alpha5_2\alpha6$(IV) trimer is a normal component of the skin epidermal basement membrane (EBM). Consequently, about 80% of males with XLAS can be diagnosed by skin biopsy based on absence of α5(IV) expression in EBM. In 60–70% of XLAS females, there is a mosaic pattern of EBM immunostaining for α5(IV). EBM expression of α5(IV) is normal in patients with ARAS and in subjects with heterozygous variants in *COL4A3* or *COL4A4*.

Diagnosis and Differential Diagnosis

AS is one potential cause of familial and sporadic glomerular hematuria. Accurate diagnosis rests on careful clinical evaluation, a precise family history, selective application of invasive diagnostic techniques and, in appropriate patients, molecular diagnosis (Fig. 16.2).

The presence of isolated microscopic hematuria in a child with a positive family history for hematuria, an autosomal dominant pattern of inheritance, and a negative family history for kidney failure strongly suggests a diagnosis of heterozygous *COL4A3/4* variants (Fig. 16.2). Less common conditions associated with familial glomerular hematuria include the autosomal dominant *MYH9* disorders (Epstein and Fechtner syndromes), in which macrothrombocytopenia is a constant feature and familial IgA nephropathy. However, there may also be overlap with heterozygous Alport syndrome and a range of glomerular pathologies; large genetic sequencing studies will help to identify these disease group intersections.

When family history for hematuria is negative, the differential diagnosis of isolated glomerular hematuria, or hematuria associated with proteinuria includes AS, IgA nephropathy, C3

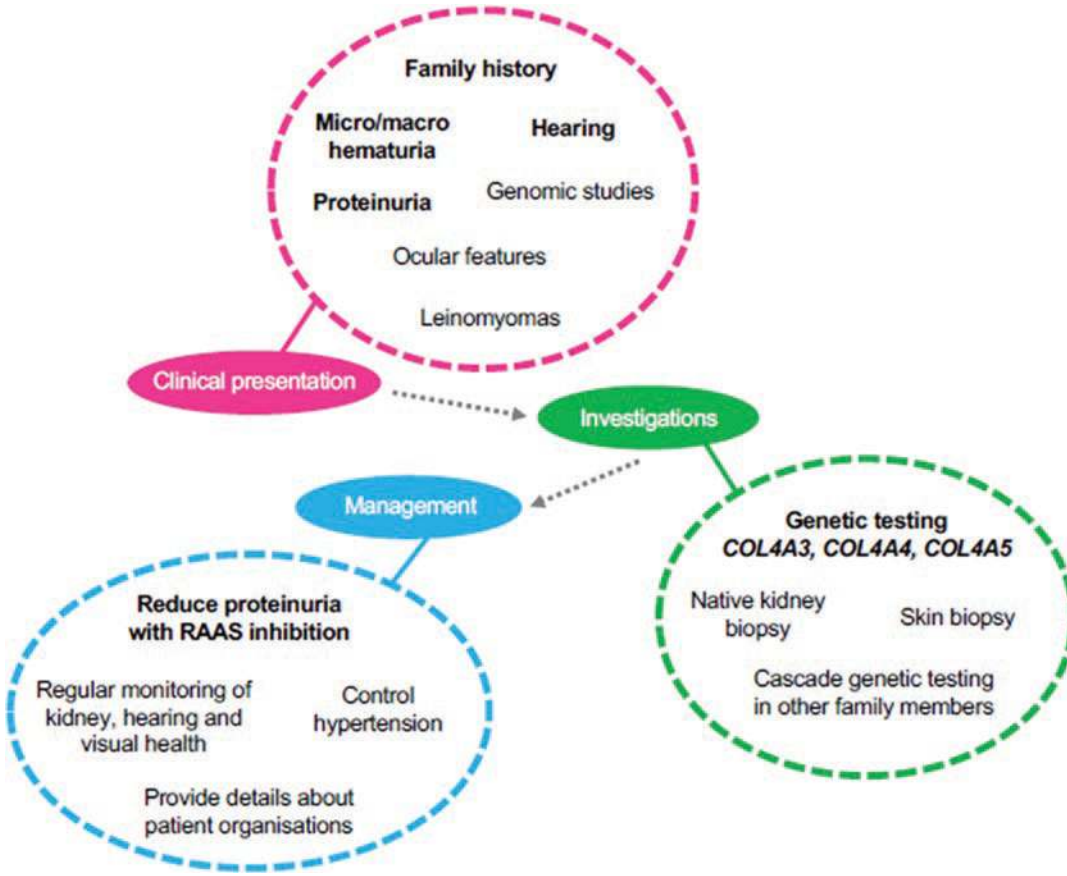

Fig. 16.2 Clinical presentation, diagnosis and management of Alport syndrome. Patients can present with a variety of clinical presentations, which should prompt investigation to confirm a diagnosis of Alport syndrome. Genetic testing for the Alport genes *COL4A3, COL4A4* and *COL4A5* is widely available and is the gold standard for diagnosis. Management includes the use of renin-angiotensin-aldosterone (RAAS) pathway inhibitors

glomerulopathy, membranous nephropathy, lupus nephritis, postinfectious glomerulonephritis, Henoch-Schönlein nephritis, and many other entities. Some of these conditions will be strongly suspected based on clinical findings (e.g., rash and joint complaints) while others will be suggested by laboratory findings, such as hypocomplementemia.

Formal audiometric and ophthalmological examinations should be considered as part of the diagnostic evaluation in children with persistent microscopic hematuria. Audiometry may be very helpful in children over age 6–8 years, especially boys, since high-frequency SNHL would point toward a diagnosis of AS. The presence of anterior lenticonus or the dot-fleck retinopathy may be diagnostic. However, these lesions are more prevalent in patients with advanced disease, and less likely to be present in the young patients in whom diagnostic ambiguity tends to be the greatest.

Genetic testing is the gold standard for diagnosing AS. Additional tissue studies are appropriate when clinical and pedigree information and genetic testing does not allow a diagnosis AS. Therefore, several options are available for confirming a diagnosis of AS, including genetic analysis, skin biopsy and kidney biopsy. Genetic analysis using Sanger sequencing is capable of identifying *COL4A5* variants in 80–90% of males with XLAS [79]. High variant detection rates in *COL4A3* and *COL4A4* in patients with ARAS are

also possible, particularly if there is parental consanguinity. Commercial genetic testing for variants in *COL4A3, COL4A4*, and *COL4A5* is available. Next generation sequencing, which allows simultaneous analysis of *COL4A3, COL4A4* and *COL4A5*, now replaces Sanger sequencing as the preferred approach. If further investigation is required, skin biopsy is often utilized as the initial invasive diagnostic procedure in patients suspected of AS it is less invasive and expensive than a kidney biopsy. On skin biopsy, the majority of subjects with XLAS will display abnormal expression of the α5(IV) chain in EBM as described above. Normal EBM α5(IV) expression in a patient with hematuria has several possible explanations: (1) the patient has XLAS, but his or her *COL4A5* mutation allows EBM expression of α5(IV); (2) the patient has ARAS, or ADAS, in which α5(IV) expression is expected to be preserved; or (3) the patient has a disease other than AS. Kidney biopsy would then provide the opportunity to diagnose other diseases, to examine type IV collagen α chain expression in kidney basement membranes, and to evaluate GBM at the ultrastructural level.

Treatment

The goal of treatment in AS is to slow the progression of kidney disease and delay the need for dialysis or transplantation. Several therapeutic approaches have demonstrated efficacy in murine ARAS, including angiotensin blockade [80–82], inhibition of TGFβ-1 [83], chemokine receptor 1 blockade [84], administration of bone morphogenic protein-7 [85], suppression of matrix metalloproteinases [34] and bone marrow transplantation [86]. Cyclosporine therapy slowed progression of kidney disease in a canine model of AS, but human studies have demonstrated significant nephrotoxicity and adverse effects and this treatment is not recommended [87–89]. Angiotensin converting enzyme (ACE) inhibition also prolonged survival in a canine XLAS model [90]. Uncontrolled studies in human AS subjects have shown that ACE inhibition can reduce proteinuria, at least transiently [91, 92]. A multicenter, randomized, double-blind study comparing losartan with placebo or amlodipine in 30 children with AS demonstrated a significant reduction in proteinuria in the losartan treated group [93]. An extension of this study showed comparable efficacy of either enalapril or losartan in reducing proteinuria in children with AS [94]. A report from the European Alport Registry, which includes 283 patients over 20 years, compared kidney outcomes in AS patients treated with ACE inhibition at various time points: at onset of microalbuminuria, at onset of proteinuria, or in chronic kidney disease (CKD) stage III-IV [95]. This retrospective review demonstrated a delay in kidney replacement therapy by 3 years in the treated CKD group and by 18 years in the treated proteinuric group [95]. These findings were confirmed in a retrospective review of kidney outcomes in men with XLAS from Japan [45]. In this study, men who received ACE inhibitors reached renal failure an average of 22 years later than those who did not receive ACE inhibitors. This beneficial effect of ACE inhibitors was also apparent in the subgroup of men with severe truncating type variants [45]. A randomized, placebo controlled trial of ramipril vs placebo in children with early Alport syndrome (microscopic hematuria alone or microalbuminuria stage) was recently reported [96]. Although not significant due to low enrollment, patients randomized to ramipril had decreased risk of progression of proteinuria and slower decline of GFR compared to patients randomized to placebo [96]. An open-label arm of this study demonstrated no safety concerns in over 200 patient years of treatment with ramipril [96].

Current clinical practice guidelines recommend treatment with an ACE inhibitor for affected males with XLAS and males and females with ARAS at the time of diagnosis if older than 12–24 months. (Table 16.2). Treatment should be started for females with XLAS and males and females with heterozygous variants in COL4A3 or COL4A4 when microalbuminuria is present [97]. Similar to other children with CKD, blood pressures

Table 16.2 Recommendations for timing of treatment with ACE inhibitors in patients with Alport syndrome

Genetic results	Indication for treatment
ARAS or male with XLAS	At time of diagnosis if age >12–24 months
XLAS female	Microalbuminuria
ADAS (heterozygous variant in *COL4A3* or *COL4A4*)	Microalbuminuria

ARAS autosomal recessive Alport syndrome; *XLAS* X-linked Alport syndrome; *ADAS* autosomal dominant Alport syndrome

should be controlled to the 50% for age, gender, and height in children with AS in order to slow the progression of kidney disease [98].

A number of additional agents are currently in clinical development for treatment of Alport syndrome kidney disease. MicroRNAs are small, highly conserved RNAs that regulate gene expression post-transcription. One of these microRNAs, microRNA-21, is upregulated in kidneys of mice with Alport syndrome and contributes to fibrosis [99]. Treatment of Alport mice with an anti-microRNA 21 agent reduces proteinuria and kidney fibrosis and prolongs lifespan [99]. This agent is undergoing testing in a randomized phase II clinical trial in adult patients with Alport syndrome (NCT02855268). Bardoxolone is a second agent currently being tested in a randomized phase II/III clinical trial in patients with Alport syndrome (NCT03019185). Bardoxolone activates Nrf-2 and inhibits NF κB to upregulate the antioxidant response and decrease proinflammatory signaling [100]. In a clinical trial in patients with kidney disease due to type 2 diabetes, bardoxolone increased eGFR; however, the trial was halted due to increased risk of hospitalization and death from heart failure in the bardoxolone treated patients [101]. Bardoxolone treated patients also demonstrated increased proteinuria [101]. It remains controversial whether patients with Alport syndrome will have sustained benefit from treatment with bardoxolone, and long-tern studies will be required to demonstrate value in slowing progression of CKD [102].

Kidney Transplantation

In general, outcomes following kidney transplantation in patients with AS are excellent [103]. Clinicians involved in transplantation of AS patients must address two important aspects of the disease. First, the donor selection process must avoid nephrectomy in relatives at risk for ESKD. Second, post-transplant management should provide surveillance for post-transplant anti-GBM nephritis, a complication unique to AS.

Informed donor evaluation requires familiarity with the genetics of AS and the signs and symptoms of the disease. In families with XLAS, 100% of affected males and ~95% of affected females exhibit hematuria. Consequently, males who do not have hematuria are not affected, and a female without hematuria has only about a 5% risk of being affected. Given an estimated 30% risk of ESKD in women with AS [46], these women should generally be discouraged from kidney donation, even if hematuria is their only symptom. A report from Germany described five women with XLAS and one ARAS carrier who served as kidney donors [104]. One donor had proteinuria prior to transplant and all had microscopic hematuria. Three donors developed new onset hypertension and two developed new proteinuria while kidney function declined by 25–60% over 2–14 years after donation in four of the donors, highlighting the increased donor risk in this population [104].

Overt anti-GBM disease occurs in 3–5% of transplanted AS males [105]. Onset is typically within the first post-transplant year, and the disease usually results in irreversible graft failure within weeks to months of diagnosis. The risk of recurrence in subsequent allografts is high. In males with XLAS, the primary target of anti-GBM antibodies is the α5(IV) chain [106, 107]. Both males and females with ARAS can develop post-transplant anti-GBM nephritis, and in these cases the primary antibody target is the α3(IV) chain [106, 108]. The α3(IV) chain is also the target of Goodpasture autoantibodies, but the epit-

ope identified by these antibodies differs from the α3(IV) epitope recognized by ARAS anti-GBM alloantibodies [109].

Hereditary Angiopathy with Nephropathy, Aneurysms and Cramps (HANAC Syndrome)

This autosomal dominant disorder results from variants in the *COL4A1* gene (Table 16.1) [9, 110, 111]. Complete absence of *COL4A1* is embryonic lethal in mice [112]. Missense variants that allow for expression of an abnormal α1(IV) chain lead to the development of HANAC syndrome. Kidney findings include gross and microscopic hematuria, cysts and CKD. Vascular anomalies include cerebral artery aneurysms and retinal arteriolar tortuosity. Affected individuals may have recurrent muscle cramps and elevated creatine kinase levels.

Pathology

No abnormalities of GBM ultrastructure or basement membrane expression of type IV collagen chains have been observed in kidney biopsy specimens from affected individuals with hematuria. Irregular thickening, lamellation and focal interruptions of Bowman's capsules, tubular basement membranes and interstitial capillary basement membranes have been described, as well as abnormalities of epidermal basement membranes and dermal arterial basement membranes.

Genetics

The reported variants in HANAC syndrome families affect highly conserved glycine residues in the collagenous domain of the α1(IV) chain, potentially affecting integrin binding sites. It is likely that a wider spectrum of disease will emerge in association with both *COL4A1* and *COL4A2* variants as well as variants in other basement membrane genes as larger cohorts of patients with kidney disease phenotypes undergo whole exome and whole genome sequencing [113].

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IgA Nephropathy

17

Rosanna Coppo and Licia Peruzzi

Introduction

Primary IgA nephropathy (IgAN) is defined by the prevalence of immunoglobulin A over the others immunoglobulins in glomerular deposits as described by Berger et al. in 1968 [1]. IgAN is the commonest glomerular disease in children and adolescents who undergo renal biopsy because of isolated microscopic hematuria or hematuria with non-nephrotic proteinuria. After its initial identification, IgAN was considered a benign renal disease in adults and even more so in children. With longer follow-up in both age groups, a significant percentage of cases had a worsening of renal function, including the need for renal replacement therapy (RRT). The interest in IgAN in children increased with the recognition that most subjects with IgAN entering a chronic dialysis program were young adults [2], who displayed a slow renal function decline over decades (about 25% of the cases need dialysis in 20 years) [3]. It was therefore clear that the primary pathogenic events occurred in many cases in children. Hence, detecting IgAN at the beginning of its natural history in childhood may offer an important opportunity for early treatment, limiting factors favoring progression and controlling the complications, maximizing the benefits during childhood, and perhaps more importantly, for adulthood.

Epidemiology

The prevalence of IgAN in children varies in different reports, mostly due to the criteria for performing renal biopsy, ranging from routine practice after detection of urine anomalies by school screening programs to limiting renal biopsy to patients that have developed proteinuria. It is likely that cases of IgAN originating in the pediatric age group are missed because most patients are asymptomatic and do not undergo regular urine screening. IgAN is more frequently reported in Japan and Korea, where screening is routine, and IgAN is reported in 32 and 40% of renal biopsies in children [4, 5]. In contrast, the percentage of renal biopsies with IgAN in children are 20–26% in Europe [6], 17–20% in China, 14% in India, 10% in North America, 14% in South America, and 2.8% in Africa [7].

Data on incidence in children vary from 5 to 140 per million children/year [6, 8], similar to adult figures. The most recent biopsy series from global registries conclude that incidence varia-

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tion is mainly due to biopsy policy and attitudes, and not secondary to true ethnic differences [9–11], although genetic studies seem to suggest a Northbound and Eastbound gradient of IgAN susceptibility [12]. Moreover, there is evidence in specific areas of China that incidence may vary based on humidity, air contaminants and other pollutants, such as polycyclic aromatic hydrocarbons [13].

Genetic Background

Familial aggregation of IgAN cases and accumulation of IgAN in certain populations suggest a complex heritable component. Familial clustering is consistent with autosomal dominant inheritance with variable penetrance of a polygenic trait, often manifesting as other autoimmune diseases. Familial cases often have a worse outcome.

Approximately 70% of IgAN subjects and 35% of their healthy relatives in different ethnic cohorts have Gd-IgA levels higher than geographically matched controls, suggesting that Gd-IgA by itself is not sufficient to induce the disease [14]. Gd-IgA has a greater specificity, sensitivity and heritability than total serum IgA [15].

Family linkage studies identified 6q22-23 as a candidate locus [16] and additional loci at 4q26-31, 17q12-22 and 2q36, but no causal underlying mutation was observed.

Next generation sequencing in the last years provided the technical tools to rapidly detect genetic variants at genome scale (whole genome sequencing) or on coding regions (whole exome sequencing), making possible large genome-wide association studies (GWAS) on multiethnic cohorts.

This powerful unbiased approach, [17, 18] supported by strict statistical methods and precise population stratification, was employed to explore disease association of common variants single nucleotide polymorphism (SNP) along the entire genome of large populations. Five large GWAS (reviewed in [17]) were carried out in populations of European and East Asian origin, evidencing strong signals in chromosome region 6p21, encoding for HLA genes involved in antigen presentation and immune response. HLA-DQA1 and DQB1 genes, which give origin to at least four HLA alleles, were associated with IgAN: DQA1*0101 and DQB1*0301 as risk alleles and DQA1*0102 and DQB1*0201 as protective [18].

A role of the non-HLA loci 1q32, encoding complement factor H (CFH), a critical inhibitor of the alternative pathway of complement, and five CFH-related (CFHR) genes [19] was identified.

The deletion in *CFHR3* and *CFHR1* genes, encoding for activators of the alternative complement pathway with a competitive effect on CFH, is associated with higher levels of CFH, lower complement activation and lower C3 deposition, with a protective effect on C3 activation in IgAN [20–22].

Other non-HLA regions associated with IgAN are 22q12, coding for several cytokines, 17p13, for TNFSF13, 8p23, for an alpha-defensin gene, 16p11, for alpha-integrins ITGAM and ITGAX, 1p13, for VAV37 and 9q34, for CARD9 [17]. In the Chinese population, other loci were found in 3q27.3, coding for ST6GAL1, in 11p11.2, for ACCS and 8q22.3, for ODF1-KLF10 [23].

Candidate genes are involved in antigen processing and presentation, innate immunity, NFkB signaling, gut mucosal immunity, IgA molecule biology and dysregulation of alternative complement pathway. Altogether these loci explain 6–8% of the risk, overlapping with other immune mediated diseases [18]. Genetic risk correlates with age at diagnosis and is associated with pathogen diversity, in particular helminths [24].

Moreover IgAN risk score varies in parallel to the geographic distribution of the disease, with a northbound and eastbound gradient, progressively increasing at growing distance from Africa and moving east [12].

Using whole exome sequencing, Zhou [25] in the Han Chinese population identified three candidate susceptibility genes in the non-HLA region (FBXL21, CCR6, and STAT3) and one in the HLA region (GABBR1), involved in modulation of IgA synthesis, response to mucosal infections and gut inflammatory response.

Pathogenesis

The development of IgAN represents the final event of a complex pathogenetic cascade, called the "four hit model", putting together the principles of an immune disease, with a genetic background and an environmental exposure. This causes inappropriate production of aberrantly glycosylated IgA1 (first hit), followed by a specific IgG response (second hit) and IgA immune-complexes circulation and glomerular deposition (third hit). The glomerular inflammatory response is elicited, with recruitment of multiple mechanisms leading to glomerular damage (fourth hit) (Fig. 17.1).

IgA is the most abundant immunoglobulin synthesized by the mucosal associated lymphoid tissue (MALT) and plays a major role for mucosal antigen defense and host commensal homeostasis.

IgA is synthetized in two subclasses, IgA1 and IgA2, which differ by an insertion of 18 amino acids in the hinge region between CH1 and CH2, which is only present in IgA1 [26] (Fig. 17.2a, b). The amino acid sequence includes three threonine and three serine residues bound to five short O-linked oligosaccharide chains. The O-glycosylation consists of a core N-acetyl galactosamine (GalNAc) which occurs alone or extended with β1,3 linked Gal or further with sialic acid in α2,3 or α2,6 linkage [27, 28]. In healthy subjects, serum IgA1 consists of a mixture of molecules with different O-glycoforms, whereas in patients with IgAN there are abnormal IgA1 O-glycoforms deficient in galactose (Gd-IgA1) with a high frequency of O-glycans

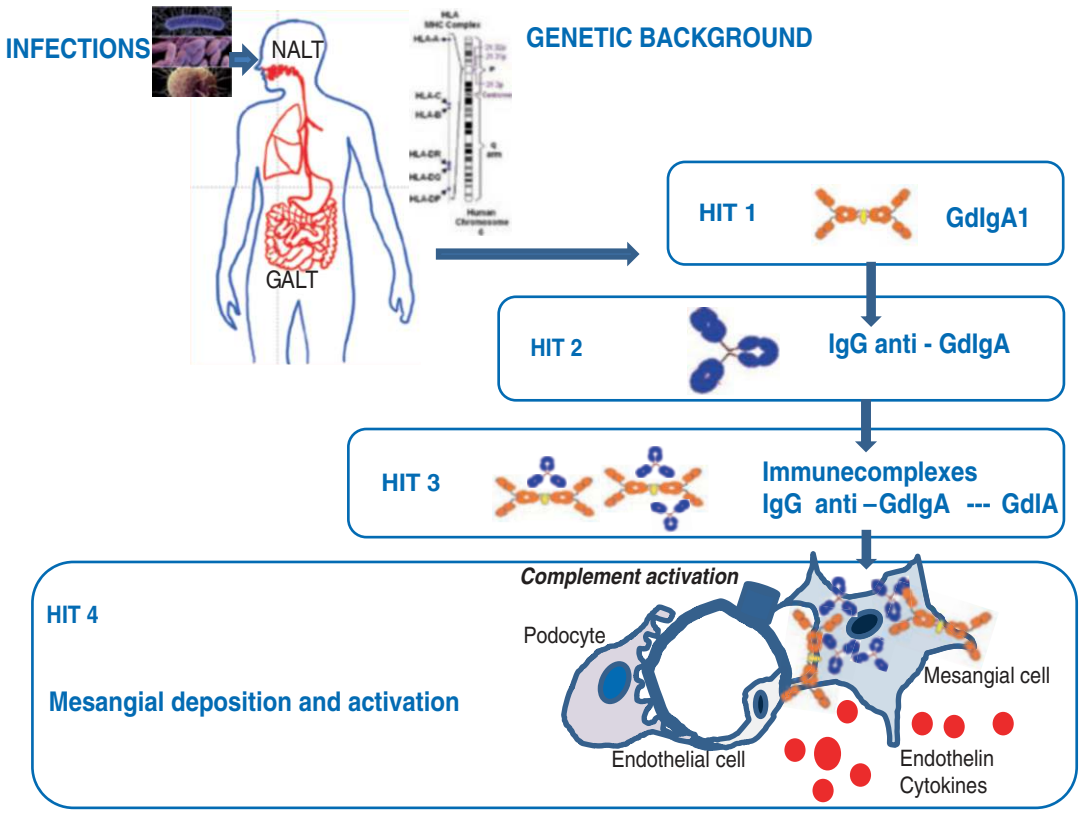

Fig. 17.1 The multihit pathogenic model

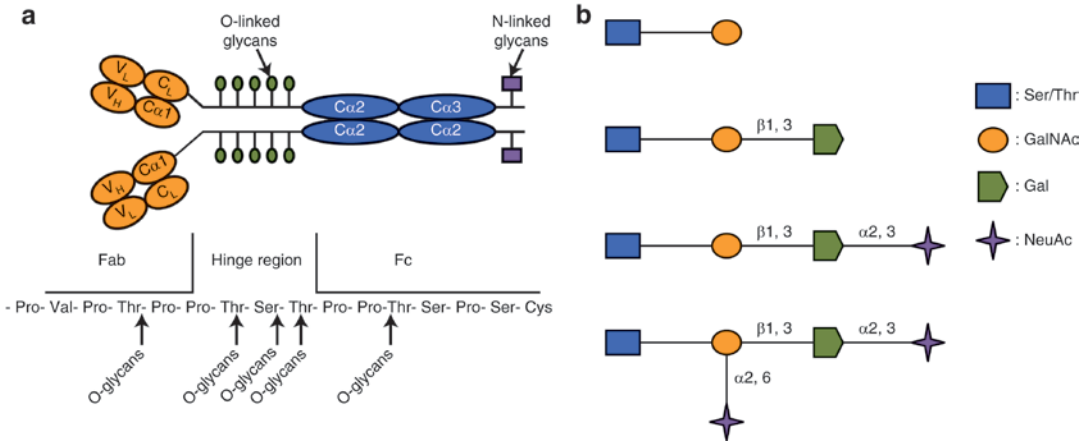

Fig. 17.2 The IgA molecule structure: (**a**) magnification of the hinge region. (**b**) Different O-glycoforms of circulating IgA1 molecules in IgAN

consisting of GalNAc alone [29–31]. Gd-IgA1 is detected in sera of up to 80% of patients with IgAN, [26, 29–33] and is prevalent in mesangial deposits of these patients [26].

The altered glycosylation of the IgA molecule confers different functional and effector mechanisms such as activation of the alternate complement pathway, Fc receptor binding, polymerization capacity and mesangial deposition due to increased affinity for matrix components. Gd-IgA1 has a hereditary component in either sporadic or familial IgAN in adults and children, [14, 15] indicated by increased plasma levels detected in healthy relatives of patients [14, 34].

Since altered glycosylation is not due to a different amino acid sequence of the hinge region [35], research efforts focused on genetically determined modifications of the B lymphocytes enzyme β 1,3-galactosyltransferase (encoded by *C1GALT1*), responsible for the terminal galactosylation of GalNAc on O-linked glycans [36] or the galactosyl transferase enzyme chaperon Cosmc (encoded by *C1GALT1C1*), but the results were not consistent with a clear genetic modification of these enzymes [34, 37].

Post-transcriptional regulation may be due to the modulatory effect of micro-RNAs, small molecules that can decrease the transcription of RNAs. Increased levels of miRNA-148b favoring the expression of the enzyme C1GALT have been detected in patients with IgAN [38].

Gd-IgA1 can circulate as monomers or participate in macromolecular self-aggregates and can elicit an IgG autoimmune response, which represents the second pathogenetic hit [29, 39]. Serum Gd-IgA1 and IgG autoantibodies have been found to correlate with disease severity and progression [40–44]. It is of interest that these IgG not only react with Gd-IgA1, but may cross-react with bacterial or viral cell-surface GalNAc-containing glycoproteins, suggesting a possible mechanism of mimicry [45]. The co-deposition of IgG in glomeruli has been demonstrated by confocal microscopy even in cases negative for routine immunofluorescence and correlates with endocapillary proliferation [46, 47].

The circulating immune-complexes (IgAIC) formed by polymeric Gd-IgA and IgG (the third hit) escape clearance by hepatic receptors and have a preferential renal deposition due to reactivity with mesangial matrix components fibronectin, laminin and collagen. The deposit on mesangial cells induces a proliferative response, matrix expansion and local inflammation [48]. IgAIC correlate with disease activity in adults and children with IgAN [40].

Macromolecular IgA also originate from interactions between human IgA1 and CD89, the soluble form of the IgA receptor [49]. The IgA1–CD89

complex binds to the transferrin 1 receptors (CD71) and transglutaminase 2 receptors (TGase2) expressed on the mesangial cell surface and increase immune deposits and inflammation [50].

The fourth pathogenic hit occurs after the renal deposition of IgAIC, Complement activation pathways are triggered and the local inflammatory process is further amplified, with release of cytokines, chemokines and inflammatory mediators acting not only on mesangial cells but also on endothelium and podocytes [51]. Mesangial cells and mesangial matrix proliferation induce a derangement of the glomerular spatial relationships, which influences podocyte behavior. Mesangial-podocyte crosstalk has been proposed as a trigger of a podocytopathy and tubulointerstitial damage, with consequent occurrence of proteinuria and disease progression [52]. In this process, activation of the RAS system also plays a crucial role [53].

The Mucosal Immunity in IgAN

The relationship between IgAN and the mucosal associated lymphoid tissue (MALT) has been considered shortly after the identification of this glomerular disease because IgA is the main product of the MALT and visible hematuria in IgAN frequently occurs during mucosal infections. About half the lymphocytes of the immune system are located in the MALT, which is at the interface with the environment [54], and thus has a major role in the defense against environmental microbes and induction of immunotolerance [55]. The principal locations of the MALT are the gut-associated lymphoid tissue (GALT) and the nasopharynx-associated lymphoid tissue (NALT), which are thought to be involved in the pathogenesis of IgAN. The GALT has up to 70% of the body's immunocytes and consists of lymphoid follicles, aggregated at the Peyer's patches, in the distal jejunum and the ileum [56]. The NALT is located in the pharynx, principally in Waldeyer's tonsillar ring, of which tonsils are the major component [57].

Peyer's patches and tonsils are the most common inductive sites where antigens prime naïve B-cells through T-cell–dependent and T-cell–independent mechanisms [58]. T cell–independent production of IgA is primarily stimulated by interleukins (IL-6 and IL-10), transforming growth factor (TGF-β), B cell activating factor (BAFF, or BLyS), and a proliferative inducing ligand (APRIL). BAFF and APRIL promote the differentiation of B cells, with class switching from IgM to IgA1 after binding to the TNF receptor homolog transmembrane activator (TACI) [59]. Activated B-cells reach the mucosal inductive sites, where they become effector cells, maturing into IgA-secreting plasma cells in the intestinal lamina propria. They produce IgA dimers, which bind a secretory component, creating secretory IgA, which is then secreted into the lumen.

The innate immune system recognizes pathogens, particularly through the activation of Toll-like receptors (TLRs), which increase IgA synthesis via promoting secretion of BAFF and enhancing lymphocytic infiltration and the expression of histocompatibility complex class II molecules on B-cells. Persistent activation of TLRs may favor increased synthesis of Gd-IgA1 in prone subjects [60].

There is a tonsil-kidney axis in IgAN. Some of the IgA eluted from renal tissue in patients with IgAN originates in tonsillar cells [61], and the number of IgA producing plasma cells in tonsils is higher in patients with IgAN than in controls [62]. These plasma cells create memory cells and may migrate to the bone marrow, where they synthesize and release Gd-IgA into the circulation. Persistent IgA deposits and hematuria were induced in mice by intranasal administration followed by systemic challenge of the respiratory Sendai virus after induction of defective mucosal tolerance [63, 64]. A microbiome study found similar tonsillar microbes in patients with IgAN and subjects with recurrent tonsillitis [65], but this observation was not confirmed [66]. Studies have shown activation of TLR9, which recognizes unmethylated DNA sequences in bacterial and

viral DNA cytosine-phosphorothioate-guanine oligodeoxynucleotides (CpG ODN) in the tonsils of patients with IgAN. TLR9 expression was associated with disease activity and clinical outcome after tonsillectomy [67]. The TLR9 ligand CpG-ODN increased the expressions of APRIL, favoring the delivery of nephritogenic IgA. Human B cells infected with Epstein-Barr virus (EBV) secrete Gd-IgA1. It has recently been hypothesized that EBV infected IgA+ cells, which have homing receptors for targeting the upper respiratory tract, may be the source of galactose-deficient IgA1. Moreover, the temporal sequence of racial-specific differences in Epstein-Barr virus infection may explain the racial disparity in the prevalence of IgAN [68].

The gut-kidney axis in IgAN [69] with dysregulated GALT was postulated after the observation of the increased association of IgAN with celiac disease [70]. This hypothesis was supported by a GWAS study showing that most loci associated with IgAN are also associated with immune-mediated inflammatory bowel diseases, maintenance of the intestinal barrier and response to gut pathogens [18]. In experimental transgenic mice overexpressing BAFF and presenting with hyper-IgA, the development of IgAN is conditioned by alimentary components and intestinal microbiota [71].

The intestinal microbiota can modulate the GALT [72] and different microbiota patterns have been reported in patients with progressive IgAN [73]. Increased intestinal permeability was detected in children and adults with IgAN [74, 75], together with high levels of IgA anti-alimentary antigens [76]. Chronic enteritis, reported in China as the second most common cause of mucosal infection, present in one third of cases of IgAN, may favor increased release of bacterial lipopolysaccharide (LPS), which can interfere with the chaperone gene Cosms, leading to production of Gd-IgA1 [77]. This hypothesis is also supported by increased expression of TLR4 (LPS specific ligand) in peripheral mononuclear cells in children and adults with IgAN [78, 79].

GALT may also be triggered by abnormal response to alimentary antigens, as detected in experimental models of oral immunization in mice [80]. Mice fed a gluten-rich diet had significantly greater IgA mesangial deposits than animals fed a gluten-free diet since birth [81]. IgA anti-alimentary components and IgAIC were reduced after a gluten-free diet in subjects with IgAN [82]. The role of gliadin was confirmed in IgA1/CD89 double transgenic mice that develop spontaneous IgAN; administration of a gluten-free diet for three generations prevented IgA deposits. The same study reported high levels of anti-gliadin IgA was associated with increased proteinuria in patients with IgAN. These data suggest a role of GALT hyper-response to intestinal alimentary components or microbes in IgAN.

Complement Activation in IgAN

The presence of C3 mesangial deposits with IgA deposits has been observed since the first description of IgAN [1], but there are many recent insights into the role of complement in IgAN. The alternative pathway is the main cascade activator in IgAN and most responsible for C3 deposition, which helps to differentiate incidental IgA mesangial deposits from true IgAN. C3 deposition is found in up to 90% of biopsies, frequently in association with C4d and usually without C1q, indicating the involvement of the alternative and lectin pathways. Properdin is present in a very high percentage of biopsies (70–100%), and CFH is present with variable frequency (30–90%) [21, 83, 84]. CFHR proteins may modulate CFH activity and *CFHR1* and *CFHR3* deletions in locus 1q32 were found to limit the alternative complement pathway activation, conferring a protective effect for development of IgAN [20]. IgAN cases with significantly higher plasma levels of FHR1 were more likely to have progressive disease [20, 85], and FHR5 plasma levels directly correlated with histologic markers of kidney injury [85–88]. Increased C3 breakdown products and IgA/C3 ratio were found to be associated with worse outcomes [89, 90].

The activation of the lectin pathway is indicated by the presence of C4d without C1q, and the detection of mannose-binding lectin (Man),

though not coincident. C4d is present in the renal biopsies of most actively progressive patients with IgAN and is a significant risk predictor for children and adults [91–94]. The terminal complement products C5-C9 may play an additive inflammatory role in the pathogenesis of IgAN; they are detected in biopsies and in in vitro experimental models [95].

Systemic oxidative stress is triggered by circulating IgAIC containing Gd-IgA1 [96]. Elevated levels of advanced oxidation protein products (AOPP) have been detected in sera of patients with IgAN and found to be correlated with the amount of proteinuria and with decreased renal function during follow-up. The association of high levels of Gd-IgA1 and AOPPs represents a risk factor for progression of IgAN.

Acquired factors which modulate the immune response are likely to be activated in some patients, with increased production of IL-4 and IL-5 by Th2 lymphocytes in IgAN leading to synthesis of Gd-IgA1 which is eventually deposited in the glomeruli.

The interaction of circulating macromolecules containing Gd-IgA1 with Fcα receptors on mesangial cells results in cellular activation and phlogistic mediator synthesis, including a variety of cytokines (IL6, platelet-derived growth factor [PDGF], IL1, TNF-α, TGFβ), vasoactive factors (prostaglandins, thromboxane, leukotrienes, endothelin, PAF, NO) and chemokines (MCP-1, IL-8, MCP-1, RANTES). The influx of monocytes and lymphocytes into the mesangium is enhanced by the C3 co-deposition.

The immune activation of mesangial cells leads to cell contraction, hemodynamic modifications and activation of the RAS [53]. Angiotensin II enhances the release of cytokines and chemokines and potentiates the actions of PDGF and TGFβ as growth factors for mesangial cells, further favoring proliferation and accumulation of extracellular matrix, and ultimately promoting sclerosis. In IgAN, there is no definite evidence of altered ACE genotype frequency, though some studies reported an association of one genotype (DD) with a faster rate of progression in IgAN and a better response to ACE-I treatment [97, 98].

Proteomics identified uromodulin as a urinary marker distinguishing IgAN from healthy controls and other glomerular diseases [99].

Renal Pathology of Children with IgAN

Light Microscopy

Primary IgAN typically presents with focal or diffuse proliferation of mesangial cells and expansion of the extracellular matrix. In both adults and children, endocapillary hypercellularity or extracapillary proliferation with crescent formation can be detected in active cases, while glomerular hyalinosis and segmental or global sclerosis or tubulointerstitial fibrosis are most prominent in patients with long lasting disease. The histological features in children are usually mild or moderate, and the rapidly progressive forms with crescents involving more than 50% of glomeruli are rare. Interstitial and arteriolar changes are infrequent. The variability of pathologic features is influenced by the approach for identifying patients to receive a renal biopsy since mild lesions are common in children with asymptomatic, microscopic hematuria detected by school screening programs [4, 5].

Several authors have proposed histological classifications of IgAN based on individual lesion intensity or extension, but all had a low clinicopathological correlation [100].

The Oxford classification for IgAN is predictive of clinical outcome in children and adults with IgAN, and it is presently universally adopted [101, 102]. It consists of a combined score of four lesions (mesangial and endocapillary hypercellularity, segmental glomerulosclerosis and tubular atrophy/interstitial fibrosis), MEST, which are predictive of outcome independent of clinical assessment (Fig. 17.3a–d). The value of crescents was not found in the original cohort, but was shown in a subsequent large study of more than 3000 patients with IgAN [103], leading to the use of the combined MEST-C score in patients of any age [52, 104].

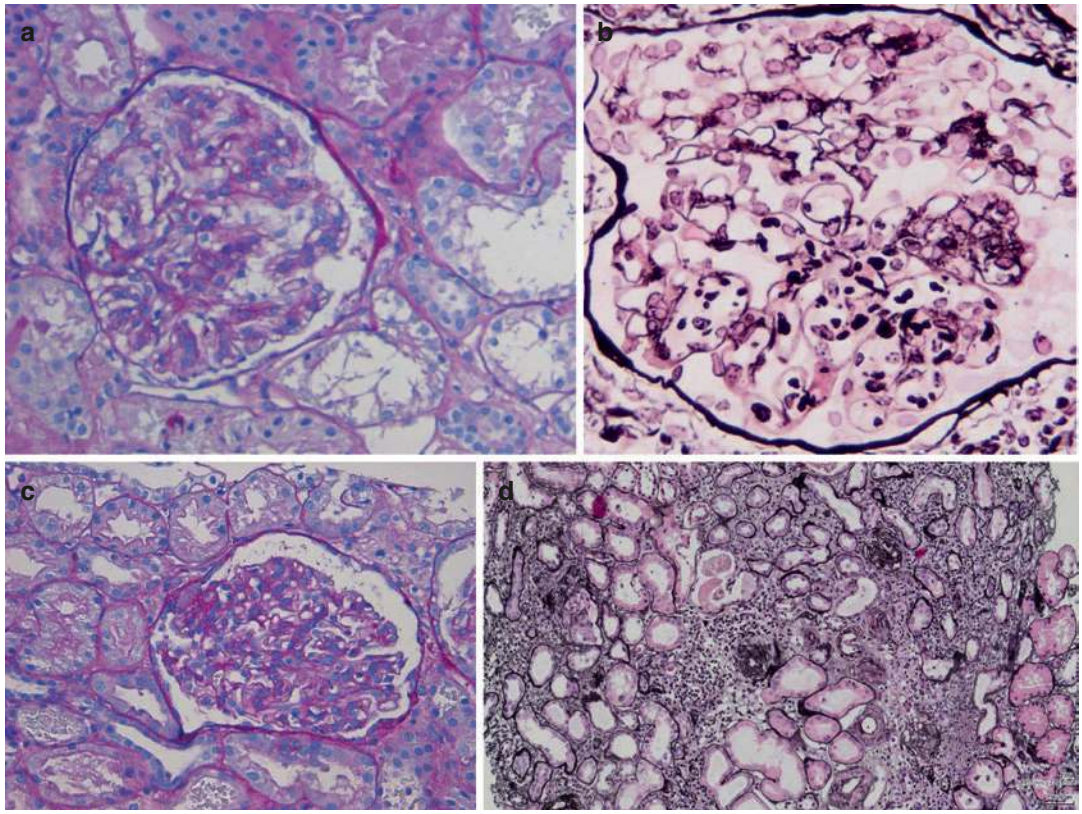

Fig. 17.3 Pathology features in IgA nephropathy. (**a**) Mesangial hypercellularity. (**b**) Endocapillary hypercellularity. (**c**) Segmental glomerulosclerosis. (**d**) Tubular atrophy/interstitial fibrosis

Although children with IgAN had more proliferative lesions and fewer chronic changes than adults, the predictive value of each lesion in the Oxford IgAN clinicopathological classification on renal survival was similar in children and adults [105].

Several validation studies have confirmed the value of the Oxford classification in children with IgAN [106–109]. The VALIGA study investigated 1147 patients with IgAN and validated the MEST score over a long-term follow-up, confirming the value of this pathology classification across the whole spectrum of IgAN, and maintaining the predictive value decades after the initial renal biopsy [110]. The search for clinical-pathological correlation and predictive value on outcome using small pediatric cohorts presents several limitations for a correct interpretation of the renal biopsy findings. The indication to perform the renal biopsy may select cases of different severity and potential risk. Moreover, the delay from clinical onset to renal biopsy detected different lesions, with higher mesangial and endothelial hypercellularity and more frequent crescents in early renal biopsies and greater segmental sclerosis and tubulointerstitial changes in delayed biopsies. The most important feature associated with outcome, in both children and adults, is tubulointerstitial damage, a marker of irreversible changes. However, much of the interest is on the predictive value of potentially reversible pathologic features, which may support a decision to initiate therapy. This occurs with podocytopathic lesions (tip lesions and podocyte hypertrophy), which, though associated with more severe proteinuria at onset and worse outcome, improved after immunosuppressive treatment [52].

Immunofluorescence

By definition, IgA is the dominant immunoglobulin present in all glomeruli (Fig. 17.4), and it is almost exclusively polymeric IgA1 with λ light chains. C3 is detectable in up to 70% of renal biopsies with the same distribution as IgA. IgG is present in 50–70% of the renal biopsies, often as intense as IgA, a feature that explains why this disease was initially called IgA–IgG nephropathy. IgM deposits are also found, but less commonly [31–66%]. The early complement components, such as C1q and C4, are rarely detected, but when present they are invariably associated with IgG and/or IgM.

The spatial distribution of IgA and C3 in mesangial deposits, studied by confocal laser microscopy, shows IgA and C3 coated by IgA in milder cases. A stronger deposition of C3c and C4d was reported in IgAN with endocapillary proliferation and active disease [91, 92, 94]. As mentioned before, the presence of C4d deposits was found to be associated with more rapid progression of IgAN in both adults and children [111]. C4d deposits often are associated with signs of thrombotic microangiopathy, suggesting that the interaction of Gd-IgA1 with endothelial cells triggers local inflammation, and activation of the complement and coagulation pathways [112].

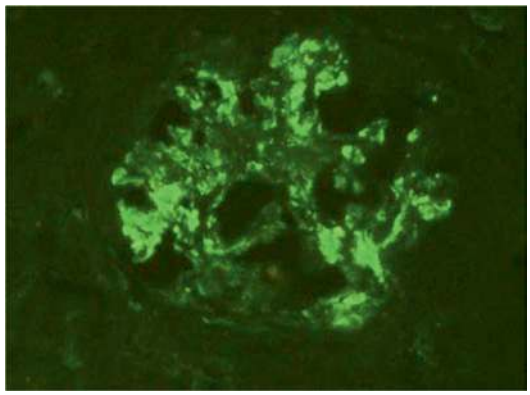

Fig. 17.4 Immunofluorescence staining with anti-IgA antibody. Mesangial distribution of IgA staining

Electron Microscopy

The most characteristic change on electron microscopy is the presence of electron-dense deposits in mesangial and para-mesangial areas.

Clinical Features

IgAN is not common in children under the age of 3 years; it is more common in adolescents [7]. In some children, the diagnosis follows an incidental finding of microscopic hematuria [113]. Asymptomatic patients may present with associated mild proteinuria (<0.5 g/day/1.73 m^2) in 3–15% of cases [114–116]. The most typical presentation of IgAN is gross hematuria coincident with upper respiratory tract infections or other mucosal inflammatory processes (in 30–40% of children). Macroscopic hematuria rarely occurs after vaccination or heavy physical exercise. The interval between the triggering event and the appearance of macroscopic hematuria is usually short (12–72 h). The macroscopic hematuria persists for few days, sometimes with flank and loin pain and fever. Some children have recurrent episodes without relevant urinary abnormalities between episodes. A transient increase in proteinuria occurs during episodes of gross hematuria. In some children, gross hematuria is associated with increased serum creatinine and hypertension [114–116], seldom with acute oliguric renal failure. In these cases, the renal biopsy shows mesangial, endocapillary and extracapillary proliferation and frequently tubular obstruction by packed red blood cells [117]. In rare cases, extensive crescentic lesions are detected [113]. Hypertension may develop during long-term follow-up or in particularly severe cases [113, 114]. An atypical presentation is nephrotic syndrome, which is reported in 7% of the cases, with podocytopathy similar to minimal change disease.

Natural History

IgAN in children is, with few exceptions, the early phase of the overall natural history of the disease. Severe clinical signs usually develop after 5–15 years, indicating the need for long follow-up, including as adults, to define the outcome of IgAN detected in childhood [118].

The natural history of IgAN varies according to the timing of performing the renal biopsy, and it differs in children diagnosed after a screening program; immediately after an onset with acute nephritic syndrome; or when there are signs of progression with decline in glomerular filtration rate (GFR). Moreover, the natural history has changed from the initial reports to the current era due to early detection and prompt therapy, including the use of RAS blockers (RASB) after diagnosis. Indeed, in the first pediatric report in 1986 of 91 French children with more than 13 years of follow-up, Levy et al. [116] reported chronic renal failure in 9%. In Finnish children, end-stage kidney disease (ESKD) from time of onset of diagnosis was found in 7% and 13% at 10 and 20 years, respectively [119]. An analysis of 500 children with IgAN in Japan showed a decrease in children reaching ESKD at 15 years from 20% to 1.2% when comparing renal biopsies before or after 1990. The European VALIGA study, over a median follow-up of 4.5 years, reported 4% of 174 children with ESKD. Since ESKD is uncommon in children, surrogate endpoints are frequently used (e.g. 50% reduction in eGFR or ESKD). In short-term follow-up studies, children seem to have a better prognosis than adults, but a 20 year survival analysis showed that IgAN in children was as progressive as in adults [115, 120]. In the long-term follow-up VALIGA study, survival to 50% reduction in eGFR or ESKD was 91% in children versus 70% in adults at 10 years and 82% in children versus 47% in adults at 20 years [110]. In a Japanese study of pediatric IgAN, patients were followed for a mean of 7 years. Clinical remission occurred in 50% and the predicted kidney survival rate was 92% at 10 years and 89% at 20 years [121]. Children with IgAN can have spontaneous remission of the urinary findings, as observed in Japanese children with IgAN and minor renal damage who did not receive medication after diagnosis [122]. Spontaneous remission could be observed after 5–8 years, but recurrences of urinary findings were detected in 20% at 5 years and in 42% at 10 years after remission. Hence, IgAN in children is a chronic disease, with phases of activity and clinical remission, rendering it difficult to provide a long-term prognosis on the basis of a short initial follow-up after renal biopsy.

Some children, usually those presenting with moderate microscopic hematuria without proteinuria and displaying the mildest lesions, do not progress to ESKD over decades of observation. In children with progressive IgAN, the clinical course is often slow and indolent. The most important factors associated with IgAN progression in adults, such as chronically reduced renal function at onset and persistent hypertension, are uncommon in children [123, 124]. The value of microscopic hematuria has been shown by some studies in adults with IgAN [125], but inter-center differences have limited the use of this urinary biomarker, so typical of IgAN, in collaborative studies.

In the VALIGA study, the clinical data, including proteinuria, blood pressure and eGFR at renal biopsy, were not significant predictors of the long-term outcome, indicating a possibility of spontaneous or drug-induced remission. Conversely, the most important risk for progression in children is the persistence of proteinuria (time-averaged) during follow-up. The threshold for time-averaged proteinuria in children is probably lower than what is accepted for adults. In adults with IgAN, only proteinuria >1 g/day is considered a significant risk for progression that deserves treatment, according to KDIGO recommendations [126]. For children, the 2021 update of KDIGO [127] suggests that those with >0.2 g/g urine protein/creatinine ratio (Up/UCr) should receive RAS inhibition. From long-term follow-up studies in children with IgAN, residual proteinuria <0.2 g/day/1.73 m^2 after treatment predicts a favorable outcome [128, 129].

In an analysis of the VALIGA cohort, only 7.5% of children with initial proteinuria >0.5 g/day/1.73 m^2 had persistent proteinuria <0.5 g/

day/1.73 m^2 at last follow-up, indicating a partial but not complete remission. The median value of proteinuria during a median 4.5 years of follow-up was 0.56 g/day/1.73 m^2, suggesting that most children with IgAN are exposed to a significant risk of progression over the following decades [106, 130].

Risk Prediction

There is a need to predict disease progression in children with IgAN to assess risk-based treatment decisions. An International IgAN Prediction Tool for adults with IgAN was recently developed to predict a 50% decline in eGFR or ESKD using clinical risk factors and MEST histology scores [131]. The variability of clinical and histological features of IgAN in children, the small number of children followed in individual centers, the short follow-up and the small number of outcome events (ESKD or 50% reduction in eGFR) represent substantial limitations for the detection of risk factors associated with progression in children. The use of the adult prediction tool was not satisfactory; hence, the global IgAN network updated the Prediction Tool for use in children using a multi-ethnic international cohort of 1060 children with IgAN followed into adulthood [132]. The Tool defined an end point of 30% reduction in eGFR or ESKD, which is considered an acceptable surrogate outcome in children with IgAN and proved to be well-calibrated. The trajectory of eGFR over time in children was different from adults: it was non-linear, with an increase in eGFR until 18 years of age followed by a linear decline similar to adults. A higher predicted risk was associated with a smaller increase in eGFR followed by a more rapid decline. This suggest that children at risk of a 30% decrease in eGFR will eventually experience a larger 50% decrease in eGFR and these two outcomes are analogous between the pediatric and adult Prediction Tools. The conclusion of this large collaborative effort was that the updated pediatric Prediction Tool could accurately predict the risk of a 30% decline in eGFR or ESKD in children with IgAN. The Prediction Tool uses a combination of commonly used clinical risk factors, and the MEST-C histology score, and is available online and in a mobile-app (www.qxmd.com/calculate-by-qxmd). The predicted risk of a 30% decline in eGFR or ESKD from the pediatric Prediction Tool at a given time horizon are similar to the predicted risks 2–3 years later of a 50% decline in eGFR or ESKD from the adult Prediction Tool.

Collectively, these results demonstrate that the Prediction Tool models are predicting clinically relevant outcomes in both children and adults and therefore can be used to identify higher-risk patients along a continuum of non-linear eGFR decline that spans the full age spectrum of patients with IgAN.

A low-risk condition according to the 2021 KDIGO recommendations, which should be the aim of treatment of children with IgAN, is persistent proteinuria <0.2 g/1.73 m^2/day and a normal GFR.

Treatment

Tonsillectomy

One of the first treatments considered for IgAN, particularly in children with recurrent gross hematuria, was tonsillectomy, which aimed to interrupt the pathogenic process initiated by an upper respiratory tract infection leading to hematuria. Tonsillectomy can eliminate a relevant source of pathogens, which multiply in tonsils, while also removing macrophages and T cells in lymphoid tonsil follicles, a potential source of aberrantly glycosylated IgA1. In children, it remains controversial whether adenotonsillectomy ultimately results in decreased serum immunoglobulins levels or, if so, whether such a decrease is associated with increased susceptibility to upper respiratory tract infections. In a randomized trial in children without IgAN [133], the IgA levels were significantly decreased after one year of follow-up; however, no relation was found between immunoglobulin levels and frequency of subsequent respiratory infections. Moreover, in children with repeated infections

despite tonsillectomy, IgA levels increased again, indicating that the remaining MALT can compensate for the loss of tonsils and adenoid tissue [133]. Even though tonsillectomy can reduce the frequency of gross hematuria and produce some benefits, this intervention lacks sufficient evidence and KDIGO 2012 suggested tonsillectomy not be performed in patients with IgAN without a clinical indication beyond IgAN [134]. This is confirmed in the 2021 KDIGO [127]; however, it takes into consideration the results of tonsillectomy in two large retrospective Japanese studies which reported benefits on renal function decline after a follow-up of more than 10 years [135]. Hence, some potential benefit in Japanese patients might be considered.

Tonsillectomy has a clear indication when tonsils are a true infectious focus and in cases of recurrent tonsillitis (>3 per year). Otherwise, the efficacy of the procedure is often supported only in association with other therapy and the benefit is unclear [136]. In Europe, a VALIGA collateral study failed to show clinical benefit on outcome of a subgroup of patients who had received tonsillectomy [137].

Inhibition of the Renin-Angiotensin System

In all children with IgAN, blood pressure (BP) control should be strict. All patients should be targeted below the 90th percentile, with a target of the 50th percentile or the maximally tolerated drug dose, if proteinuria is present. This is consistent with the target recommended in adults by KDIGO [126]. The drugs of choice for BP control in IgAN are RASB, either angiotensin converting enzyme inhibitors (ACE-Is) or angiotensin receptor blockers (ARBs).

Children with IgAN and heavy proteinuria are at risk for progressive disease. RAS inhibition has a strong rationale for use in IgAN, not only because it improves two risk factors for progression (hypertension and proteinuria), but also because it can inhibit the long series of potentially negative effects caused by angiotensin II on mesangial cells, particularly in the presence of mesangial immune deposits.

A European multicenter randomized controlled trial (RCT) included children and young patients (3–35 years old) with a constant level of proteinuria (>1 < 3.5 g/day/1.73 m^2 over the 3 months before enrolment) and normal or moderately reduced renal function. Patients were randomized to receive benazepril 0.2 mg/kg/day or placebo and the primary outcome of renal disease progression was >30% decrease in eGFR and/or worsening of proteinuria to nephrotic range. Treatment with ACE-I was an independent predictor of prognosis, while no effect on the progression of renal damage was found for gender, age, baseline GFR, systolic or diastolic blood pressure, mean arterial pressure, or proteinuria [138].

On the basis of this study, KDIGO suggested to treat children with IgAN and persistent proteinuria >0.5 and < 1 g/day/1.73 m^2 with RAS inhibition. There are no data to indicate a preference between ACE-Is or ARBs, except for fewer side effects with ARBs. No significant additional benefit was found in a RCT that investigated the addition of ARB to ACE-I in children with IgAN [139].

No RCT has addressed the effects of ACE-I in children with minimal proteinuria <0.5 g/day/1.73 m^2, but some benefits were suggested by a single-arm, collaborative, Japanese study that treated patients for 2 years [140].

Glucocorticoids

Based on the results of RCTs in adults [135, 141, 142], KDIGO in 2012 suggested that if proteinuria >1 g/day persisted unchanged after 3–6 months of RAS inhibition, glucocorticoids should be considered for treatment of IgAN in children and adults [126, 143]. In children, lower levels of persistent proteinuria (>0.5 g/day/1.73 m^2) may be considered for initiating treatment. Protocols included months regimens, using either 3 intravenous pulses of methylprednisolone (1 g) and oral prednisone (0.5 mg/kg on alternate days) [141], or oral prednisone 0.8–1 mg/kg/day for 2 months, with weaning

over 6 months [142, 144]. However, subsequent RCTs in adults suggested a more cautious approach in cases with slow decline of GFR. The STOP-IgAN trial [145] did not show a superior effect of immunosuppressive therapy (monotherapy with methylprednisolone pulses for 6 months in patients with eGFR >60 ml/min; oral prednisone and cyclophosphamide followed by azathioprine in patients with lower eGFR) versus supportive care for three years. The Therapeutic Evaluation of Steroids in IgA Nephropathy Global (TESTING) study [144] compared oral methylprednisolone versus placebo. Recruitment was discontinued after 2 years because of excess serious adverse events (mostly infections, including two deaths) in patients receiving corticosteroids. The preliminary results were consistent with potential renal benefit, but early termination of the study did not allow any conclusions. These recent studies have focused attention on the potential side effects and limited benefits of corticosteroids in adults with IgAN.

In children, evidence-based reports on the effect of corticosteroids in treating IgAN are scarce [146]. Small studies in children with variable baseline data and different treatment regimens provided conflicting results [147–149]. The best results were reported in severe cases with crescentic IgAN successfully treated with pulse steroid therapy [149].

A US RCT using prednisone (60 mg/m^2 every other day for 3 months, then 40 mg/m^2 every other day for 9 months, then 30 mg/m^2 every other day for 12 month) or fish oil (4 g/day for 2 years) failed to find significant benefit of treatment [150]. However, the relatively short follow-up period, inequality of baseline proteinuria, and small numbers of patients precludes a definite conclusion.

The only RCT proving efficacy of corticosteroids in children with IgAN was performed in Japan; it enrolled children with severe mesangial proliferation. The children were randomized into two groups, with one arm (intervention) receiving prednisone, azathioprine, heparin-warfarin and dipyridamole and the other arm (control) receiving heparin-warfarin and dipyridamole. This 2 year study reported a significant reduction in proteinuria, serum IgA concentration, mesangial deposition, and prevention of increased number of sclerosed glomeruli in the intervention arm [151]. After a follow-up of 10 years free of additional treatment, the children in the intervention arm (who had previously received the immunosuppressive combination therapy) were less likely to reach the end point of GFR <60 ml/min/1.73 m^2. In another study, a similar combination therapy produced a disappearance of IgA mesangial deposits after 2 years of treatment [152]. These reports suggest that early aggressive treatment in children with modifiable histologic risk factors for progression can in the long-term protect the kidneys from sclerosis. Side effects and limited if any benefit of prolonged anticoagulation and anti-platelet therapy suggest that they do not provide a net benefit when added to immunosuppressive therapy [153]. In adults, the addition of azathioprine to corticosteroids failed to add further benefits and caused side effects [154].

A French, uncontrolled, retrospective study [155] reported the outcome of children with severe clinical presentation and acute histologic features who received therapy with either corticosteroids (sometimes in association with cyclophosphamide) and RASB, or RASB alone. Although the two groups had different baseline proteinuria and time from onset to treatment, a large benefit of corticosteroid/immunosuppressive therapy was reported, with improvement in eGFR and decrease in proteinuria after a short follow-up of 6 months.

According to KDIGO 2021 [127], in children with proteinuria >1 g/day/1.73 m^2 and/or mesangial hypercellularity, most pediatric nephrologists will treat with glucocorticoids in addition to RAS blockade from time of diagnosis. The most common protocol is oral prednisone 1–2 mg/kg/day for 4 weeks tapered over 4–6 months. Regimens including intravenous methylprednisolone are also used. KDIGO 2021 reports that there is scarce evidence for the additional use of non-glucocorticoid immunosuppressants, but this approach may be considered in more severe cases. Children with nephrotic syndrome and his-

tological features of minimal change disease (MCD) associated with IgAN should be treated as MCD. IgAN with rapidly progressive renal deterioration of renal function (>50% decline in eGFR in <3 months) irrespective of the percentage of glomeruli involved with crescents, may benefit from steroids and cyclophosphamide, analogous to the treatment of ANCA vasculitis [126]. Prompt use of aggressive immunosuppressive treatment, sometimes in association with plasmapheresis, has shown some benefit in slowing the progression of these difficult cases [156]. Finally, KDIGO 2021 suggests to strictly follow children with IgAN even when they enter remission, since recurrence is always possible.

New Formulations of Corticosteroids

An enteric formulation of budesonide has been developed that targets release of the drug in the distal ileum, an area within the GALT. The NEFIGAN trial compared this targeted release formulation (TRF) of budesonide with placebo in patients with persistent proteinuria despite optimized RAS blockade [157]. At 9 months, mean proteinuria decreased significantly more in TRF budesonide-treated patients than in placebo–treated patients. GFR stabilized with TRF budesonide, but decreased in the placebo arm. No increase in serious adverse events, and particularly infections, were reported in treated patients. Results from further RCT and studies in children are expected.

Mycophenolate Mofetil

The KDIGO guideline does not recommend mycophenolate mofetil in IgAN, even though some benefit in Asian adult patients have been reported [158]. A RCT that included children, young subjects and adults investigating the effects of corticosteroids, mycophenolate mofetil and fish oil was prematurely terminated for lack of benefit [159].

Cyclophosphamide

This drug, in combination with corticosteroids, was tested in adults with particularly active disease in several uncontrolled case series, and a small RCT [160] reported a protective effect of cyclophosphamide in progressive cases of adult IgAN. However, this was not confirmed by the STOP-IgAN study [145].

Rituximab

Rituximab, in a small RCT in adult patients with IgAN, had no effect on eGFR decline or remission of proteinuria [161].

Calcineurin Inhibitors

A recent meta-analysis reported limited effects of calcineurin inhibitors when used in addition to small doses of prednisone, while reporting more adverse events [162].

Future Therapies for IgAN

Hydroxychloroquine combined with RAS inhibitors significantly reduced proteinuria over 6-months in adults with IgAN [163]. Results in children are expected.

Inhibitors of endothelin-1 receptors combined with RASB are being tested in a large collaborative phase III RCT. A study in children is in preparation.

Explorative trials with Inhibitors of the BAFF-TNF receptor family (BAFF, APRIL TACI) using humanized monoclonal antibodies against these mediators are ongoing [164]. Several complement inhibitors are under investigation, including anti-C5 (eculizumab), anti-C5a receptor inhibitors (CCX168), anti-C3 (compstatin), anti-factor D (lampalizumab), and MASP-2 inhibitors (OMS721) [164, 165].

A Practical Approach to Treatment of Children with IgAN

The prospects for successful treatment of IgAN to improve long-term outcomes in children seems promising since children are more likely than adults to be treated in the early stages of the disease, when mesangial proliferative lesions/endocapillary proliferations are more prominent than sclerosis, and when proteinuria is not massive. On the other hand, we must take into consideration the toxicity and side effects of these treatments, which are particularly undesired in patients with mild disease, and the possibility of spontaneous remission in mild cases [166].

According to KDIGO 2012 and 2021 [127] recommendations, the treatment of IgAN in adults and in children is mostly driven by the level of proteinuria at renal biopsy, with advanced renal failure suggesting disease beyond "the point of no return" being the only limitation. These recommendations originate from the available evidence in the literature. However, proteinuria can be associated with active lesions, but also be the clinical manifestation of sclerotic lesions. The recent identification of pathologic features that are risk factors independent from proteinuria, blood pressure and GFR at renal biopsy, strongly suggests that there should be an integrated approach to treatment as indicated in Fig. 17.5.

For all children with IgAN, a general approach is to carefully follow BP and to target blood pressure < 50th percentile for sex and age or maximally tolerated dose using RAS inhibition if proteinuria is present.

For children with mild IgAN disease, presenting with normal GFR, urinary protein/creatinine ratio (Up/Ucr) <0.2, normal BP and with all MEST (see legend for Fig. 17.5) negative [0], watchful waiting is suggested, and prescribing a RASB if Up/Ucr exceeds 0.2 g/g.

For children with IgAN and moderate/severe IgAN (Up/Ucr >0.5), at risk of GFR decline by the prediction tool, which considers MEST scores, without severe irreversible sclerotic changes, glucocorticoids should be added to a RASB. Methylprednisolone pulses [143] or oral steroids over 6 months can be adopted.

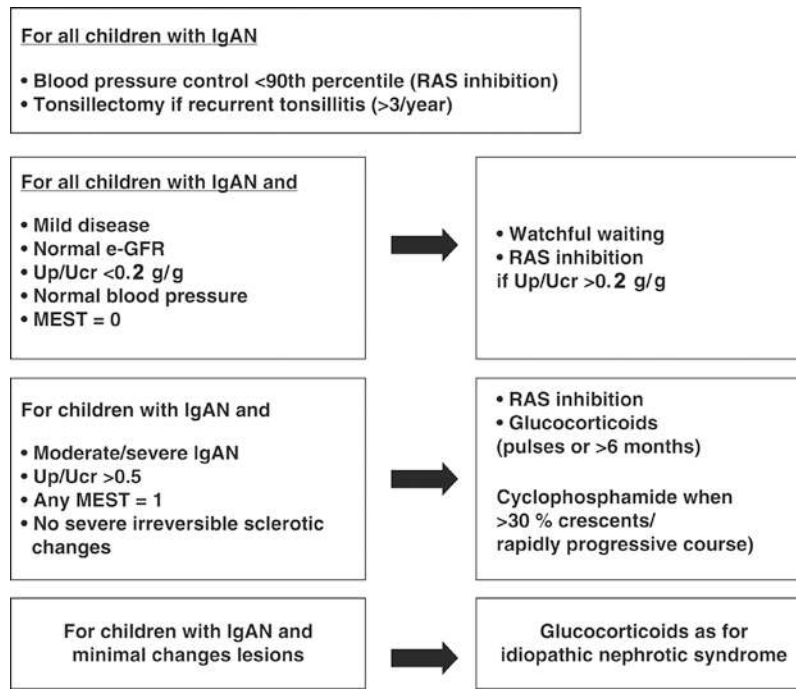

Fig. 17.5 Treatment of children with primary IgA nephropathy (IgAN). e-GFR: estimated glomerular filtration rate in children calculated with updated Schwartz formula; up/Ucr: urinary protein/creatinine ratio; normal blood pressure: <90th percentile corrected for height, sex and age; MEST: scores derived from Oxford classification of IgAN [101, 102]; M: mesangial hypercellularity; E: endocapillary hypercellularity; S: segmental sclerosis; T: tubular atrophy/interstitial fibrosis

In cases with severe endocapillary proliferation, or with crescent formation involving >30% of the glomeruli, with a rapidly progressive course, cyclophosphamide may be effective. In children with IgAN, minimal change lesions and nephrotic syndrome, a protocol similar to that recommended for idiopathic nephrotic syndrome is a suitable choice.

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Membranous Nephropathy

18

Myda Khalid and Laurence H. Beck Jr

Incidence

Nephrotic syndrome is one of the most common kidney disorders encountered in a pediatric nephrology practice. In children, it occurs at a reported incidence of 2 per 100,000 per year and a cumulative prevalence of 16 per 100,000 children. Compared to other more commonly occurring nephrotic disorders in this population, such as minimal change disease and primary focal and segmental glomerulosclerosis, membranous nephropathy (MN) is relatively rare. Given its rarity in children, there is scant data on the true incidence, prognosis and best management practices. It is estimated that 1.5% of children with nephrotic syndrome will have membranous nephropathy based on the International Study of Kidney Disease in Children report in 1978 [1]. The incidence is higher, approaching 22%, in adolescents compared to younger children [2].

Because many children with steroid-sensitive nephrotic syndrome will never be biopsied, the relative prevalence of MN versus minimal change disease and focal segmental glomerular sclerosis is unclear. Another bias is the age range of the population. In a series from Pakistan that investigated 538 children who underwent biopsy for idiopathic nephrotic syndrome, there was a significant difference between the 3% rate of MN in children aged <13 years and the 18.5% rate found in adolescents aged 13–18 years [3]. Similar rates were found in adolescents in two different cohorts [2, 4].

MN has been reported to account for only 0.6% of pediatric chronic and end-stage kidney disease (ESKD) cases, with a median age at onset of ESKD being 16 years based on the 2007 North American Pediatric Renal Trials and Collaborative Studies report and 2012 United States Renal Data System. This is in contrast to adults, in whom MN is one of the more common forms of nephrotic syndrome and a significant contributor towards ESKD (Table 18.1).

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Table 18.1 Differences between pediatric and adult membranous nephropathy

Feature	Pediatric MN	Adult MN
Disease type/subtype		
Proportion of all primary nephrotic syndrome cases that is MN	<5% (children); 5–20% (adolescents)	15–30%
MN that is primary ("idiopathic")	Minority	Majority
Proportion of primary MN that is PLA2R-associated	Less than 50% (more frequent in adolescents)	70–80%
Demographic and clinical features		
Male predominance	Variable	Yes
Full nephrotic syndrome	40–75%	75%
Microscopic hematuria	70–90% (can be macroscopic)	50%
Hypertension	<10%	30%
Thromboembolic events	<5%	10–20%
Spontaneous remission	Common	30%
Progressive renal impairment	<25%	30–40%
Pathologic features		
Mesangial deposits	Up to 50%	30%
Segmental distribution of deposits	Occasional	Very rare

MN membranous nephropathy

Etiology

The etiologies of MN have traditionally been divided into *idiopathic* (now more commonly called *primary*) and *secondary* causes. While primary MN typically reflects a kidney-specific autoimmune response, the glomerular process in secondary MN can usually be attributed to a systemic disease process, a drug or toxin, infection, or malignancy. Recent progress in the identification of target autoantigens and other specific biomarkers of MN has led the field to consider moving toward an antigen-based approach to naming the different types of MN, irrespective of primary or secondary designations [5]. However, it is still useful to subdivide MN into its autoimmune (idiopathic or primary) forms in which there is loss of tolerance to a self-antigen within the glomerulus, and secondary forms in which the antigen may be exogenous. Distinction between primary and secondary MN relies on a systematic evaluation of historical (drugs, exposures), clinical, laboratory, and histological features, as will be discussed further later in this chapter.

Although primary autoimmune forms of MN can occur in children, there has historically been a higher frequency of secondary forms of MN when compared to adults. The main etiologies of secondary disease in the pediatric population are infections and systemic autoimmune diseases such as lupus (Table 18.2). Drugs and malignancy are less frequent causes of MN in children,

Table 18.2 Secondary causes of membranous nephropathy

A. Autoimmune diseases
1. Systemic lupus erythematosus
2. Autoimmune thyroiditis
3. Sarcoidosis
4. Sjögren syndrome
5. Rheumatoid arthritis
B. Infectious diseases
1. Hepatitis B
2. Other viruses (HIV, HCV, EBV, CMV)
3. Quartan malaria
4. Schistosomiasis
5. Filariasis
6. Congenital syphilis
C. Drugs and exposures
1. Non-steroidal anti-inflammatory agents
2. Penicillamine
3. Bucillamine
4. Mercury salts
5. Recombinant enzymes used in enzyme replacement therapy
D. Neoplastic
1. Rare associations with disparate types of malignancy
E. Other conditions
1. Familial truncating mutations in *MME* (gene for NEP)
2. Anti-cationic BSA antibodies
3. *De novo* MN after kidney transplantation
4. Immune dysregulation or deficiency syndromes (IPEX, CVID)
5. Stem cell transplantation

Abbreviations: *HIV* human immunodeficiency virus; *HCV* hepatitis C virus; *EBV* Epstein Barr virus; *CMV* cytomegalovirus; *MME* membrane metalloendopeptidase; *NEP* neutral endopeptidase; *BSA* bovine serum albumin; *IPEX* immunodysregulation, polyendocrinopathy, enteropathy, X linked; *CVID* common variable immunodeficiency

but certainly need to be considered as potentially causal when the processes are concurrent. As in adults, sarcoidosis and autoimmune thyroid disease can be linked to MN in children. Hepatitis B has historically been a major cause of secondary MN in parts of the world in which the virus is endemic [6]. Fortunately, the incidence of hepatitis B-associated MN has decreased worldwide with the introduction of vaccination programs [7–9]. Additional viral infections such as HIV, EBV, and CMV have been reported as secondary causes of childhood MN. Infections such as schistosomiasis, filariasis, malaria and congenital syphilis are also important conditions linked to development of MN in areas where these diseases are prevalent.

Dysregulation of the immune system as occurs in IPEX syndrome [10] or combined variable immunodeficiency [11] can be a cause of MN in children. Exposure of host immune system to donor antigens (or vice versa), as occurs following hematopoietic stem cell transplantation or after kidney transplantation, can lead to alloimmune forms of membranous nephropathy [12, 13].

Pathophysiology

Despite the various etiologies that can lead to the histological pattern of membranous nephropathy and its consequent clinical manifestations, much of the underlying pathophysiology is fundamentally similar. Pediatric disease has provided important conceptual information about adult disease, and vice versa.

Membranous nephropathy (also variably called membranous glomerulonephritis or glomerulopathy) is named due to a thickening of the glomerular capillary walls as visualized by light microscopy, especially with prolonged disease [14]. The thickening is a result of (1) immune complexes that deposit beneath the basal surface of the podocyte at the outer aspect of the glomerular basement membrane (GBM) and (2) the additional extracellular matrix material that is eventually secreted between and around these deposits. The subepithelial immune deposits reliably consist of several components, which aid in the pathological detection and classification of MN. The immune complexes themselves are formed by immunoglobulin (most often IgG) and a target antigen, the identities of which have been identified over the past 15 years. Components of the complement system such as C3, C4, and C5b-9, activated by these immune complexes, are also consistently found within the immune deposits. These factors are routinely identified by immunostaining of the kidney biopsy (see Pathology below). The ability to detect the specific antigen within the deposits has been an important development in both pediatric and adult MN.

The field of MN has benefited both from well-studied animal models such as Heymann nephritis as well as important insights from human disease such as the identification of a growing number of targets antigens (Fig. 18.1). The ability to detect and follow circulating antibodies against these target proteins has opened up a new era for the diagnosis and monitoring of MN from early infancy to adulthood [15, 16].

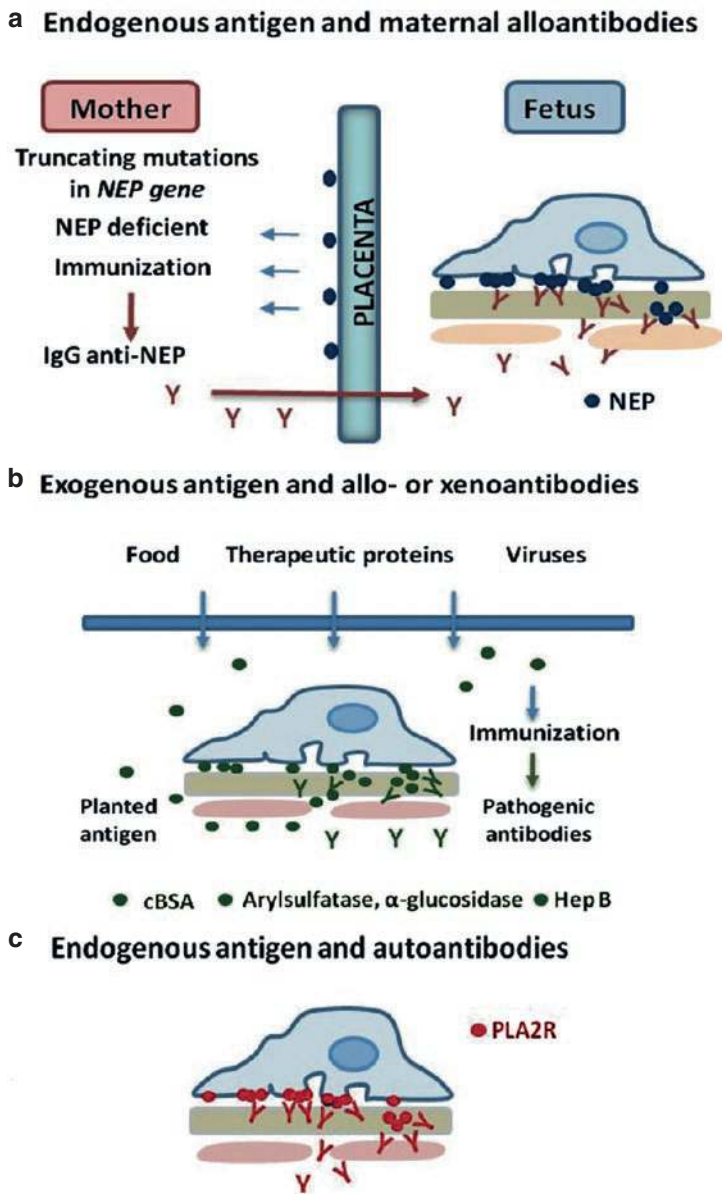

Fig. 18.1 Schematic representation of membranous nephropathy etiologies in children. (**a**) NEP-related alloimmune glomerulopathy. Neutral endopeptidase (NEP, blue dots) serves as a pathogenic antigen in the podocyte cell membrane. Antibodies to this protein originate in women who genetically lack NEP because of truncating mutations in *MME* (which encodes NEP). Immunization occurs during pregnancy when the mother's immune system is first exposed to NEP, which is strongly expressed by placental cells and by fetal cells entering the mother's blood. From about the 18th week of gestation, maternal antibodies are actively transported across the placenta to the fetus, where they bind to the NEP antigen expressed on podocytes. (**b**) Exogenous antigen-induced membranous nephropathy. Foreign, exogenous proteins commonly induce the production of antibodies. Owing to unusual physicochemical properties, these antigens can be trapped in the glomerular basement membrane where they can serve as a target for circulating antibodies, leading to the formation of in situ immune complexes. Green dots represent cationic bovine serum albumin (cBSA) from the diet, infused enzymes used in enzyme replacement therapy (arylsulfatase, α-glucosidase in this example) or hepatitis B antigens (Hep B). (**c**) PLA2R-associated primary (autoimmune) membranous nephropathy. The phospholipase A2 receptor (red dots), an integral membrane glycoprotein of podocytes, is a target antigen for circulating autoantibodies

Heymann Nephritis, the First Experimental Model of MN

An important animal model that established much of the pathophysiological paradigm for human membranous nephropathy was developed in 1959 by Walter Heymann, a pediatrician from Cleveland (Ohio, USA) and his colleagues. Lewis rats were immunized with crude extracts of rat kidney in what became known as the active Heymann nephritis model [17] and yielded a glomerular and clinical phenotype that was remarkably similar to human MN. As the experimental disease could be induced by immunizing rats with a rat proximal tubular brush border fraction, the source of the predominantly glomerular deposits was initially felt to be due to glomerular trapping of circulating immune complexes of brush border antigens and corresponding anti-brush border antibodies. Development of passive Heymann nephritis models in which the anti-brush border antibodies were first raised in rabbits or sheep and then transferred to the rats raised doubts about the importance of circulating immune complexes. Two groups, using ex vivo and isolated perfused rat kidney systems, independently demonstrated that the anti-brush border antibodies bound to an antigenic target on the podocyte (in fact, common to the podocyte and brush border) and suggested that the subepithelial deposits formed locally [18, 19].

The antigenic target common to the rat podocyte and tubular brush border was ultimately identified by Kerjaschki and Farquhar in the early 1980's as gp330 or megalin [20, 21], now known as the low-density lipoprotein receptor-related protein 2 (LRP2). Megalin was found to reside in clathrin-coated pits at the basal surface of the podocyte foot process, where it could interact with circulating anti-megalin antibodies from the capillary lumen to form immune complexes in situ. The subepithelial deposits were felt to grow in size as polyclonal anti-megalin antibodies formed highly-cross linked immune complexes with megalin that continued to be delivered to the basal surface in clathrin-coated vesicles [22]. Cleavage of the transmembrane protein megalin to release its large extracellular region into the immune complexes likely facilitated deposit formation [23].

These findings established the paradigm that a podocyte derived antigen that was expressed at the basal surface of the podocyte could react with anti-podocyte antibodies to initiate the pathologic process of MN. The search began for anti-megalin antibodies as an explanation for human MN, but it was quickly realized that human podocytes do not routinely express megalin and that other target antigens were likely the cause of human disease. Of note, anti-brush border antibodies (ABBA, now known to be anti-LRP2) are responsible for an autoimmune form of human interstitial nephritis that consistently exhibits segmental subepithelial deposits that may be due to limited megalin/LRP2 expression by aging podocytes [24].

Alloimmune Neonatal MN: Neutral Endopeptidase as the Target Antigen

The first podocyte-expressed target antigen in human MN was identified in a neonate born with MN [25] (Fig. 18.1, upper). Under the assumption that maternal antibodies might have caused this immune-complex disease in the developing fetal kidney, both maternal and infant serum was used to screen human, rat, and rabbit kidney tissue for candidate antigens. The culprit protein was identified as neutral endopeptidase (NEP) and NEP was found to co-localize with IgG and C5b-9 within the immune deposits of the infant's kidney biopsy [25, 26]. Experiments in which a pregnant rabbit was injected with IgG from the mother demonstrated that these anti-NEP antibodies were able to transfer disease [27].

NEP (which has several alternative names such as neprilysin, CD10, common acute lymphoblastic leukemia antigen or CALLA) is a product of the *MME* (membrane metalloendopeptidase) gene. It is an enzyme responsible for the degradation of biologically active peptides such as enkephalin, natriuretic peptides, endothelin, bradykinin and substance P in the vicinity of their receptors. It is present in many organs as

well as granulocytes; in the kidney, NEP is detected at the podocyte surface, in the brush border and in vessel walls.

The mother of the index case of NEP-associated antenatal MN, despite having high titers of circulating anti-NEP, did not exhibit any manifestations of nephrotic syndrome herself, and it was hypothesized that she might be genetically deficient in NEP. This was confirmed at the genetic level, showing the presence of truncating mutations within the *MME* gene [26] and by the lack of reactivity of her granulocytes with a panel of anti-NEP antibodies [25]. This mother was likely alloimmunized to NEP from a prior pregnancy which was miscarried, during which NEP of paternal origin was expressed by the syncytiotrophoblastic cells of the placenta. Four additional families with maternofetal alloimmune MN were identified. All immunized mothers were NEP deficient as a result of truncating mutations in exon 7 and exon 15 of the NEP gene [26, 28].

Since the anti-NEP antibodies were of maternal origin, transferred to the fetal circulation across the placenta, it is not surprising that most of these infants exhibited rapid improvement in their renal function and proteinuria as the maternal IgG cleared from their system and the glomerular deposits were resorbed. An exception was one newborn with a long duration of kidney failure requiring dialysis. The mother in this case possessed anti-NEP with an abundance of the IgG1 subclass, in contrast to other cases in which IgG4 was predominant. In addition to possibly better activating the complement system within the glomerular deposits, this IgG1 anti-NEP was also shown to possess enzyme inhibitory function that may have had renal vascular consequences [28].

NEP-associated alloimmune antenatal MN therefore represents the first type of human MN in which an intrinsic podocyte protein was implicated as the target antigen. Furthermore, anti-NEP IgG from the mother could induce disease when transferred to a rabbit. This maternofetal incompatibility due to a maternal genetic defect leading to an alloimmune response in the fetus is rare, but could be considered in other situations in which the mother is genetically deficient in a podocyte protein normally expressed in the fetal kidney.

M-Type Phospholipase A_2 Receptor-Associated MN

As idiopathic MN in adults did not demonstrate the presence of antibodies to either megalin/LRP2 or to NEP, the search continued to identify target antigens in this disorder. A breakthrough came in 2009 with the identification of the M-type phospholipase A_2 receptor, now better known as PLA2R [29]. Sera from adult cases of MN possessed circulating IgG4 that could detect a distinct glycoprotein by immunoblotting extracts from human glomeruli. Through the use of a candidate antigen approach that was informed by a mass spectrometric analysis of human glomerular proteins immunoprecipitated with IgG from MN cases, the target antigen was ultimately identified as PLA2R [30]. Seventy to 80% of idiopathic (primary autoimmune) MN are PLA2R-associated.

Like NEP and megalin (in the rat), PLA2R is expressed as a transmembrane receptor on the podocyte surface, where circulating antibodies can target one or more epitopes in the extracellular domain (Fig. 18.1, lower). It is of interest that, like megalin, it is also delivered to and recycled from the plasma membrane in clathrin-coated vesicles. Its role within the podocyte is not clear, but it may help to internalize small secreted phospholipase A_2 enzymes that pass through the GBM. PLA2R is not expressed by podocytes in small animals and thus passive administration of human anti-PLA2R antibodies (PLA2R-ab) has not been able to transfer disease as was the case for anti-NEP alloantibodies.

The identification of PLA2R as the target antigen in the majority of adult idiopathic MN cases paved the way for a new era of diagnosis, monitoring, and understanding in MN. As the major target antigen in adult MN and after more than a decade of study, this autoantibody-target antigen system now represents the paradigm by which we understand the link between circulating antibod-

ies and clinical manifestations of disease. We will focus on this system in the paragraphs below.

The identification of specific target antigens (like PLA2R) in MN allows for immunodetection of that antigen within immune deposits of the kidney biopsy, as well as assaying for the presence of autoantibodies to the target antigen in the circulation. Serologic assays to detect PLA2R-ab are based on ELISA using the recombinant human PLA2R protein or by immunofluorescence (IFA) of PLA2R transfected cells and are now commercially available in most countries as a clinical test. The specificity of the presence of circulating PLA2R-ab for MN is nearly 100%, especially when borderline or low-titer positive ELISA tests are confirmed by the more-sensitive IFA. Normal individuals or those with other proteinuric disease will not have PLA2R-ab. Such specificity has even led to the suggestion that a positive test for PLA2R-ab is diagnostic and may obviate the need for kidney biopsy, especially in those with normal kidney function and without other potential secondary causes of MN [31]. In situations where biopsy is difficult due to a patient already being on anticoagulation due to thromboembolic events resulting from the nephrotic state, a solitary kidney, or other relative contraindications to kidney biopsy, seropositivity for PLA2R-ab may be diagnostically sufficient to guide subsequent therapy.

A low prevalence of PLA2R-ab has been observed in forms of MN found in association with SLE, infectious disease, therapeutic drug use (especially nonsteroidal anti-inflammatory agents) or malignancy [32–35]. It is often quite difficult to assign causality of the MN to the associated condition, and in many cases a coincidental occurrence of the PLA2R-related MN and underlying disorder cannot be excluded. There may be exceptions: indeed, patients with MN associated with active sarcoidosis or replicating hepatitis B appear to have a high prevalence of PLA2R-related disease, which suggests that the immunologic setting of sarcoidosis and hepatitis B might trigger or enhance immunization against PLA2R [34, 36, 37]. Because therapeutic strategies are different for patients with idiopathic and secondary MN, discriminating between these two groups of patients is of utmost clinical importance.

In the majority of cases of MN, a biopsy is performed, and immunodetection of the PLA2R antigen (by immunofluorescence or immunohistochemistry) within the deposits in a fine, granular peripheral capillary wall pattern can establish the type of MN as PLA2R-associated. Immunostaining for PLA2R is more sensitive for PLA2R-associated MN than are the serologic assays [38], for the reason that circulating PLA2R-ab may not be detectable or not be present due to rapid absorption in the kidney in early disease or due to the achievement of immunological remission in individuals who remain proteinuric in later disease. This test enables the retrospective diagnosis of MN in archival, paraffin-embedded biopsy specimens, which is crucial for the monitoring of patients who will benefit from a kidney graft. It should be noted that there is a small proportion of cases of biopsy-proved MN who have detectable circulating PLA2R-ab, but do not stain for PLA2R within the deposits [37, 38]. It is possible that the antigen is masked by abundant PLA2R-ab targeting multiple epitopes or by another protein that limits detection with the commonly used commercial antibodies. Further study of such cases is needed to see if the circulating PLA2R-ab correlate with disease activity (see below) or if the PLA2R-ab might be non-pathogenic and an epiphenomenon.

A fundamental observation that has revolutionized the manner in which we monitor disease activity in MN is that, as the disease resolves, circulating PLA2R-decline and disappear in a manner that precedes and predicts clinical disease activity [39]. First noted in MN patients treated with the B cell depleting agent rituximab [40], the relationship between declining autoantibody and declining proteinuria holds for all immunosuppressive agents used in the treatment of PLA2R-associated MN [41–43]. PLA2R-ab are typically present in the setting of active nephrotic syndrome, are absent at clinical remission, and return with disease relapse [44] or in the setting of recurrent PLA2R-associated MN in the kidney allograft [45, 46]. Because high titers of

PLA2R-ab tend to take longer to disappear and/or are more resistant to therapy, they are associated with a lower chance of spontaneous [47] or immunosuppressive therapy induced remission [48] and with a higher risk of deterioration of kidney function [49]. A proposal for using PLA2R-ab in clinical practice was published several years ago [50] and the main tenets were incorporated into the 2021 Kidney Disease: Improving Global Outcomes (KDIGO) guidelines for glomerulonephritis (www.kdigo.org).

Role of PLA2R-ab-Related MN in Childhood

Although the bulk of the studies investigating the utility of monitoring PLA2R-ab has been from the adult MN literature, it is likely that the same principles could be applied to pediatric MN. However, the incidence of this type of MN is clearly less in children than adults, although exact numbers are not yet known. It is exceptionally rare for a child younger than 10 years old to have PLA2R-associated MN.

With more clinical and pathological testing for PLA2R and some of the more recently described types of MN, the field is gaining an understanding of the relevance of these forms of "adult" MN within the pediatric MN population. A number of studies have assessed the prevalence of PLA2R-associated MN by immunostaining pediatric MN biopsy registries in a retrospective manner for the presence of PLA2R within immune deposits. An early study focused on 22 MN cases with no clinical evidence for secondary disease, most between the ages of 10 and 17, and identified 10 cases (45%) positive for PLA2R [51]. In contrast, the PLA2R-negative cases were more likely to have secondary features on biopsy, although no clear secondary causes emerged with 3 years of follow up. These findings suggest a more diverse and currently incompletely explained set of etiologies in pediatric MN.

International studies confirm this prevalence of PLA2R-associated MN, largely within an adolescent population. A study of adolescents (10–19 years old) with primary MN in India revealed that the majority had PLA2R staining on biopsy, circulating PLA2R-ab antibodies, or both [52]. A higher prevalence of PLA2R-associated MN in children over the age of 10 is also suggested by a report from Japan [53]. These authors found 2 cases of PLA2R-associated disease amongst 19 patients in the 10 to 15-year-old group, but no cases amongst 15 patients aged 3–9 years old. A study from China also confirms a prevalence of PLA2R-associated disease in adolescents that is similar to that found in adult primary MN and a lower rate in younger children [54]. In this cohort, the mean age of the 16 PLA2R-associated cases was 12.9 years old, while that of the PLA2R-negative cases was 6.8 years old. Nearly 82% (9/11) of MN within the older 13–17-year-old group was PLA2R-associated, whereas only 7 of 27 (25.9%) were PLA2R-associated in the 1–12-year-old group. The authors do not report the ages of these seven PLA2R-positive cases. A final case series from Japan may have identified the youngest known patient with PLA2R-associated MN. Amongst 11 pediatric (age 4–14) patients with MN, a 6 year old with 2 g/day of proteinuria was found to have PLA2R-positive granular immune deposits on biopsy; serology was not available to confirm [55].

Pregnancy and Exposure to Maternal PLA2R-ab

Although no infants have ever been shown clinically to mount an autoimmune response to PLA2R, they can rarely be exposed to maternal PLA2R-ab in utero. This scenario is theoretically similar to the cases of alloimmune fetomaternal MN with anti-NEP in which podocyte-reactive antibodies cross the placenta to cause glomerular deposits in the fetus. There are several reports of pregnancy and active PLA2R-associated MN in a 39-, 43-, 33-, and a 21-year-old mother [56–59]. In three cases, the infant was born without any evidence of proteinuria or other abnormalities. In the fourth case [58], the infant was delivered early due to worsening oligohydramnios and at birth had similar PLA2R-ab levels as the mother, but levels subsequently declined. However, with

close follow-up, the infant was subsequently found to have developed proteinuria and an increased PLA2R-ab titer, which was felt to be due to ingestion/absorption of PLA2R-ab secreted in the mother's breast milk. A retrospective review of 27 women with primary MN and pregnancy showed adverse maternofetal outcomes in 10 cases [60]. Seropositivity for PLA2R-ab and the inability to achieve remission during pregnancy were risk factors for adverse events.

Additional Target Antigens Since PLA2R

Although PLA2R is the most common target antigen in primary adult MN, comprising about 80% of cases, more recent findings have implicated other target antigens or biomarkers that reveal other types of MN, some with different clinical and histologic phenotypes than the more common PLA2R-associated MN.

Thrombospondin Type-1 Domain-Containing 7A (THSD7A)

Five years after the description of the major target antigen PLA2R in adult MN, a second podocyte antigen called THSD7A was identified [61]. This protein is a well-conserved transmembrane protein that sits at the basal surface of the podocyte, directly beneath the slit diaphragms [62]. In much the same manner as the PLA2R/PLA2R-ab system, circulating antibodies to THSD7A were found in a minority of adult patients with primary MN, and appeared to correlate with disease activity [61]. THSD7A and IgG co-localized within immune deposits. Remarkably, several cases of malignancy associated MN were found to have been caused by tumor overexpression of THSD7A and regional lymph node activation of the humoral response [63, 64]. The association with malignancy may not be as common as initially reported, but is clearly greater than seen in PLA2R-associated MN [65].

THSD7A-associated MN is not common in children but can occur. The youngest patient identified with anti-THSD7A antibodies is a 4-year-old whose anti-THSD7A declined in response to treatment with rituximab [66]. A small retrospective cohort study from Germany demonstrated that 50% of their 12 pediatric MN cases had circulating antibodies to PLA2R [67], with tissue staining for PLA2R on biopsy also confirmed in two of these cases. The other six had no identifiable circulating antibodies to THSD7A, cationic bovine serum albumin (see below), or neutral endopeptidase. When Zhang and colleagues stained their 22 PLA2R-negative pediatric cases for THSD7A, none was positive [54]. It is not clear at this time what the overall prevalence of THSD7A-associated disease may be in children or adolescents.

Additional Antigens with Potential Secondary MN Associations

New findings may help to characterize cases of pediatric MN that fail to exhibit staining for PLA2R or THSD7A. Using the technique of laser capture microdissection followed by mass spectrometric analysis, Sethi and colleagues have identified a number of novel target antigens in forms of MN that were previously uncharacterized [5]. Several recent additions to the MN target antigen (or biomarker) repertoire have been identified using this methodology. NELL1 has been identified as a target of circulating antibodies in adults with MN [68]. The median age of patients diagnosed with this form of MN was nearly 67 years old, and one third of the cases were associated with underlying malignancy [69]. Several other markers or target antigens have been found that can point to underlying lupus as the etiology behind the MN. The exostosins 1 and 2 (EXT1/EXT2) are partners of a glycosyltransferase complex and seem to be a specific biomarker within deposits in a subset of secondary MN associated with lupus and other autoimmune diseases [70]. In similar manner, neural cell adhesion molecule 1 (NCAM-1) has

been found as the antigen targeted by circulating antibodies and present within immune deposits in more than 6% of class V (membranous) lupus nephritis cases and in a smaller percentage of apparently primary cases. The median age for these two types of MN was in the 30's so it is possible that adolescents and young adults could develop these types of disease.

Semaphorin 3B

Another of these novel target antigens is semaphorin-3B (Sema3B), which seems to associate with a type of MN more commonly found in the pediatric population [71]. In the initial report, 73% of cases were pediatric, with a mean age of 6.9 years old, while the remainder were adults with a still younger-than-average mean age of 36.3 years old. Nearly half of the pediatric cases were under the age of 2. Unique features of this type of MN are the presence of tubular basement membrane immune deposits (exclusively found in those cases <2 years old), a predominance of the IgG1 subclass within the deposits, and a possible inherited pattern of disease in some cases [71]. In addition, although circulating anti-Sema3B antibodies could be detected in the serum, these antibodies could only recognize the recombinant human Sema3B protein under conditions in which disulfide bonds were reduced, suggesting the presence of a cryptic epitope that would not normally be available to the anti-Sema3B antibodies when the protein is in its native state. It is hoped that similar techniques as the laser capture microdissection followed by mass spectrometry method will be useful for identifying further antigens specifically in the subset of pediatric MN.

Food Antigen-Related MN

An unusual form of secondary MN in children has been described in which there are high levels of antibodies to bovine serum albumin (BSA) [72]. These four children (all younger than 5) were found to have BSA in their bloodstream that was cationic (positively charged) which is atypical for the normal protein. BSA was detected in subepithelial immune deposits only in children with both anti-BSA antibodies and high levels of circulating cationic BSA, and it colocalized with IgG, in the absence of PLA2R-ab. IgG eluted from kidney biopsy samples of children with BSA deposits belonged to IgG1 and IgG4 subclasses and specifically reacted with BSA, but not with human serum albumin.

The source of BSA in children is most likely cow's milk, and the increased permeability of the intestinal barrier in infants and young children, possibly exacerbated by episodes of gastroenteritis, is hypothesized to have allowed the entry of the whole molecule into the circulation. The precise source of the cationic form of BSA is unknown, but this modification could be due to differences in food processing (powdered infant formula) or intestinal microbiota. It is of pathophysiological and historical interest that this type of human MN seems to be equivalent to a rabbit experimental model of MN developed in the 1980's in which cationic (but not the normally anionic) BSA could induce MN [73] (Fig. 18.1, middle). These clinical findings strongly support the scenario of "planted" antigens in human disease. Based on this finding, other ingested antigenic sources or non-dietary antigens from the environment should be considered as potential targets in cases of pediatric MN in which the cause remains a mystery.

Enzyme Replacement Therapy as a Cause of Alloimmune MN

The administration of recombinant enzymes or factors as replacement therapy for a genetic deficiency can lead to the development of alloantibodies. Alloantibodies may be without clinical significance or may lead to hypersensitivity reactions and decreased bioavailability and efficacy of the therapeutic proteins. They may also lead to formation of immune complexes of the replacement enzyme and alloantibody that occasionally become planted in a glomerular subepithelial location. This has occurred in a patient with

mucopolysaccharidosis type VI treated with human recombinant arylsulfatase B (rhASB) [74] (Fig. 18.1, middle). The clinical circumstances in this case, particularly the resolution of proteinuria when ERT was suspended, and the finding that IgG eluted from the biopsy specimen reacted specifically with rhASB, strongly suggested that the alloimmune response to the recombinant enzyme was the cause of the disease. Other cases of nephrotic syndrome associated with MN-like subepithelial immune complexes have been induced by immune-tolerance induction regimens in which increasing doses of enzyme replacement therapy (e.g., alpha-glucosidase in Pompe disease) [75] or factors (factor IX in hemophilia B) [76] have been administered in an attempt to limit inhibitory alloantibodies.

The Case of Secondary MN

Despite our knowledge of specific proteins that comprise the immune deposits in the forms of MN described earlier in the chapter, the molecular characterization of the immune complexes in most secondary forms remains limited. Hepatitis B, hepatitis C and Helicobacter pylori antigens, tumor antigens, thyroglobulin, and DNA containing material have been detected by elution from or immunostaining of subepithelial deposits in patients with secondary MN [77, 78]. These antigens may have been trapped in the GBM owing to unusual physicochemical properties similar to what is thought to occur with cationic BSA. Alternatively, small-sized, circulating, non-precipitating IgG4 complexes containing these antigens could become deposited in the GBM as in the chronic serum sickness model, although there is no experimental evidence yet supporting this hypothesis in humans.

Genetic Factors

The finding that different forms of MN occur in different age groups may imply a complex interaction of environmental influences with an underlying genetic susceptibility. It has long been known that European Caucasians show a strong association of MN with the HLA-B8 DR3 haplotype and other HLA class II loci [79, 80]. The first genome-wide association study (GWAS) conducted, with only 556 white European adults with idiopathic MN was, sufficient to show a highly significant interaction with *HLA-DQA1* as well as *PLA2R1* [81]. The risk variants at these sites displayed an unusually strong genetic interaction such that homozygosity for the risk variants at both locations conferred an almost 80-fold increase in the risk of MN [82]. These genetic data were confirmed in ethnically distant populations from Europe [49, 83] and Asia [84–86]. Robust genetic data are lacking in children, where the prevalence of PLA2R-related MN is much lower.

Subsequent work has raised appealing suggestions as to what might underlie the robust genetic interaction between the HLA class II locus and PLA2R1 [87]. Although there was early speculation that rare single nucleotide variants leading to amino acid changes in the sequence of PLA2R might alter protein conformation, this theory was felt not to be the case as there were not specific coding variants that associated with disease [88]. A higher resolution GWAS conducted across 3782 cases of MN and over 9000 controls points to an intronic, regulatory region of *PLA2R1* that might confer increased expression of the antigen within the kidney or potentially other tissues [89]. Since the PLA2R protein is highly disulfide bonded and difficult to express in experimental settings, a genetically driven overexpression of the protein could lead to protein misfolding and presentation of aberrant protein to the immune system.

A series of studies largely from China have pointed to discrete regions within the class II MHC molecules that have been genetically linked to MN [90–92]. The most significant genetic associations can be pinpointed to variants that are predicted to alter the amino acid sequence within the binding groove of the molecule that presents peptides to the T cell receptor [89, 91, 92]. Such variants are predicted to allow PLA2R fragments to bind with potentially higher affinity to the HLA molecules encoded by the risk variants than

to molecules encoded by other alleles and may therefore represent a mechanism why some genetically-predisposed individuals might more easily mount an autoimmune response to the self-protein PLA2R.

Other non-HLA alleles also predispose individuals towards the development or progression of MN such as the tumor necrosis factor (TNF) allele G308A, and polymorphisms in genes encoding IL-6, STAT4, nephrin, and plasminogen activator inhibitor type-1 (PAI1) [93]. A very large GWAS performed in MN on an international scale [89] points to two other risk loci: one in the gene for interferon response factor 4 (*IRF4*) and one within nuclear factor kappa B (*NFKB1*), suggesting that susceptibility lies in other arms of the immune regulatory system as well. Overall, these data suggest that a combination of gene variants initiates disease, and that modifier genes controlling glomerular permeability, inflammation and fibrosis might be involved in the pathogenesis of MN.

Clinical Presentation

Children with MN classically present with nephrotic syndrome or isolated sub-nephrotic proteinuria. They have hypoalbuminemia, proteinuria and may have edema on presentation. Most children have normal renal function at presentation. Microscopic hematuria is commonly seen in children, and in one study was found in 77% of children at the time of biopsy [94]. In the same pediatric cohort, eGFR at the time of biopsy was normal at 107 mL/min/1.73 m^2 [94]. The incidence of thromboembolism in children at presentation and during the course of the illness was significantly lower compared to adults [94].

It is not uncommon for children to be initially treated with oral corticosteroids for presumed minimal change disease and for MN to be discovered instead when a biopsy is performed weeks later after the patient is found to be partially responsive or non-responsive to steroids. It is also possible that there is a subset of children with MN that either spontaneously remit or respond to prednisone, who do not get biopsied. This would be another factor limiting the determination of the true incidence of MN in children.

Distinguishing primary from secondary MN based on presentation alone is challenging. A detailed history for concomitant illnesses, recent travel and review of systems may elicit signs and symptoms of an underlying illness that would predispose a child to secondary MN. The incidence of serum PLA2R-ab and tissue PLA2R positivity at diagnosis in children has not been widely studied, with reports showing a varying degree of seropositivity, ranging from 45 to 70% [51, 95], and the specificity of these findings for primary disease is not known. Secondary etiologies of MN may present in similar fashion to primary disease, and an underlying cause is not always readily apparent. Due to the decreasing prevalence of HBV-associated MN, SLE is now the most common secondary etiology of pediatric MN. Very importantly, class V lupus nephritis can be the initial presentation of SLE in the absence of extra-renal manifestations and of serological manifestations of SLE such as hypocomplementemia or anti-double stranded DNA antibodies, which will often appear later.

Laboratory Investigations

The child or adolescent that presents with features of nephrotic syndrome should undergo standard serological workup that includes antinuclear antibodies (with anti-double stranded DNA as a confirmatory test), levels of C3 and C4, and screening tests for hepatitis B infection. Due to the possibility of finding serologically active MN, testing for PLA2R-ab should be considered in the older child or adolescent even before a biopsy diagnosis of MN is available. Specialized tests, such as anti-Sema3B, may one day be clinically available, but are currently limited to the research setting.

Children with hepatitis B-related MN show positivity of hepatitis B surface antigen (HBsAg) and usually hepatitis B surface antibody is not detected. The hepatitis B early antigen (HBeAg) can be detected in serum of 90% of patients.

Hypocomplementemia (low C3 and C4) is observed at disease onset, but levels of C3, C4 return to normal later in the disease course. Circulating immune complexes are detected in 80% of patients. Serum levels of transaminases may be elevated on presentation, which may lead the clinician to order viral hepatitis testing.

Histopathology

The glomerular features seen in membranous nephropathy are a result of the subepithelial deposits and the extra basement membrane material that is laid down surrounding them in response.

The typical *light microscopic* findings in adult MN are normal-to-enlarged glomeruli with thickened and rigid capillary walls, but without elements of proliferation. With the use of Jones' methenamine silver stain, which stains the GBM, there are spikes of new matrix between deposits in the GBM, and the deposits may be suggested by craters of absent staining (Fig. 18.2). Biopsy features in pediatric disease are similar, although mesangial deposits and a segmental distribution of the deposits seem to be more common in pediatric versus adult disease (Table 18.1). Features such as mesangial hypercellularity should prompt the clinician to look for evidence of secondary causes of disease, especially lupus.

In addition to the glomerular findings, it is important to also consider the overall health of the renal parenchyma, as prolonged glomerular disease may lead to tubulointerstitial injury. As with many glomerular diseases, the presence of glomerulosclerosis, interstitial fibrosis and tubular atrophy are signs of chronicity and tend to be associated with diminished GFR and a poorer prognosis. Intraparenchymal B cell infiltrates forming tertiary lymphoid structures have been demonstrated in human MN and other autoimmune glomuleronephritides [96, 97].

Immunofluorescence (IF) can be the most sensitive of the imaging modalities to suggest the presence of MN. In most cases of MN, there are fine granular deposits of IgG (Fig. 18.3) and C3 decorating the peripheral capillary walls in a global and diffuse distribution. The degree of complement deposition has been correlated with

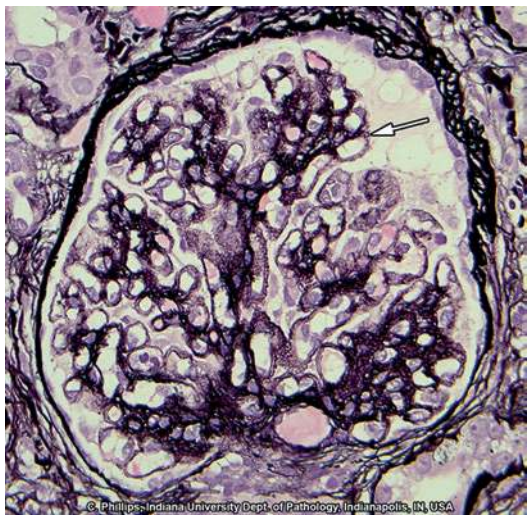

Fig. 18.2 Brightfield microscopy. Jones' silver stain of a paraffin tissue section reveals pinholes (arrow) and spikes along glomerular basement membranes (GBM) from a patient with membranous nephropathy. This GBM distortion is in response to deposition of immunoglobulin and complement (original magnification 400×). Image courtesy of Carrie L. Phillips MD, Department of Pathology, Indiana University School of Medicine

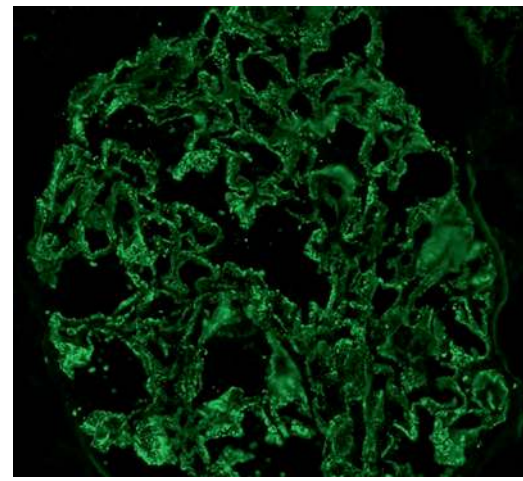

Fig. 18.3 Widefield epifluorescence microscopy. Direct immunofluorescence using fluorescein-tagged antibody to IgG shows granular deposits along glomerular capillary loops in a patient with membranous nephropathy (original magnification 400×). Image courtesy of Carrie L. Phillips MD, Department of Pathology, Indiana University School of Medicine

the rate of progression of kidney dysfunction [98]. The detection of additional immunoreactants such as C1q, IgA, and IgM can point to the presence of a secondary cause of MN. The target antigen (PLA2R, THSD7A, Sema3B) when assayed will yield a similar pattern, and co-localization studies on the same tissue section will show nearly identical patterns. Although most cases of adult MN demonstrate the global pattern in which all capillary loops exhibit immune deposits, a minority of pediatric cases will show a segmental pattern, with some loops apparently being spared from the deposits. The pathophysiological reasons behind this are currently not known.

Electron microscopy (EM) can be helpful, but it is often not needed to make the diagnosis (Fig. 18.4). EM is useful for detecting the size and distribution of deposits, and the degree of matrix reaction to the deposits. In primary disease, the deposits are largely subepithelial, while the presence of significant mesangial and/or subendothelial deposits suggests secondary etiologies of disease. The presence of tubuloreticular inclusions in endothelial cells, a sign of the interferon response, usually points to the presence of lupus or a viral infection such as HIV. Features of podocyte injury and simplification on EM are the effacement of the basal foot processes and loss of slit diaphragms, as well as the microvillous change on the apical side of the podocyte cell body.

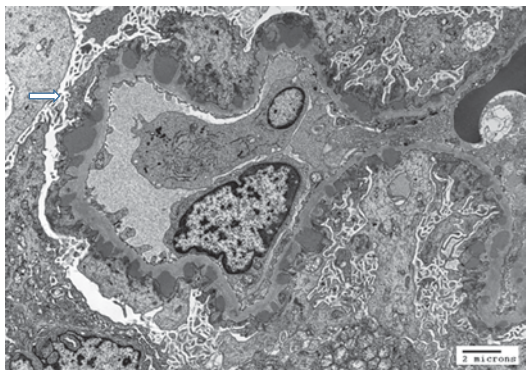

Fig. 18.4 Transmission electron microscopy. This electron micrograph shows subepithelial electron dense deposits (white arrow) along glomerular basement membranes, including some separated by spikes of extracellular matrix. Podocyte foot process effacement is extensive. Image courtesy of Carrie L Phillips MD, Department of Pathology, Indiana University School of Medicine

The appearance of the electron-dense subepithelial deposits has traditionally been described as morphological stages [99]. The stages based on combination of light and electron microscopic features are as follows:

- Stage 1. Light microscopy shows normal GBM in thickness and appearance. The electron dense deposits are small and flat but discrete. The smaller deposits are often located at the site of the slit diaphragm, while larger deposits are located immediately adjacent to the effaced foot processes.
- Stage 2. Thickening of the GBM is discernible by light microscopy. The electron dense deposits are increased in number and size. These deposits are flanked by prominent spikes in almost every capillary loop.
- Stage 3. The basement membrane material (spikes) completely surrounds the deposits. These are larger and acquire intramembranous position. The capillary wall is irregular and has a moth-eaten appearance.
- Stage 4. The GBM is severely altered and irregularly thickened. The deposits are only a few in number or completely absent. The vacuolated appearance of GBM is discernible. The loss of deposits is seen as electron-lucent areas in the basement membrane.

Investigators have more recently introduced a Stage 0 in the setting of recurrent MN in the kidney allograft [100], often seen in early protocol biopsies or biopsies conducted to explore delayed graft function. In this stage, fine granular deposits of IgG can be seen by the highly sensitive IF technique, while there are no discernible electron dense deposits by EM. This stage is virtually never seen in native kidney biopsies, as there are no clinical features present at that time that would warrant a kidney biopsy.

The most common stages of deposits found in childhood MN are stages 2 and 3, which can occur in a mixed (heterogeneous) pattern. Since children with nephrotic syndrome are often tri-

aled on corticosteroid therapy prior to consideration of biopsy, the potentially longer duration between clinical symptoms and biopsy may explain the paucity of earlier, stage 1 lesions.

Specific Diagnostic Approaches

Many of the etiologies of pediatric MN are rare, and it may be difficult to rule out all potential causes. Research or clinical groups who specialize in such disorders should be contacted for further advice on testing. Much of the testing will rely on immunostaining the biopsy in a validated protocol to look for the presence of potential antigens (e.g., Sema3B, NEP, BSA) within the immune deposits. It is not expected that a renal pathologist will have the resources to look for all such antigens, and thus the availability of extra sections to send to specialized centers might be critical in making a diagnosis. Serological tests may be available for certain antigens in the future. Some specialized centers have the ability to query the proteins that accumulate over time in the deposits by means of laser capture microdissection of the glomeruli from tissue sections and then to perform mass spectrometry. This methodology is not clinically available, but may provide a diagnosis in the research setting.

Prognosis and Predictors

The course of primary MN is variable. As a general rule in adult MN, approximately one third of patients undergo spontaneous remission and maintain normal renal function with or without occasional relapses [101, 102]. Another third of patients display persistent proteinuria of variable degree, with normal or mildly impaired but stable GFR. The remaining patients develop progressive chronic kidney disease (CKD) eventually leading to ESKD. In a review of natural history studies in untreated adults with MN, 50% of these patients either died or developed ESKD within 10 years of disease onset [101].

Children seem to have a relatively better overall outcome than adults [103], with some of the more recent series reporting overall remission rates of 75% [1, 104] (Table 18.1); however, most studies still show decreased kidney function in about 20% of patients at final follow-up [1, 105]. It should be emphasized that the existing information about natural history and treatment outcomes of primary MN in children is not only uncontrolled, but there is considerable variability regarding the therapeutic protocols used and the definition of remission. Individually based decisions are therefore of paramount importance to minimize the risk of progression to renal failure.

Unfortunately, predictors of outcome are still poorly defined in children. The degree of proteinuria seems to be a valuable predictor of outcome, which is excellent in children with asymptomatic, non-nephrotic proteinuria; however, approximately 25% of those with nephrotic syndrome will develop renal failure within 1–17 years. In one cohort, all patients who developed CKD presented with nephrotic syndrome [106]. Hypertension and interstitial fibrosis on biopsy might be further predictors of adverse outcome [104, 105].

In adults, there is no difference in response to treatment between PLA2R-ab positive and PLA2R-ab negative patients, although the response rate to rituximab appears to be lower in patients with a high titer of antibodies [107]. Further studies are required to assess how the PLA2R-ab antibody titer may be of prognostic value in children.

As with adults, in children with PLA2R-related MN the quantification of PLA2R-ab antibodies will likely become an invaluable tool for the monitoring of disease immunological activity and the titration of immunosuppressive treatments. Antibodies disappear before proteinuria in patients treated with rituximab [40, 42], which leads to consideration of withdrawal of immunosuppressive treatment at the time of immunological remission before renal remission is achieved. The time-lag between immunological and renal remission most likely corresponds to the time required for restoration of the glomerular capillary wall. PLA2R-ab antibody levels at the end of therapy may also predict the subsequent course. In a series of 48 patients treated with immuno-

suppressive agents, 58% of antibody-negative patients were in persistent remission after 5 years compared with none of antibody-positive patients [107]. However, further prospective studies on large cohorts of patients are needed before drawing definitive conclusions and extrapolating them to children.

Treatment

The first step in the approach to treatment is ruling out secondary causes of MN including lupus, hepatitis B and C. In cases of secondary MN, treating the underlying condition results in resolution of MN. Removal of potential offending agents such as non-steroidal anti-inflammatory agents or cow's milk in infants with suspected cationic-BSA induced MN is required in these specific situations. The treatment of pediatric membranous (class V) lupus nephritis is discussed at the end of this section.

Currently, no randomized controlled trials addressing treatment of primary MN in children exist, which represents a substantial limiting factor in determining the most efficacious regimens for children with MN. Even studies predicting natural disease course are scant and limited to small cohorts. Therefore, most treatment recommendations stem from the data in adult patients. This also has its limitation given that the underlying disease mechanism in children may be different from that in adults. Serum PLA2R positivity in children appears to be lower at around 45% in children compared to 70% in adults, suggesting different pathophysiological mechanisms are often present in the pediatric population [51].

A second step in deciding on treatment course is to determine the degree of nephrosis. As with adults, it seems reasonable that children with asymptomatic, non-nephrotic presentation may be treated conservatively with angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs) for their anti-proteinuria and nephroprotective effects, along with lifestyle modifications, including a low-salt diet. This would give time for spontaneous remission to occur, which would obviate the need for immunosuppression. Therapy in children who subsequently develop or initially present with the nephrotic syndrome and nephrotic range proteinuria remains controversial. In the adults, there is a growing body of literature suggesting superiority of treatment with rituximab compared to other immunosuppressive agents. Regimens described in the pediatric and adult cohorts are discussed in detail below.

The 2021 KDIGO guidelines (www.kdigo.org) are based on evidence from adult studies and recommend conservative treatment in subjects with eGFR >60 mL/min/1.73 m^2 and proteinuria <3.5 g/day. If, however, one or more risk factors for disease progression are present then treatment with rituximab, a combination of cyclophosphamide and steroids for 6 months, or tacrolimus therapy for at least 6 months should be considered. Risk factors for disease progression include: eGFR <60 mL/min/1.73 m^2, proteinuria >4 g/day and no decrease in 6 months despite conservative therapy, high PLA2R-ab titers, significant low-molecular-weight proteinuria or life-threatening complications of nephrotic syndrome. There are no specific treatment recommendations in children, but it is noted that therapy is often initiated prior to 6 months and that rituximab and calcineurin inhibitors have been used as treatment options in children.

There is a report of the clinical characteristics and treatment response in a large, prospectively followed cohort of 48 children in India ranging from age 1 years to 20 years with primary MN [95]. The median follow-up was for 29 months and the median age was 17 years. All except one patient, who underwent a spontaneous remission, were treated with immunosuppression. The predominant treatment regimens were a minimum of 3 months with a combination of cyclophosphamide with glucocorticoids (GC; 53%), tacrolimus with GC (21%), and rituximab (15%). Response rates in the three treatment groups were similar. Remission rates were 62.2% and 70.5% at 6 and 12 months, respectively. Of note, in subjects with resistant or relapsing disease, rituximab was used as a second agent with considerable success. Serum PLA2R-ab positivity was similar to that in adult primary MN patients at 72.9% (35

subjects). In addition, the study observed a reduction in PLA2R-ab titer with disease remission/resolution [95].

Another study reported treatment patterns in a North American and European cohort of 37 children with primary MN enrolled in the Cure Glomerulopathy Network [94]. The median age was 14 years with a follow-up of 23 months. Children who had received 6 weeks or less of glucocorticoids prior to the time of the kidney biopsy were considered immunosuppression naïve and enrolled in this cohort since most children are initially treated for presumed minimal change disease without a biopsy. Twenty-three (59%) of the children were placed on immunosuppressive therapy (IST) within 6 months after biopsy, with 70% initiating IST at any time within the follow-up period. The median time to initiate IST after the diagnostic biopsy was 1.9 months. It is important to highlight that 30% of the children did not receive any IST, which is in line with some other studies. Renin-angiotensin-aldosterone system (RAAS) inhibitors were used in 73% of the children [94]. Interestingly, the first line IST treatment most commonly prescribed in children in this group were calcineurin inhibitors (46% of the 26 children that were treated). However, since this cohort included children who may have had GC prior to biopsy, it is possible that the true exposure to GC is higher if including the treatment pre-biopsy. Seven children (27%) received 30 days or more of GC as a first line single agent. Rituximab as a single agent and first line therapy was used in 4 children (11%) and mycophenolate mofetil in two children. As part of combination therapy, up to 76% of children in the treated group received GC. Seven of 26 children were transitioned to a second-line agent [94]. There were no differences observed in clinical characteristics at presentation in the group of children that were treatment with IST compared with the children who were managed with conservative therapy. This study emphasizes the variability in approach in treating children with MN, probably a result of limited evidence for a specific treatment strategy.

In an older series that predated the use of rituximab, Valentini et al. reported treatment outcomes in 12 children with biopsy proven primary MN [108]. The mean age was 11.9 years and mean follow up time was 27 months. Two patients did not receive any IST and entered spontaneous remission. The remaining ten patients were treated with oral prednisolone 2 mg/kg/day (maximum 80 mg/day) for 4–8 weeks. One patient responded to GC alone, four patients were partially-responsive, and five patients did not respond. Cyclophosphamide (2 mg/kg/day orally for 12 weeks) was used as a second line agent for the five steroid resistant patients, one partial responder, and one initially steroid-responsive subject who became steroid dependent. This treatment regimen resulted in complete remission in all but one subject who was steroid resistant [108]. There were no clinical differences at presentation in the nine children that ultimately demonstrated complete remission compared to three children that did not.

A report from India described five children with MN, ages 5–13 years. All had positive PLA2R staining of the kidney biopsy tissue and three had a positive PLA2R-ab titer at the time of biopsy [52]. These subjects had already received oral prednisolone prior to the biopsy. Two patients were treated with the modified Ponticelli protocol (pulse methylprednisolone, oral prednisone and cyclophosphamide). The remaining three were treated with tacrolimus and oral prednisone [52]. All 5 patients were in complete or partial remission by 12 months. PLA2R-ab disappeared at 6 months in one patient with complete remission and declined by 50% in another patient with a partial remission. This study suggests that in children with PLA2R-ab positivity, a response to therapy will trend with reduction in PLA2R-ab titer.

A retrospective study performed before the era of PLA2R-ab described a Korean cohort of 19 children with primary MN with a median age of onset of 11 years [1]. There were 11 children with nephrotic syndrome at presentation and eight children with proteinuria without nephrotic syndrome at presentation. In the latter group, children without nephrotic syndrome, three children did not receive any IST and entered spontaneous remission. Five children received varied

regimens of corticosteroids and some also received cyclosporine. All five subjects eventually entered remission at time ranging from 1 month to 33 months after treatment. In the second group, 11 children with nephrotic syndrome, all were treated with either prednisone alone (varied regimens) or in combination with cyclosporine. Six entered remission by a median time of 12 months, but five children did not demonstrate any response to steroids or additional IS. The authors report that at last follow-up, in the 11 children with nephrotic syndrome at presentation, six had entered remission, two had persistent proteinuria with normal renal function, one patient had CKD and two patients had progressed to ESKD [1].

Current treatment recommendations are from clinical trials in the adult MN population. The GEMRITUX trial in France compared nonimmune antiproteinuric therapy (NIAT), defined as ACE inhibitor or ARB, for 6 months to the use of rituximab 375 m/m^2 given IV for two doses 1 week apart along with NIAT [109]. When this trial was initiated, there was no objective evidence of the benefits of rituximab outside of smaller pilot studies [110–112] and a large observational cohort [42, 113] in adult patients with primary MN. The patients in the GEMRITUX study were age 18 years or older, had biopsy proven primary MN and nephrotic syndrome at the time of enrollment. At 6 months, 35% of the patients receiving NIAT-rituximab combination therapy had achieved remission compared to 21% in the group receiving NIAT alone. A significant reduction in PLA2R-ab was noted in the group receiving NIAT-rituximab. By 6 months, 50% of the NIAT-rituximab group had achieved immunologic remission (disappearance of PLA2R-ab) compared to only 12% in the NIAT alone group [109]. Complication rates in the two groups were similar.

In 2019, the MENTOR trial compared the use of rituximab 1000 mg IV given in 2 doses 14 days apart, with an additional cycle at 6 months if needed, to cyclosporine taken orally at a dose of 3.5 mg/kg/day for 12 months [114]. Study subjects had 5 g/day or more of proteinuria, a GFR of 40 mL/min/1.73 m^2 or greater and had received ACE inhibitor or ARB for at least 3 months. At one-year, complete or partial remission rates were similar: 60% in the rituximab group and 52% in the group receiving cyclosporine. However, at 24-months, complete or partial remission was more common in the rituximab group (60%) than in the cyclosporin group (20%) [114] due to a high number of relapses during cyclosporine tapering and withdrawal.

The results of the GEMRITUX and MENTOR trials support the effectiveness of rituximab in adults with primary MN. In addition, rituximab likely has a more favorable side effect profile than cytotoxic agents and glucocorticoids. The most effective dosing regimen for treating MN with rituximab is not clear. This was partially addressed by a comparison of the use of two infusions of 1 g of rituximab at a 2-week interval in 28 patients from the NICE cohort to rituximab dosed at 375 mg/m^2 given as two infusions at a 1-week interval in 27 participants from the GEMRITUX cohort [115]. Patients in both cohorts had high levels of PLA2R-ab. At 6 months, 64% of the participants from the NICE cohort were in remission compared to 30% from the GEMRITUX cohort [115]. The median time to achieve remission in the NICE cohort was 3 months and 9 months in the GEMRITUX cohort. Participants in the NICE cohort had lower levels of PLA2R-ab at 6 months compared to those in the GEMRITUX study. This suggests that a higher dose of rituximab in adults with primary MN may result in an improved response. The ideal dosing regimen in children remains unknown.

One of the main drawbacks of the use of calcineurin inhibitors such as cyclosporine and tacrolimus for treatment of MN is the high proportion of patients that experience a relapse once the medication is tapered and discontinued. The dramatic drop in the number of clinical remissions observed between 12 and 24 months in the MENTOR trial provides recent evidence of this limitation [114]. The STARMEN trial sought to reduce the rate of relapse by introducing a single dose of rituximab prior to tapering the calcineurin inhibitor [116]. In this study, 6 months of the modified Ponticelli regimen (6 months of alter-

nating corticosteroids and cyclophosphamide) was compared to a 6 month course of tacrolimus, with 1 g rituximab given at 6 months, before a 3 month tacrolimus taper. The corticosteroid-cyclophosphamide group had a significantly higher rate of clinical remissions at 24 months (83.7% partial or complete remissions) versus the 58.1% rate in the tacrolimus-rituximab group [116]. Despite this outcome, there were fewer (12%) relapses after tapering tacrolimus than the 30–40% rate that is typically observed.

The RI-CYCLO trial was a randomized trial in adults comparing rituximab 1 g on days one and 15 to a 6 month cyclic regimen of corticosteroids alternated with cyclophosphamide every other month [117]. The cumulative dose of cyclophosphamide per patient was 180 mg/kg. Outcomes and adverse events in both groups were similar. At 12 months, 62% of the patients in the rituximab group were in complete or partial remission, and this number increased to 85% at 24 months. In comparison, 73% of patients in the cyclic treatment group were in complete or partial remission at 12 months, and by 24 months the percentage had increased to 81% [117].

Safety

The potential adverse effects of immunosuppression should be considered when deciding on treatment. Since pediatric MN may undergo spontaneous remission more often than in adults, many patients can be monitored, with the clinical course and PLA2R-ab levels in some patients determining whether the disease is likely to improve without intervention or cause long-term morbidity. For cyclophosphamide, gonadal toxicity and bone marrow suppression leading to heightened infection risk are major concerns. Peripheral blood counts should be monitored and the cytotoxic agents withheld at a total leukocyte count <3000/mm^3 or in the presence of an active infection. Calcineurin inhibitors have less of an infection risk, but there is a risk of nephrotoxicity with prolonged blood levels above the therapeutic range. Trough blood levels be monitored regularly, and whenever there is an unexplained rise in the serum creatinine (>20%) during therapy. Rituximab can be associated with infusion reactions, reactivation of latent infections, or hypogammaglobulinemia. Potential long-term adverse effects, especially in the pediatric population, are not yet known due to its relatively recent introduction for treatment of nephrotic syndrome in children.

Class V (Membranous) Lupus Nephritis

Nearly half of pediatric lupus cases will be complicated by some form of lupus nephritis, and of these, more than one quarter have class V lesions, either in isolation (16%) or in combination with other (class II, III, or IV) lesions (12%) [118]. GFR tends to be better preserved over time in patients with pure class V lesions than in patients with overlap with class III or IV, but the vast majority of pediatric lupus MN patients are treated with immunosuppressive therapy, often due to the presence of additional extrarenal features. Data from the Childhood Arthritis and Rheumatology Research Alliance, representing a majority of pediatric rheumatology centers across the United States, demonstrate that most patients with class V lupus nephritis receive hydroxychloroquine (98.7%), mycophenolate (91.9%) and daily corticosteroids (96%), with smaller proportions treated with cyclophosphamide, rituximab, calcineurin inhibitors or azathioprine [118]. As with primary MN, the majority of patients are nephrotic, and one center reported that 78% of cases received RAAS inhibitors [119]. In this single center cohort, 74% of isolated class V lupus nephritis patients achieved complete remission at 24 months, but no specific predictors of clinical outcome were found [119]. Achievement of complete remission was not rapid, and can take years. Proliferative features were present in 13% on repeat biopsy; close monitoring of renal function, proteinuria and hematuria is essential.

Conclusions

Membranous nephropathy is an uncommon cause of nephrotic syndrome in children. The diagnosis is usually established when the children are biopsied after being labeled as steroid resistant. It is imperative to evaluate the patient for secondary etiologies since these may respond to treatment of the underlying condition. The initial management of idiopathic MN should generally be conservative. Immunosuppressive therapy should be considered in those children with nephrotic-range proteinuria and/or progressive renal dysfunction. We anticipate that advances from adult disease, including the identification of new target antigens and experience using circulating autoantibodies to guide treatment decisions, will be incorporated into improved treatment and monitoring of pediatric MN.

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19 Postinfectious and Infectious Glomerulopathies

Velibor Tasic and Mignon McCulloch

Introduction

It is important to utilize the correct terminology when describing the infection-related glomerulopathies. In order to better understand the pathogenesis and management of infection-related glomerulonephritis (GN), Nadasdy and Hebert suggest classification as either postinfectious GN or the GN of active infection [1]. Acute postinfectious GN (APIG) is the most common pathology in lower and middle-income countries (LMIC) and is due to a wide spectrum of infective agents. The prototypical APIG is acute poststreptococcal GN (PSGN). In APIG the infection is mild and has usually resolved spontaneously or with antibiotics at the onset of GN, 1–3 weeks later.

Conversely, in GN due to active infection the patient develops infection which does not resolve spontaneously; very often antibiotics are not administered since the infection is not recognized or not considered to be serious. Several weeks after infection begins the patient develops GN, which manifests with hematuria, proteinuria, acute nephritic syndrome or kidney failure. In contrast to postinfectious GN, where antibiotics have no effect on the course of the GN, administration of antibiotics in GN due to active infection eliminates antigen production, ultimately leading to resolution of the GN.

The clinical presentation of infection-related GN varies from subclinical disease to severe acute kidney injury, with the majority of patients having a mild clinical course. There is a growing list of organisms which may cause infection-related GN (Table 19.1).

The pathogenesis of infection-related GN is secondary to (1) formation and deposition of circulating immune-complexes in glomeruli (2) implantation of the antigen in glomerular structures, initiating immunologic reactions and formation of immune-complexes in situ or (3) modifications of native glomerular structures, which become autoantigens [2]. The end result is activation of the complement system and coagulation cascade, and production of proinflammatory cytokines, adhesion molecules and chemoattractants. This leads to proliferation of glomerular cells and infiltration with polymorphonuclear cells.

The most common histological presentation of infection-related GN is diffuse endocapillary or proliferative GN (group A Streptococcus, Streptococcus viridans, Staphylococcus aureus, Diplococcus, Brucella melitensis, measles, mumps, varicella, Cat scratch disease and others),

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Table 19.1 Etiological agents associated with infection-related glomerulonephritis

Bacterial	Viral	Fungal	Parasites
• Streptococcus group A, C, G • Streptococcus viridans • Staphylococcus (aureus, albus) • Pneumococcus • Hemophilus • Neisseria meningitis • Mycobacteria • Salmonella typhosa • Klebsiella pneumoniae • E.coli • Yersinia enterocolitica • Legionella • Brucella melitensis • Listeria • Leptospira • Treponema pallidum • Corynebacterium bovis • Actinobacilli • Cat-scratch bacillus	• Coxsackievirus • Echovirus • Cytomegalovirus • Epstein Barr virus • Hepatitis B, C • HIV • Rubella • Measles • Varicella • Vaccinia • Parvovirus • Influenza • Adenovirus • Rickettsial scrub typhus • Mumps • Hantavirus • Rotavirus	• Coccidioides immitis • Candida • Histoplasma	• Plasmodium malariae • Plasmodium falciparum • Schistosoma mansoni • Leishmania • Toxoplasma gondii • Filariasis • Trichinosis • Trypanosomes • Echinococcus

but it may present as focal or diffuse crescentic GN (Streptococcus, Staphylococcus aureus, varicella, Treponema pallidum). Mesangiocapillary GN is associated with hepatitis C virus and Streptococcus viridans infections. Membranous GN occurs in infections with hepatitis B virus, syphilis, filaria, schistosoma, mycobacterium, and Plasmodium falciparum. Mesangioproliferative GN (focal or diffuse) is associated with Diplococcus, salmonella, hepatitis B virus (childhood vaccination has resulted in decline in this condition), influenza virus, and adenovirus infections. Focal segmental, necrotizing and sclerosing GN is seen in bacterial endocarditis; mesangiolytic GN occurs with ECHO virus infections.

The initial infection may be mild or severe. Examples of more severe infections include pneumonia, meningitis, sepsis, endocarditis, and ventriculoatrial shunt infection [3–6]. The GN may be mild (asymptomatic proteinuria and hematuria), but patients may develop hypertension, circulatory congestion, nephrotic syndrome, and acute kidney injury. Usually there is transient hypocomplementemia.

As previously reviewed, kidney biopsy shows various glomerular lesions, of which the most common is acute endocapillary and proliferative GN, but tubulointerstitial injury also may be present [7]. Immunofluorescence (IF) studies show granular deposits of immunoglobulins and complement. Treatment of GN of active infection must include antibiotics to address the infection, with additional interventions if the infection does not respond to antibiotics alone (e.g., shunt removal, abscess drainage).

In LMIC countries, the incidence of PSGN has declined over the past 5 decades, although is still commonly seen in some countries. In contrast, Staphylococcus aureus, including methicillin-resistant strains, has increased as an etiology of GN, particularly in older adults and diabetics, who often have worse clinical features and outcomes [1, 8–10]. Kidney biopsy shows diffuse glomerular endocapillary hypercellularity with neutrophil infiltration on light microscopy; dominant IgA deposits on IF; and the presence of subepithelial humps on electron microscopy (EM). This entity was entitled IgA dominant postinfectious GN and should be differentiated from classic IgA nephropathy because of different treatment strategies. Kimata et al. [11] described the youngest patient, a 6 year old girl with methicillin-resistant Staphylococcus aureus-

associated GN who initially presented with pneumonia. Vigorous antibiotic treatment resulted in resolution of the GN; in contrast, corticosteroid treatment failed.

It is believed that the outcome of infectious related GN is benign, but in the case of acute kidney injury and crescents on biopsy, corticosteroids, methylprednisolone pulses and cyclophosphamide may be benefitial [6]. The literature is sparse with studies dealing with long-term prognosis of non-streptococcal GN. In a study from Milan 50 adult patients with infection associated GN have been followed for 90 ± 78 months; at the last observation 37% had renal insufficiency or were on hemodyalisis [12]. The unfavourable outcome was due to the underlying disease and presence of interstitial infiltration on kidney biopsy.

Post-streptococcal Glomerulonephritis

PSGN is still the most common glomerulopathy in LMIC countries. The disease is characterized by the acute onset of nephritis, potentially including hematuria, edema, hypertension, oliguria and azotemia [2, 13]. It was recognized as a complication of scarlet fever in the eighteenth century. Due to improved living standards and medical care, PSGN is uncommon in western countries, mainly occurring as sporadic cases [14–16].

Epidemiology

PSGN occurs worldwide [17–22]. It is a complication of pyoderma due to hot climate and high humidity in tropical countries. Skin injuries, insect bites (such as scabies), and poor hygiene and sanitation predispose to infection with group A beta hemolytic streptococcus (GABHS) [23]. In countries with moderate and cold climates, PSGN is usually a complication of upper respiratory tract infections (pharyngitis) during the winter months. Streptococcal M types 2, 47, 49, 55, 57, 60 are associated with PSGN following pyoderma, while M types 1, 2, 3, 4, 12, 25 and 45 are associated with PSGN following pharyngitis. While typically seasonal, isolated cases of PSGN may be seen throughout the year. In the past, epidemics of PSGN following impetigo were reported. In some areas (Trinidad, Maracaibo), epidemics occurred every 5–7 years; there is no satisfactory explanation for this phenomenon [13]. Populations at increased risk include children and soldiers, due to intimate contact, overcrowded living conditions, and poor hygiene and sanitation. The ratio male:female is up to 2:1, but when subclinical cases are included, there is no male predominance. The disease is most common in children aged 3–12 years, although PSGN has been reported in infants [24, 25]. The risk for developing PSGN after infection with a nephritogenic strain of GABHS is about 15%; for M type 49, it is 5% after pharyngeal infection and 25% after pyoderma [26]. Rarely, PSGN occurs as a complication of piercing [27] or circumcision [28]; presents in a kidney allograft [29]; or occurs secondary to immune reconstitution inflammatory syndrome in pediatric HIV infected patient [30]. Besides GABHS, streptococci from group C and G can also cause acute GN [31–33], but the concept of a common nephritogenic antigen is questionable [34].

Pathogenesis

There is clear evidence that PSGN is an immune complex disease, but the identity of the nephritogenic antigen is uncertain [35, 36]. The proposed mechanisms are;

1. Deposition of circulating immune complexes containing nephritogenic antigen in glomeruli
2. Implantation of the nephritogenic antigen into glomerular structures and in situ formation of immune complexes
3. Molecular mimicry between streptococcal antigens and glomerular antigens, which react with antibodies against streptococcal antigens
4. Direct activation of the complement system by implanted streptococcal antigens. Many proteins such as endostreptosin, preabsorbing antigen, nephritis strain–associated protein,

streptococcal pyrogenic exotoxin B (SPEB), nephritis-associated plasmin receptor (NaPlr) have been considered as potent nephritogenic antigens in PSGN [37–45].

There are several reports of infection-related GN caused by pathogens other than Streptococcus group A and C with detection of NAPlr in kidney biopsies. This lists includes Streptococcus pneumoniae, Staphylococcus aureus, Mycoplasma pneumoniae and Aggregatibacter actinomycetemcomitans [46–49].

Evidence that an antigen is nephritogenic should include identifying the antigen in kidney biopsy specimens from patients with PSGN; extracting the same antigen from streptococci obtained from PSGN patients; not identifying the antigen in streptococci cultured from patients with rheumatic fever; and demonstrating significant titer of antibodies against the nephritogenic antigen in sera from PSGN patients in the convalescent phase. Lange and his group [39, 40] considered that endostreptosin (ESS) was an ideal nephritogenic antigen because it fulfilled the these criteria. Interestingly, ESS was identified in early but not in late biopsy specimens,. In animal experiments, ESS was implanted on the glomerular basement membrane very early in the course of the disease. Late in the disease course, there was production of anti-ESS antibodies, which bound ESS and thus enabled detection of the ESS. The two main disadvantages of this theory are (1) endostreptosin is an anionic antigen and this cannot explain its implantation on the glomerular basement membrane (GBM) (2) injection of ESS has never induced histological changes and clinical features compatible with PSGN.

Vogt et al. provided evidence that cationic antigens were responsible for the immunopathogenesis of PSGN; they identified cationic antigens in 8 out of 18 biopsy specimens from PSGN patients and confirmed that streptococci cultured from PSGN patients produced cationic antigens [42]. Later this antigen was confirmed to be streptococcal pyrogenic exotoxin B (zymogen, SPEB), a plasmin binding membrane receptor. Glyceraldehyde phosphate dehydrogenase (GAPDH) and NAPlr/Plr are also candidate nephritogenic antigens [36, 44, 50, 51]. Both antigens induce long lasting antibody responses; antibodies against NaPlr can be detected 10 years after an acute episode. This may explain why second attacks of PSGN are rare. Nephritogenic potential is not limited to GABHS, but extends to groups C and G, with sporadic and epidemic cases of PSGN reported after infection with these streptococcal groups. The common pathway for both antigens is binding to plasmin, which activates complement, and promotes chemotaxis and degradation of GBM components. Bound plasmin can cause tissue destruction by direct action on the GBM, or by indirect activation of procollagenases and other matrix metalloproteinases. This allows circulating immune complexes to transit the damaged GBM and accumulate in the subepithelial space, seen as humps by EM.

NAPlr has been isolated from both groups A and C streptococci, and was considered as a putative antigen in the Japanese population with serum antibodies detected in 92% of convalescing PSGN patients and in 60% of patients with uncomplicated streptococcal infections [44]. Glomerular deposits and serum antibodies against these two putative antigens were examined concurrently in biopsies and sera from PSGN patients [45]. This study suggests that SPEB is the most likely major antigen involved in the pathogenesis of PSGN in patients from Latin America, US and Europe. Subsequently, a genome study of *S. equi* subsp. *Zooepidemicus* strain MGCS10565, a Lancefield group C organism that caused an epidemic of nephritis in Brazil, found that this organism lacked a gene related to SPEB and challenges the hypothesis that SPEB or antibodies reacting with it singularly cause PSGN [34].

Immune complexes deposited from the circulation or formed in situ activate the complement cascade. This leads to production of various cytokines and other cellular immune factors which initiate an inflammatory response manifested by cellular proliferation and edema of the glomerular tuft [52, 53].

In some PSGN patients, rheumatoid factor, cryoglobulins, and antineutrophil cytoplasmic antibodies (ANCA) are present [54–58]. The significance is unknown.

Pathology

The typical presentation on light microscopy is diffuse enlargement of all glomeruli due to hypercellularity (Fig. 19.1). There is swelling of the endothelial cells, which leads to the obliteration of the capillary loops. The number of mesangial cells is increased. There is recruitment of numerous inflammatory cells in the glomeruli, mainly polymorphonuclear leukocytes and monocytes; thus, this pathological picture is termed exudative proliferative GN. Polymorphonuclear leukocytes may be seen in the tubular lumen. If the mesangial proliferation is axial, then the glomerulus has a lobular appearance. Capillary walls are not thickened. Arterioles and tubules are not affected. There may be edema of the interstitium and infiltration with inflammatory cells. Rarely, proliferation of parietal cells of Bowman's capsule may result in formation of crescents; a high percentage is associated with a rapidly progressive course.

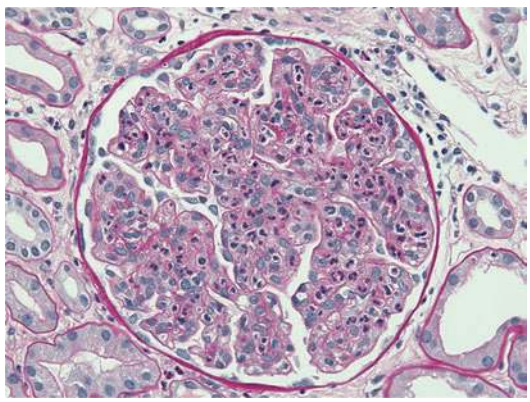

Fig. 19.1 Acute post-streptococcal glomerulonephritis, light microscopy. The glomerulus is enlarged and hypercellular; capillary loops are obliterated; and there is infiltration with polymorphonuclear leukocytes (hematoxylin and eosin, ×400). Courtesy of Prof. N. Kambham, MD, Dept. of Pathology, Stanford University

By IF, the most common finding in the acute phase is an irregular, granular capillary and mesangial staining for complement alone, or complement and immunoglobulins. During the resolving phase, there is only mesangial staining (Fig. 19.2). The predominant finding is C3 and IgG, but C4, C1q, IgM, fibrinogen and factor B may be found. Sorger et al. described three types of immune deposits in PSGN [59]. Starry sky pattern is the fine granular deposition of C3 and IgG along the capillary walls in the first week of the disease (Fig. 19.2a). Mesangial pattern is found between the fourth and sixth week after the disease onset; the only immune reactant is C3, which is found in a mesangial location (Fig. 19.2b). The garland pattern is characterized by dense, confluent deposits along the capillary loops, while mesangial and endocapillary locations are preserved (Fig. 19.2c). Subepithelial location of the deposits correlates with the humps seen on EM. The garland pattern is associated with massive proteinuria and does not correlate with the time of kidney biopsy [60]. In clinically atypical cases with acute kidney injury and nephrotic syndrome, NAPlr staining of the glomeruli is a useful tool for confirmation of the diagnosis of PSGN [61].

The typical finding on EM in the acute phase is deposits on the subepithelial side of the GBM (humps), Fig. 19.3. These deposits disappear after the sixth week of disease onset [62].

Parallel to the clinical resolution of the disease, there is marked improvement of the histological picture, with resolution of exudative and endocapillary changes; there is residual mesangial proliferation in the convalescent phase (resolving mesangioproliferative GN). Subepithelial deposits disappear or decrease in number after the sixth week; immune deposits decrease in parallel. Complete histologic resolution usually occurs by 1 year after onset.

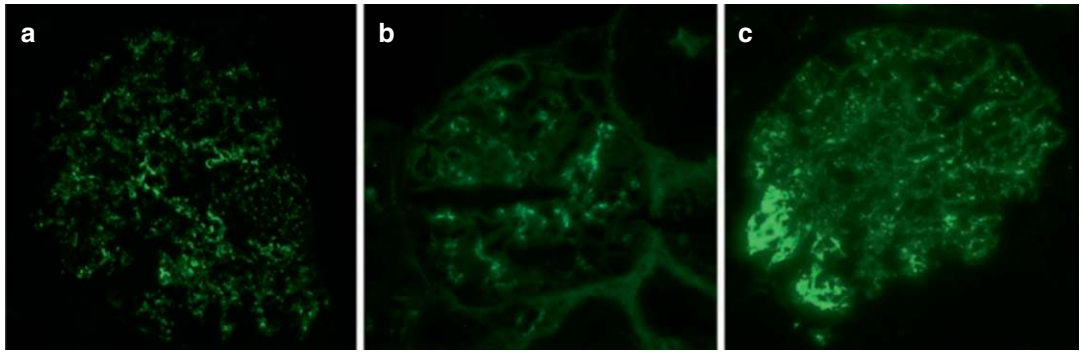

Fig. 19.2 Immunofluorescent study in acute poststreptococcal glomerulonephritis showing intensive immune deposit of C3. (**a**) Starry sky pattern (×400). (**b**) Mesangial pattern (×400). (**c**) Garland pattern (×400). Courtesy of Prof. N. Kambham, MD, Dept. of Pathology, Stanford University

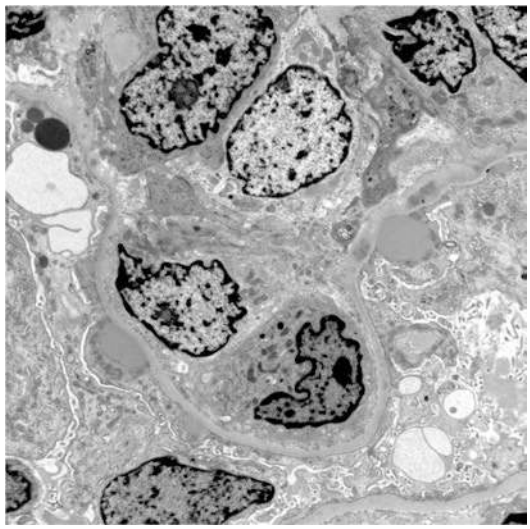

Fig. 19.3 Acute poststreptococcal glomerulonephritis, electron microscopy. Typical electron dense deposits (humps) located on the subepithelial side of the glomerular basement membrane (electron micrograph, ×8000). Courtesy of Prof. N. Kambham, MD, Dept. of Pathology, Stanford University

Clinical Features

The latent period between the upper respiratory infection (pharyngitis) or pyoderma and nephritis is usually 10–14 days or 2–4 weeks, respectively. One third of PSGN patients develop discrete microscopic hematuria and/or proteinuria in the latent period. Usually the disease has acute onset, with development of nephritic syndrome (oedema, oliguria, azotemia hematuria, hypertension). Evidence of nephritis within 2–3 days of the onset of an upper respiratory tract infection is suggestive of other etiologies such as IgA nephropathy or Alport syndrome. At the onset of the disease, non-specific symptoms may be present, such as pallor, malaise, low-grade fever, lethargy, anorexia and headache.

Gross hematuria is present in 30–70% of patients with PSGN, while microscopic hematuria is present in all patients. Microscopic examination of the urine reveals dysmorphic red blood cells and casts. The urine is described as being smoky, cola colored, tea colored or rusty. Gross hematuria may be present continuously or only a few hours during the day. Usually it resolves after 1–2 weeks and transforms into microscopic hematuria. Once gross hematuria has seemingly resolved, it may reappear after physical exercise or intercurrent infections. A few patients have minimal urinary finding (few red blood cells/per high power field), yet have a severe clinical presentation of the disease [63, 64].

Edema in PSGN results from retention of salt and water. Despite the sodium retention, the increased level of atrial natriuretic peptide in plasma of PSGN patients indicates unresponsiveness of the kidneys to its action [65]. Parents often do not appreciate the edema, but it becomes clear during the diuretic phase when there is a marked weight loss. Most children have mild morning periorbital edema; this location is due to reduced tissues resistance. There may also be pretibial edema or generalized

edema (anasarca), including pleural effusions and ascites. Early salt and water restriction may prevent the consequences-circulatory congestion and hypertension.

Hypertension occurs in up to 70% of hospitalized children. Hypertension in PSGN is low renin type due to retention of water and salt, which leads to expansion of the extracellular fluid volume with consequent suppression of the renin-angiotensin-aldosterone axis. Usually it is mild and has a biphasic character. Hypertension that is severe and associated with retinal changes is suggestive of pre-existing renal disease. Normalization of the blood pressure correlates with diuresis and recovery of the kidney function. Hypertension beyond 4 weeks after disease onset may indicate rapidly progressive disease or chronic GN.

Complications

Circulatory congestion is the most common complication in hospitalized children with PSGN. If severe, it can lead to pulmonary oedema, which represents an emergency state and requires prompt and appropriate therapy. The signs and symptoms of circulatory congestions are tachycardia, dyspnoea, orthopnea, rales and cough. Sometimes clinical signs may be subtle, but a chest radiograph shows signs of congestion. Since children and young individuals have healthy cardiovascular systems, cardiac failure is rarely seen.

Posterior reversible encephalopathy syndrome (PRES), previously called hypertensive encephalopathy, is another serious complication found in 0.5–10% of hospitalized patients [13]. The most common clinical signs and symptoms are nausea, vomiting, headache, and impairment of consciousness, which varies from somnolence to coma. The children may manifest seizures, hemiparesis, amaurosis and aphasia. These symptoms are a consequence of sudden elevation of the blood pressure that impairs cerebral autoregulation leading to vasogenic edema. Electroencephalography has non-specific changes, which resolve in parallel with resolution of the neurological symptoms. Analysis of the cerebrospinal fluid may reveal the presence of protein, but no cells. On magnetic resonance imaging, there is alteration of the posterior white matter, which is termed reversible posterior leukoencephalopathy syndrome [66, 67]. The images show edematous lesions primarily involving the posterior supratentorial white matter and corticomedullary junction. Neurological complications in PSGN cannot be attributed exclusively to hypertensive encephalopathy or abnormal serum biochemistry, particularly in those patients with normal blood pressure during the incident (e.g. seizures). With advances in neuroimaging, there is evidence that some children develop cerebral vasculitis [68, 69]. This has practical implication because it may require different treatment.

PSGN may also be complicated by severe acute kidney injury. Some patients require dialysis.

Clinical Variants

Approximately 90% of patients have subclinical disease and never seek medical care due to absence of symptoms [13]. Rarely, patients may have nephrotic syndrome (0.4%) or rapidly progressive disease (0.1%). The incidence of subclinical disease (expressed as ratio subclinical: clinical disease) varies from 0.03 to 19.0 [70, 71]. This is most likely due differences in methodology and study populations, which has included epidemic contacts [26], family contacts [70, 72, 73], or patients with well-documented streptococcal infections [71, 74]. The population at risk has been tested for urinary abnormalities and hypocomplementemia once or sequentially. More frequent testing increases the likelihood of detecting abnormalities, which may be transitory and normalize within a week.

Sagel et al. followed 248 children from New York 4–6 weeks after well-documented streptococcal infections [71]. Abnormal urinalysis with hypocomplementemia was detected in 20 children, but only one had symptomatic disease. The incidence of nephritis after streptococcal infection in this report was 8.08% and the

ratio subclinical/clinical nephritis 19.0. Kidney biopsy was performed in all 20 children and showed histological lesions varying from mild focal cellular proliferation to classical exudative and proliferative GN. Only one child had normal histology and lack of immune deposits. The authors concluded that only a minority of PSGN cases are detected. Yoshizawa et al. performed a similar study in Japan; 12 out of 49 patients with well-documented streptococcal infection developed subclinical nephritis (24%) and all 12 patients had abnormal kidney biopsies [74].

In a study of family contacts from Macedonia, the incidence of nephritis in parents and siblings was 0% and 9.4%, respectively [73]. It seems that parents are "protected" from developing PSGN. The ratio of subclinical/clinical nephritis in contacts was 1.28. An additional number of family contacts had glomerular type microhematuria and elevated ASO titre; thus, it is possible that they also had subclinical PSGN and that their complement levels normalized before occurrence of nephritis in the index cases. Lange et al. pointed that the finding of significant titers of endostreptosin antibodies in patients with chronic GN or on hemodialysis suggested the possibility of previous undetected subclinical PSGN [39, 75].

Nephrotic syndrome occurs in 4–25% of hospitalized children with PSGN. It usually resolves within 2–3 weeks; persistence beyond 3 weeks is associated with a poor outcome. Less than 1% of hospitalized children develop rapidly progressive disease, which is characterized by prolonged oligo-anuria, uremia, hypertension, anemia and persistent nephrotic syndrome. Crescents are present on kidney biopsy, and the percentage of crescents correlates with the severity of the disease and the outcome.

Cerebral vasculitis is an infrequent complication; cutaneous and gastrointestinal vasculitis have also been reported in PSGN patients and may mimic Henoch-Schönlein purpura [76]. PSGN is uncommonly associated with rheumatic fever [77, 78]. An unusual or atypical course of the disease is reported in patients with concurrent IgA nephropathy, diabetes mellitus, hemolytic uremic syndrome, reflux nephropathy and bilateral kidney hypoplasia [79–82]. Simultaneous occurrence of acute immune thrombocytopenia has also been reported in a few PSGNpatients [83–85]. The most likely mechanism is production of autoantibodies that cross-react against GABHS and platelets [84].

Laboratory Findings

Proteinuria and hematuria are found in almost all patients with PSGN. The presence of red blood cell casts and dysmorphic erythrocytes confirms the glomerular origin of the hematuria. In a few patients, minimal urinary findings occur despite a severe clinical presentation.

A mild dilutional anemia may be seen at the onset of the disease and is due to expansion of the extracellular fluid volume. Thrombocytopenia is extremely rare; its presence suggests the possibility of systemic lupus erythematosus or hemolytic uremic syndrome. If there is no significant impairment of glomerular filtration rate (GFR), blood chemistry is almost normal; severe reduction of renal function leads to hyperkalemia, uremia and acidosis. Hypoproteinemia, hypoalbuminemia and hyperlipidemia are evident if there is associated nephrotic syndrome.

Patients should be evaluated for evidence of previous streptococcal infection. Cultures from the throat or skin should be obtained, depending on the site of the initial infection. Antibodies against streptococcal antigens (antistreptolysin O, antihyaluronidase, anti-DNA-se B titer), or combination of antigens (streptozyme) should be measured serially during the course of the disease. Of note, in postpyodermal disease there is an insignificant rise in antistreptolysin O titres. Testing anti-zymogen titres is very sensitive and specific for diagnosing streptococcal infection in PSGN patients, but this test is not available for routine practice. A high titre of antibodies against glyceraldehydes phosphate dehydrogenase is found in PSGN patients.

There is marked depression of CH50 and C3 due to activation of the alternative pathway. In some patients, there is also depression of C2 and C4, suggesting activation of both classical and

alternative pathways [86]. Usually, complement levels normalize within 6–8 weeks; persistence of hypocomplementemia beyond 3 months suggests an alternative diagnosis (membranoproliferative GN).

Complement activation was analyzed in 34 children with APIG and low C3 level at onset [87]. They demonstrated that anti-factor B (anti-FB) antibodies enhance alternative pathway convertase activity in vitro, confirming their pathogenic effect. They identified anti-FB autoantibodies in 31 of 34 (91%) children with APIG and in four of 28 (14%) children with C3 GN. Sensitivity and specificity of anti-FB antibodies for APIG diagnosis were 95% and 82%, respectively. Anti-FB antibodies were not detected in 15 patients with IgA nephropathy and 26 with lupus nephritis. During the follow-up, the anti-FB antibody levels became negative or decreased in children with APIG. The authors hypothesize that streptococcal infection may cause transient anti-FB autoantibodies, which overactivate complement with subsequent deposition of complement C3 breakdown products in the glomeruli.

Kozyro et al. tested children with PSGN for the presence of antibodies against C1q and found that 8 of 24 were positive for anti-C1q [88]. They found that anti-C1q positive children had more severe disease (hypertension, proteinuria) and unfavourable resolution of the disease.

Kidney Biopsy and Differential Diagnosis

Usually children with PSGN have a favourable disease course and outcome; thus, kidney biopsy is not necessary. In cases of severe or atypical clinical presentation or delayed recovery, then kidney biopsy is mandatory. Indications for kidney biopsy are summarized in Table 19.2.

PSGN should be differentiated from the following diseases: IgA nephritis (short latent period from infection), hereditary nephritis (family history, short latent period), MPGN (persistent hypocomplementemia and unresolving nephritic syndrome), lupus nephritis (persistent hypocomplementemia, systemic manifestations), GN in acute and chronic infections (evidence for other non-streptococcal infection), vasculitides (polyarteritis nodosa, Henoch Schönlein purpura), haemolytic uremic syndrome (hemolysis, thrombocytopenia).

Table 19.2 Indications for kidney biopsy

Early stage	Recovery phase
• Age <2 years • Short latent period • Anuria • Rapid progressive course • Hypertension >2 weeks • Depressed GFR >2 weeks • Normal complement levels • No elevation of antistreptococcal antibodies • Extrarenal manifestation	• Depressed GFR >4 weeks • Hypocomplementemia >12 weeks • Persistent proteinuria >6 months • Persistent microhematuria >18 months

Vernon et al. described a girl who developed chronic kidney disease and persistent hypocomplementemia after streptococcal throat infection. Kidney biopsy 1 year after presentation revealed features of C3 glomerulopathy while genetic studies detected a heterozygous mutation in the complement factor H-related protein 5 gene (CFHR5) [89]. A group from the Mayo Clinic presented a series of 11 patients who had atypical postinfectious GN [90]. Kidney biopsy was performed due to persistent proteinuria, hematuria, and depressed C3. On light microscopy, there were exudative and proliferative changes; IF studies revealed dominant C3 mesangial deposits while EM revealed subepithelial humps. In 10 of 11 patients, there was an underlying defect in the alternative pathway of the complement, either autoantibodies against C3 convertase or mutations in complement regulatory genes.

Treatment

Bedrest and limited activity are indicated in the early stage of the disease, particularly if circula-

tory overload and hypertension are present. There is no evidence that prolonged bedrest hastens recovery.

In most cases, fluid and salt restriction are sufficient to prevent edema and hypertension. Salt intake should be limited to less than 1.0 g/day. Usually protein intake should be limited to 1.0 g/kg/day. In case of marked azotemia, calories should be provided from carbohydrates and fats. to the diet is individualized based on clinical and biochemical indices. Diuresis and body weight should be monitored every day. Loop diuretic (furosemide 1–2 mg/kg/day) is indicated if there is moderate circulatory congestion. Higher doses, up to 5 mg/kg per dose intravenously are indicated if there is pulmonary oedema, although caution is indicated if there is severe azotemia because of potential ototoxicity.

Moderate hypertension should be treated with diuretics and oral antihypertensive drugs; amlodipine is now widely used. Angiotensin converting enzyme inhibitors (ACEIs) may be effective, but there is concern for worsening hyperkalemia [91]. In a hypertensive emergency, options include intravenous labetalol (bolus or continuous infusion) and nicardipine or nitroprusside by continuous infusion. Short-acting, oral or sublingual nifedipine has been used for hypertensive emergencies without encephalopathy. However, given the occurrence of serious and fatal adverse effects in adults, it should be administered in children with great caution [92]. Amlodipine or isradipine may be safer options.

Hyperkalemia should be prevented with restricted potassium intake. If present, conservative treatment should be started immediately to prevent fatal complication. Severe hyperkalemia, azotemia, acidosis, uncontrolled hypertension, cardiovascular insufficiency and pulmonary edema are indications for urgent dialysis.

There is no clear evidence that immunosuppressive therapy has a beneficial effect in children with crescentic PSGN. In those with >30% crescents one may attempt pulse methylprednisolone 0.5–1.0 g/1.73 m^2 for 3–5 days. In ten children with crescentic PSGN, five were given quintuple therapy (including immunosuppressive drugs) and five were given only supportive treatment [93]. At the end of the follow-up, the outcomes were similar, though patients who received quintuple treatment had faster normalization of serum creatinine and decreased duration of hospitalization. Nevertheless, based on efficacy in other forms of rapidly progressive GN, some clinicians administer high-dose intravenous methylprednisolone for 3–5 days for severe disease based on kidney function and percentage of crescents.

Antibiotic therapy is indicated if there are still signs of streptococcal infection (pharyngitis, pyoderma) or patients have positive throat or skin culture. Oral penicillin V (or erythromycin for allergic patients) is preferred over parenteral treatment. Antibiotic treatment does not alter the course of the PSGN, but it is important to prevent spread of nephritogenic strains of GABHS. Long-term antibiotic prophylaxis is not justified since second attacks of PSGN are rare [94].

Prognosis

The prognosis of PSGN in children in the acute phase is excellent, with mortality less than 1% due to improved conservative management and availability of dialysis. There are conflicting data regarding long-term outcomes. For example, Baldwin et al. [95, 96] suggest unfavourable outcomes in many patients while Potter et al. [97] describe excellent outcomes. This is mainly due to different criteria for selection of patients for prognostic studies, excellently reviewed in detail by Cameron [98]. It is important to remember that only clinical cases (10%) are included in the analysis, while those with subclinical and mild disease may escape medical attention. Moreover, some are not referred to a nephrologist and only a small percentage have a kidney biopsy. The series describing prognosis differ in respect to following parameters: pediatric/adult, sporadic/epidemic, evidence/no evidence for previous streptococcal infection, with/without kidney biopsy, and with/without crescents on kidney biopsy.

In a study by Vogl et al. [99], 36 children and 101 adults had biopsy and serological confirmation of PSGN and had been followed for 2–13 years; none of the children reached end stage kidney disease (ESKD), but 10% had elevated serum creatinine between 1–2 mg/dL. Clark et al. provided excellent information concerning long-term outcome of PSGN in children [100]. Although their series was small, it was exclusively pediatric, with adequate documentation of streptococcal infection and initial biopsy in all children and rebiopsies in some of them. Thirty children were followed for 14.6–22 years (mean: 19 years). Urinary abnormalities were present in 20% of patients during the follow-up, but none had reduced GFR, assessed with creatinine clearance. Clark et al. questioned the role of kidney biopsy for diagnosis and follow-up of children with typical PSGN.

Baldwin et al. reported unfavourable data on long-term prognosis of PSGN [95, 96]. In their series, 37 out of 126 patients were children; 11 patients progressed to terminal uremia, nine in the first 6 months. During the follow-up of 2–15 years, proteinuria, hypertension and reduced GFR were documented in half of the patients. A total of 174 kidney biopsies were performed; in the first years after the acute episode there was a prevalence of proliferative changes, while in 2/3 of the late biopsies there were sclerosing lesions, which Baldwin considered as an indicator of chronicity. The poor outcomes may seem likely secondary to a highly selected patient population; 20% presented with nephrotic syndrome. Patients who died or rapidly progressed to uremia had crescentic nephritis at biopsy. A substantial number of patients were lost to follow-up, with selection of those who had more severe disease. Furthermore, GFR in this study was not corrected for sex, age and body surface area. The same group reported six patients with PSGN who progressed to terminal uremia 2–12 years after resolution of acute nephritis and normalization of the GFR [101]. Of note, five of six patients had nephrotic syndrome at the disease onset. Gallo et al. presented data on the morphologic alteration in kidney biopsies from patients who recovered from PSGN; they found that the incidence of glomerular and vascular sclerosis increased with time [102]. The clinical consequence of this healing process is reduced kidney functional reserve after a protein loading test [103, 104].

The two studies from Maracaibo, Venezuela also pointed to the progressive character of PSGN [105, 106]. One hundred and twenty patients (101 children) who had survived the epidemics in 1968 were evaluated between 1973–1975. Proteinuria, microhematuria, hypertension or reduced GFR were found in 36.7% of adult patients compared with 8.7% of pediatric patients. Kidney biopsies showed advanced glomerulosclerosis in all patients with abnormal findings. Mild to moderate mesangial proliferation and glomerulosclerosis were present even in those patients who had no history of any clinical abnormality.

Herrera and Rodríguez-Iturbe investigated the incidence of ESKD among Goajiros Indians, a semi-nomadic tribe that live in the northwestern part of Venezuela [107]. The incidence of ESKD was 1.7 times higher than the incidence for the country. Also, the attack rate of PSGN was double compared with the general population in the neighboring Maracaibo city. Low birth weight was common among Goajiros Indians (23% of newborns weigh less than 1000 g). The authors concluded that high attack rate of PSGN and low nephron endowment were responsible for the increased risk of ESKD in this population.

In contrast, Dodge et al. [108] and Travis et al. [109] reported excellent clinical and histological healing of the disease in their pediatric series. Dodge et al. found that the presence of proteinuria was present despite histological healing; it had an orthostatic character before definitively cleared [108]. In a study from Macedonia, 40 post-nephritic children were investigated 3 months to 10 years after the acute episode, but no increase in proteinuria was found after moderate to strenuous physical activity [110]. Perlman et al. reevaluated 61 children 10 years after the original epidemics in 1963 [111]. All children had normal GFR, 3 had proteinuria >100 mg/24,

but all had normal morphology on kidney biopsy. Sixteen children had a kidney biopsy; four patients had minimal focal proliferation, but no sclerosing lesions were seen.

Three studies from Trinidad evaluated medium and long-term prognosis of PSGN. These are the largest studies, predominantly pediatric, with excellent outcomes concerning presence of urinary abnormalities, hypertension or impaired renal function [97, 112, 113]. Renal biopsy was not performed in many studies for diagnosis and follow-up of PSGN in children, but the diagnosis was based on firm clinical and serological documentation of previous streptococcal infection and transitory hypocomplementemia. Results of these studies confirmed the benign course of PSGN in children, with very low percentage having persistent urinary abnormalities, hypertension or reduced GFR [114–116]. Besides clinical healing, there was complete functional recovery in almost all patients. Drukker et al. found that natriuretic response was excellent in post-nephritic children after intravenous saline loading [117].

In some indigenous communities in Australia and New Zealand, there is still high attack rate of PSGN [118, 119]. Repeated episodes of PSGN and low number of nephrons due to higher rate of prematurity contribute to higher prevalence of chronic kidney disease in the Aboriginal population [120, 121].

From analysis of multiple studies, the following risk factors for unfavourable outcome were identified: older age, high serum creatinine at presentation, nephrotic syndrome and crescents on kidney biopsy. Even after initial normalization of the kidney function, impairment of the GFR may ensue many years after disease onset; thus, children who present with crescents need indefinite follow-up [122].

Prognosis of PSGN caused by group C *Streptococcus zooepidemicus* appears less favorable. After a mean follow-up of 5.4 years after epidemics in Brazil, a relatively high percentage of patients had microalbuminuria, hypertension and reduced GFR [123]. However, it was impossible to draw conclusions for children since few were evaluated.

Shunt Nephritis

Immune complex GN associated with infection of a ventriculoatrial shunts was first reported in 1965 by Black et al. [124]. Shunt infections are common, but few patients develop GN (2%). Ventriculoperitoneal shunts are now preferred over ventriculoatrial shunts because of lower rates of complications, including shunt infections. The clinical features of shunt nephritis are variable and include proteinuria, hematuria, hypertension, nephrotic syndrome, anemia and compromised kidney function [10, 125]. Symptoms of shunt infections may be present and include fever, anemia, malaise, hepatosplenomegaly and cerebral symptoms. Colonization of the shunt may persist for months and years in otherwise asymptomatic patients. Low-grade fever may be the only sign of active shunt infection and this may result in delay of diagnosis. Staphylococcus epidermidis is the most common pathogen, occurring in 75% of shunt infections. This is a skin contaminant most likely introduced during the surgical procedure. Other isolated pathogens are S. aureus, corynebacterium, listeria, pseudomonas, Propionibacterium acnes, and Bacillus species [10, 126].

Laboratory investigations demonstrate low C3 in 90%, elevated erythrocyte sedimentation rate, cryoglobulinemia and positive blood or cerebrospinal fluid cultures. Patients with ventriculoatrial shunt with unexplained hematuria, proteinuria, or compromised kidney function should have prompt diagnostic work up for subacute shunt infection, even in the absence of fever and leukocytosis [10]. Kidney biopsy shows membranoproliferative pattern on light microscopy in the majority of patients [125]. IF studies demonstrate granular deposits of C3, IgM and IgG in subendothelial and mesangial locations. Persistent antigenemia is responsible for immune complex formation, but it is unclear whether the immune complexes are formed in the circulation or *in situ*. Their presence induces complement activation through the classical pathway, which further mediates injury to glomerular cells (through the C5–9 complex) and generates che-

motactic peptides (C3a, C5a) that perpetuate local inflammation.

The prognosis of shunt nephritis is excellent, with normalization of kidney function and resolution of proteinuria, if the infected shunt is removed and appropriate antibiotic treatment is administered. Kidney function normalizes within a few weeks and hypocomplementemia also resolves [10, 125]. Delayed removal of the infected shunt may result in progressive worsening of kidney function and lead to ESKD.

Endocarditis-Associated Glomerulonephritis

Infective endocarditis (IE) is mainly a complication of congenital or rheumatic heart disease in children and still has high mortality despite appropriate antibiotic therapy. Kidney involvement occurs in about 25% of the patients with IE and manifests as kidney infarcts, GN, and interstitial nephritis [10]. The most common pathogen is Streptococcus viridians, whose indolent clinical course enables prolonged antibody response and formation of circulating immune complexes which predispose to the development of GN. Other causative pathogens include S. epidermidis, enterococcus, Hemophilus influenza, actinobacillus, chlamydia, Bartonella henselae, and Coxiella burnetti [126].

Children with acute IE) present with severe illness, including fever, anemia, heart murmur, hepatosplenomegaly, skin purpura and retinal hemorrhages (Roth spots). In contrast, those with a subacute course may have subtle symptoms that are only recognizable during the work-up of the GN. GN usually ensues within 7–10 days of clinical illness. Duration of endocarditis does not increase the risk of developing GN. The clinical presentation is variable, from mild proteinuria and hematuria to a severe, rapidly progressive course. The most common presentation is acute nephritic syndrome. Laboratory investigation reveals hypocomplementemia, which correlates with the severity of kidney disease and infection. Rheumatoid factor may be positive and some patients have ANCA that react against proteinase 3.

The kidney biopsy findings are diverse; focal segmental proliferation is the most common finding, followed by diffuse endocapillary proliferation. Exudative features are similar to those seen in PSGN. Crescents and glomerular necrosis may also be present, and in some patients may affect >50% of glomeruli [6]. The tubular atrophy and fibrosis correlates with the extent of glomerular necrosis and crescents. Rarely, membranoproliferative GN resembling MPGN type I may be present and the biopsy shows diffuse mesangial and endocapillary proliferation, lobular accentuation, and GBM reduplication. During resolution of the GN, mesangial proliferation is the dominant histological pattern. IF studies show dominant deposits of C3 and less intense IgG and IgM in mesangial areas and in capillary walls. EM detects subepithelial deposits in the early phase; latter in the course of the disease they are located in subendothelial and mesangial areas.

Endocarditis-associated GN represents an immune complex disease with deposition of circulating immune complexes in the glomeruli, but also there is evidence for in situ formation of complexes. The nephritogenic bacterial antigens were identified within the affected glomeruli in S. aureus and streptococcal infections. Treatment consists of antibiotic therapy and surgery to remove valvular vegetations and eradicate the infection. Most infective and non-infective complications of IE resolve on treatment with appropriate antibiotics. In a few patients with proliferative lesions and no improvement with antibiotics, corticosteroids and cyclophosphamide may be useful [6, 127]. Patients with crescentic GN may benefit from plasmapheresis.

HIV Related Kidney Disease

The implementation of the successful prevention of mother-to-child transmission (PMTCT) program has dramatically decreased new cases of pediatric HIV infection [128, 129]. Highly active antiretroviral therapy (HAART) has significantly

decreased the mortality rate in children who acquire perinatal HIV infection. However, managing and preventing contraction of HIV remains a major part of adolescent care in Africa [130]. HIV is currently the second leading cause of death among adolescents worldwide. Women aged 15–24 are the group with the highest rate of new HIV infection in sub-Saharan Africa [131].

Increased survival and complications of therapy have led to a variety of non-infectious complications in patients with HIV infection, and kidney disease is an important concern. Chronic kidney disease in children with perinatal HIV infection is the consequence of primary HIV infection, antiretroviral therapy, and other nephrotoxic drugs, including aminoglycosides. The spectrum of kidney disease includes chronic glomerular disorders, such as HIV associated nephropathy (HIVAN) and HIV immune complex kidney disease (HIVICK); the thrombotic microangiopathies (atypical haemolytic uremic syndrome and thrombotic thrombocytopenic purpura); disorders of proximal tubular function; and acute kidney injury [132].

The histology of HIVAN in children is classical FSGS, with or without mesangial hyperplasia. Other features may include microcystic tubular dilatation and interstitial inflammation. This contrasts with adults, where collapsing FSGS is the typical histological finding. Two pediatric studies have reported collapsing FSGS in 14% and 32.5% [133, 134]. This has important prognostic implications in children since collapsing FSGS has more rapid and progressive course towards ESKD compared with the classical form.

In the pathogenesis of HIVAN, the initial event is infection of the kidney epithelial cells by HIV-1, but it is still enigmatic how the virus enters the epithelial cells since podocytes and renal tubular cells do not express CD4 or other co-receptors. The injured podocytes undergo proliferation and apoptosis; then the remaining podocytes hypertrophy and leave bare segments of basement membrane that promotes the development of the sclerotic lesions that characterize HIVAN. HIV nef and tat genes are implicated for the glomerular pathology while vpr genes are responsible for tubular lesions. Host genetic factors predispose to development of HIVAN and progression to ESKD [132]. This is supported by the observation that African-Americans have a higher incidence of HIVAN, with a rapid and unfavorable course. Polymorphisms G1 and G2 in the APOL-1 gene are highly associated with FSGS and HIVAN. These risk polymorphisms are found with increased frequencies in African populations.

HIVICK occurs as the result of deposition of circulating immune complexes in the glomeruli or their formation in situ. Immune complexes contain viral core and envelope antigens. In addition, HIV patients may have other immune complex mediated diseases (IgA nephropathy, membranous GN or membranoproliferative GN, very often associated with hepatitis A, B and C coinfection). Lupus-like GN may be also found by IF and EM studies in the absence of clinical and serological features of systemic lupus erythematosus.

In Black and Hispanic populations, FSGS with or without collapsing glomeruli and microcystic tubular dilatation are common while mesangial hyperplasia and immune complex-type disease predominates in whites. Other glomerular pathologies may be also detected in HIV infected children and adults such as postinfectious GN, minimal change disease, diabetic nephropathy, amyloidoses, and thrombotic microangiopathies.

Persistent proteinuria ($\geq$1+ on dipstick; urinary protein/creatinine ratio > 2.0 mg/mg) and microhematuria point to HIVAN [132, 135–137]. Additional suggestive features are finding of microcysts (shed epithelial cells) in the urinary sediment, highly echogenic kidneys, Black race, and nephrotic range proteinuria with or without edema or hypertension. These criteria are suggestive but not confirmatory; definitive diagnosis of HIVAN should be established by a kidney biopsy [132].

Children with perinatal HIV infection can shave tubulointerstitial nephritis, and may present with non-specific manifestations of acute kidney injury. It may be secondary to medication

exposure, including non-steroidal antiinflammatory drugs, trimethoprim-sulfamethoxazole, indinavir, and ritonavir. Various electrolyte and acid-base disturbances may be found in children with perinatal HIV infections because of malnutrition, gastroenteritis, pneumonia, intracranial infections, and syndrome of inappropriate antidiuretic hormone. Antiretroviral agents such as tenofovir can cause proximal tubular dysfunction, nephrogenic diabetes insipidus, and acute kidney injury.

Children with HIVAN should be treated with HAART. If already receiving HAART, it may suggest inadequate disease control, which is supported by low CD4 counts and a high viral load. Resistance testing enables selection of the optimal HAART regimen. Since many antiretroviral drugs are excreted via the kidneys, modification of dosages based on GFR is necessary. Nephrotoxic drugs should be avoided or carefully monitored.

Although there are no controlled, randomized trials, ACEIs and angiotensin receptor blockers have been used with HAART therapy in many centers in order to decrease proteinuria. Steroids and immunosuppressive agents are not recommended for treatment of children with HIVAN [132].

Both dialysis modalities are used in HIV infected children with ESKD. Those on peritoneal dialysis have increased risk of recurrent peritonitis and worsening of malnutrition, while those on hemodialysis with central venous lines have high risk of tunnel infections and thrombosis. In the pre-HAART era, there were major concerns about transplantation in otherwise immunocompromised patients. With better control of the disease with HAART, improved prophylaxis and treatment of opportunistic infections, transplantation, as in other causes of pediatric CKD, is now the optimal treatment modality for these children. However, this requires stability on HAART with undetectable viral load for 6 months and an adequate CD4 count, and an understanding of the need for adherence to a combination of life-long HAART and immunosuppression [138].

A shortage of donor kidneys has led to adult HIV-positive kidney donors being utilized in adult HIV kidney positive transplants recipients with good success [139]. In pediatrics, this has not been described in kidney transplantation, although a successful case of living donor liver transplantation from an HIV-positive mother to her HIV-negative child has been performed as a lifesaving measure [140, 141].

Conclusion

Infectious and post-infectious GN have been important causes of pediatric kidney disease. However, there has been a decrease in these etiologies of GN due to the introduction of successful vaccines (hepatitis A and B, meningococcus, varicella and COVID-19); public health initiatives (efforts to decrease transmission of malaria and scabies); and maternal screening (HIV and syphilis).

The management of post-infectious GN focuses on addressing the clinical consequences of the GN. In contrast, eradicating the underlying infection is critical for infectious GN, although management of the GN is also important.

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Rapidly Progressive Glomerulonephritis

20

Shina Menon and Arvind Bagga

Rapidly progressive glomerulonephritis (RPGN) is a rare syndrome in children, characterized by clinical features of glomerulonephritis (GN) and rapid loss of renal function. Histology shows crescentic extracapillary proliferation in Bowman space affecting the majority of glomeruli. This may be seen in any form of GN, including post-streptococcal GN, renal vasculitis, IgA nephropathy, systemic lupus erythematosus (SLE) and membranoproliferative GN. RPGN is a medical emergency, which if untreated rapidly progresses to irreversible loss of renal function. Prompt evaluation and specific therapy is necessary to ensure satisfactory outcome in most cases.

Definition

RPGN is a clinical syndrome characterized by an acute nephritic illness accompanied by rapid loss of renal function over days to weeks [1]. The histological correlate is the presence of crescents (crescentic GN), usually involving greater than 50% of glomeruli. The presence of crescents is a histologic marker of severe glomerular injury, which may occur in a number of conditions, including postinfectious GN, IgA nephropathy, SLE, renal vasculitis and membranoproliferative GN [1, 2]. The severity of clinical features correlates with the proportion of glomeruli that show crescents. While patients with circumferential crescents involving more than 80% of glomeruli present with more severe acute kidney injury (AKI), those with non-circumferential crescents in less than 50% of glomeruli have a relatively milder course.

Although the terms RPGN and crescentic GN are used interchangeably, similar presentation, with rapidly evolving AKI, might occur in conditions without crescents, including hemolytic uremic syndrome, diffuse proliferative GN and acute interstitial nephritis. Table 20.1 lists common conditions that present with RPGN in childhood.

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Table 20.1 Causes of rapidly progressive glomerulonephritis (RPGN)

Immune complex GN
Post infectious GN. Poststreptococcal nephritis, infective endocarditis, shunt nephritis, *Staphylococcus aureus* sepsis, other infections: HIV, hepatitis B and C, syphilis
Systemic disease. Systemic lupus erythematosus, IgA vasculitis, cryoglobulinemia, mixed connective tissue disorder, juvenile rheumatoid arthritis
Primary GN. IgA nephropathy, MPGN, membranous nephropathy, C1q nephropathy
Pauci-immune crescentic GN
Microscopic polyangiitis, granulomatosis with polyangiitis (Wegener granulomatosis), renal limited vasculitis, eosinophilic granulomatosis with polyangiitis (Churg-Strauss disease)
Idiopathic crescentic GN
Medications: penicillamine, hydralazine, hydrocarbons, propylthiouracil
Anti-glomerular basement membrane GN
Anti-GBM nephritis, Goodpasture syndrome, post-renal transplantation in Alport syndrome
Post-renal transplantation
Recurrence of IgA nephropathy, IgA vasculitis, MPGN, systemic lupus erythematosus
RPGN without crescents
Diffuse proliferative GN

GN glomerulonephritis; *MPGN* membranoproliferative GN; *GBM* glomerular basement membrane; *HIV* human immunodeficiency virus

Pathophysiology

Crescent formation is usually secondary to a nonspecific inflammatory response to severe injury to the glomerular capillary wall. While the underlying inciting reason may differ, the final pathway is often similar.

Underlying Triggers for Crescent Formation

In immune complex crescentic GN, the underlying trigger is deposition of immune complexes in the glomerular capillary tufts. These immune complexes may be formed in the circulation or within the glomerular capillary wall.

Pauci-immune crescentic GN is associated with anti-nuclear cytoplasmic antibody (ANCA), directed against either myeloperoxidase (MPO), proteinase 3 (PR3), or both. While the mechanisms by which ANCA arise have not been clearly elucidated, there is increasing evidence that they activate neutrophils and set off an inflammatory cascade which subsequently causes endothelial and microvascular injury, and damage to the glomerular capillary wall. Activated neutrophils result in the extrusion from the cell of neutrophil extracellular traps (NETs) containing entrapped MPO, PR3, and complement components [3, 4]. Subsequently, neutrophils undergo a form of cell death, NETosis. NETs also mediate direct injury to endothelium, transfer MPO/PR3 to vascular endothelium for antigen presentation and activate the alternate complement pathway. Involvement of the alternate complement pathway, specifically anaphylatoxin C5a and C5a receptor (CD88), has been shown in studies from an anti-MPO model in complement-deficient mice [5]. It has been proposed that there may be an amplification loop wherein activated neutrophils release properdin, activate the alternate pathway and generate C5a, resulting in additional neutrophil priming and activation. There is also emerging evidence that ANCA-negative pauci-immune GN maybe secondary to activation of the alternate and terminal pathway of complement caused by a genetic or acquired defect in the alternative pathway [6].

Finally, in anti-glomerular basement membrane (GBM) GN, there are circulating IgG antibodies directed against the non-collagenous domain of the α3 chain of type IV collagen that is present in the GBM and alveolar basement membrane.

Pathogenesis of Crescent Formation

Crescents are defined as the presence of two or more layers of cells in Bowman space. The chief components of crescents are coagulation proteins, macrophages, T cells, fibroblasts and parietal and visceral epithelial cells [1, 7]. There is evidence that podocytes also have a role in crescent formation [8]. Perturbations of humoral

immunity as well as the T helper type 1 cellular immune response contribute to the pathogenesis [1, 2]. Various pathways involving T cells, including disturbances in regulatory T cell function and stimulation of toll-like receptor 4, have been described [9, 10].

Initiation of Crescent Formation

The initial event in formation of crescents is the occurrence of a physical gap in the glomerular capillary wall and the GBM, mediated by macrophages and T lymphocytes. Breaks in the integrity of the capillary wall lead to passage of inflammatory mediators and plasma proteins into Bowman space with fibrin formation, influx of macrophages and T cells, and release of inflammatory cytokines (*e.g.,* interleukin-1 (IL-1) and tumor necrosis factor-α). Similar breaks in Bowman capsule allow cells and mediators from the interstitium to enter Bowman space and for contents of the latter to enter the interstitium, resulting in inflammation. It is proposed that podocytes, which are terminally differentiated and stationary cells, change into a migratory phenotype and contribute to crescent formation [11].

Formation

The development of a crescent results from the participation of coagulation factors and different proliferating cells, chiefly macrophages, parietal epithelial cells and interstitial fibroblasts. The presence of coagulation factors in Bowman space results in formation of a fibrin clot and recruitment of circulating macrophages. Activated neutrophils and mononuclear cells release procoagulant tissue factor, IL-1 and tumor necrosis factor-α (TNF-α), serine proteinases (elastase, PR3) and matrix metalloproteinases. The proteases cause lysis of the GBM proteins and facilitate entry of other mediators in Bowman space. Release of IL-1 and TNF-α result in expression of adhesion molecules, leading to macrophage recruitment and proliferation. Apart from macrophages, major components of the crescents are proliferating parietal and visceral epithelial cells [12].

Resolution of Crescents

The stage of inflammation is followed by development of fibrocellular and fibrous crescents. The expression of fibroblast growth factors and transforming growth factor-β is important for fibroblast proliferation and production of type I collagen, responsible for transition from cellular to fibrocellular and fibrous crescents. Transition to fibrous crescents, which occurs over days, is important since the latter is not likely to resolve following immunosuppressive therapy. The plasminogen-plasmin system is responsible for fibrinolysis and resolution of crescents.

Causes and Immunopathologic Categories

Based on pathology and immunofluorescence (IF) staining patterns, crescentic GN is classified into three categories, which reflect different mechanisms of glomerular injury [1].

1. Immune-complex GN with granular deposits of immune complexes along capillary wall and mesangium
2. Pauci-immune GN with scant or no immune deposits, and associated with systemic vasculitis
3. Anti-GBM GN with linear deposition of anti-GBM antibodies

Immune Complex Crescentic GN

These patients form a heterogeneous group in which multiple stimuli lead to proliferative GN with crescents. Immunohistology shows granular deposits of immunoglobulin and complement along capillary walls and in the mesangium. The causes include infections, systemic diseases and primary GN.

Systemic infections Poststreptococcal GN can rarely present with crescentic histology. While most patients recover completely, the presence of

nephrotic range proteinuria, sustained hypertension and crescents is associated with an unsatisfactory outcome [13, 14]. Other infectious illnesses associated with crescentic GN include infective endocarditis, infected atrioventricular shunts and visceral abscesses. Crescentic GN might be associated with infection with methicillin resistant *Staphylococcus aureus,* hepatitis B and C virus, leprosy and syphilis.

Systemic immune complex disease RPGN with glomerular crescents might be seen in patients with IgA vasculitis (formerly known as Henoch Schönlein purpura) and lupus nephritis (class IV more commonly than class III).

Primary GN Patients with IgA nephropathy, immune complex mediated membranoproliferative GN (MPGN), C3 glomerulopathy (C3 GN and dense deposit disease), and membranous nephropathy may occasionally present with rapid loss of renal function and crescentic GN [13–16].

Pauci-immune Crescentic GN

This form of GN is characterized by few or no immune deposits on IF microscopy [2, 17]. This includes renal-limited vasculitis, and the renal manifestations of microscopic polyangiitis (MPA), granulomatosis with polyangiitis (GPA; formerly Wegener granulomatosis), or eosinophilic granulomatosis with polyangiitis (formerly Churg-Strauss syndrome). Most (80%) show antineutrophil cytoplasmic autoantibodies (ANCA) in blood and are collectively classified as ANCA-associated vasculitides (AAV). Some cases of ANCA positive disease might be induced by drugs, including penicillamine, propylthiouracil, minocycline and hydralazine.

In addition, approximately 10–30% of patients with pauci-immune crescentic GN are ANCA negative [18]. These patients have fewer constitutional and extrarenal symptoms than those who are ANCA-positive. Studies show differences in outcome between these groups, suggesting a different pathophysiological basis.

Anti-GBM Crescentic GN

This condition is uncommon in childhood, accounting for less than 10% of RPGN cases in children [1, 14, 19–21]. The nephritogenic autoantibody is directed against a 28 kDa monomer located on the α3 chain of type IV collagen (Goodpasture antigen). Pulmonary involvement (Goodpasture syndrome) is uncommon. Approximately 5% of patients with Alport syndrome who receive a renal allograft develop anti-GBM autoantibodies and anti-GBM nephritis within the first year of the transplant [22]. Unlike de novo anti-GBM nephritis, pulmonary hemorrhage is not observed in post-transplant anti-GBM nephritis because the patient's lung tissue does not contain the antigen. The risk of post transplantation anti-GBM nephritis is low in subjects with normal hearing, late progression to end-stage kidney disease (ESKD), or females with X-linked Alport syndrome.

Idiopathic RPGN

This group includes patients with immune complex crescentic GN who do not fit into any identifiable category, and those with ANCA-negative pauci-immune disease. While both conditions are uncommon, the proportion varies across different regions.

Epidemiology

The incidence of RPGN in children is not known. Crescentic GN comprises about 5% of unselected renal biopsies in children. While there are no population-based studies in children, a report from Romania suggested an annual incidence of 3.3 per million adult population [23]. The 2010 NAPRTCS Annual Transplant Report shows that idiopathic crescentic GN contributes to 1.7% of all transplanted patients [24]. This figure is an underestimate since other conditions in the database, including membranoproliferative GN (2.5%), SLE (1.5%), systemic immune disorders (0.3%), GPA (0.6%), chronic GN (3.2%) and IgA

Table 20.2 Causes of crescentic glomerulonephritis in children (%)

	Mayer et al. [14] (N = 60)	Sinha et al. [20] (N = 36)	Piyaphanee et al. [11] (N = 67)	Maliakkal et al. [19] (N = 305)	Alsaad et al. [21] (N = 37)
Immune complex disease					
Unspecified	6	–	–		13.5
Systemic lupus erythematosus	17	11.1	30	21	54.1
Poststreptococcal/Post infectious GN	12	8.3	51		16.2
IgA vasculitis (Henoch-Schönlein purpura), IgA nephropathy	43	11.1	6	42	
Membranoproliferative GN		5.5			
ANCA associated Vasculitis/Pauci immune GN	17	52.7	7.5	17	8.1
Idiopathic crescentic GN		11.1	1.5	6	
Anti-glomerular basement disease	2		1.5	3	
Others	3		3		8.1

GN glomerulonephritis

nephropathy and IgA vasculitis (2.4%), might present as RPGN.

Table 20.2 outlines the underlying conditions in five series of crescentic GN reported from India [25], United States [19], Thailand [21], Germany [20] and Saudi Arabia [26]. Immune complex GN is the most common pattern of crescentic GN in children, accounting for 75–80% cases in most reports. Pauci-immune crescentic GN, while common in adults, is less frequent in children, accounting for 15–20% cases. The decline in the incidence of postinfectious GN has resulted in a change in the profile of crescentic GN.

Clinical Features

The spectrum of presenting features in RPGN is variable, and includes macroscopic hematuria (60–90% patients), oliguria (60–100%), hypertension (60–80%) and edema (60–90%) [14, 17]. The illness is often complicated by occurrence of severe hypertension with end organ involvement, pulmonary edema and cardiac failure. Occasionally, RPGN may have an insidious onset with initial symptoms of fatigue or edema. Nephrotic syndrome is rare and seen in patients with less severe renal insufficiency. Systemic complaints, involving the upper respiratory tract (cough, sinusitis), skin (vasculitic rash), musculoskeletal system (joint pain, swelling) and nervous system (seizures, altered sensorium) are common in patients with pauci-immune RPGN, with or without ANCA positivity. Relapses of systemic and renal disease occur in one-third of patients with vasculitis [2, 17]. Patients with anti-GBM antibody disease may present with hemoptysis and, less often, pulmonary hemorrhage. Similar complications are found in GPA, SLE, IgA vasculitis and severe GN with pulmonary edema. The kidney disease is most severe with anti-GBM disease, followed by pauci-immune GN and finally immune complex crescentic GN [1, 24].

Investigations

Hematuria, characterized by dysmorphic red cells and red cell casts, is seen in all patients; most also have gross hematuria. A variable degree of non-selective proteinuria (2+ to 4+) is present in more than 65% of patients. Urinalysis also shows leukocyte, granular and tubular epithelial cell casts. Severe AKI is often present at diagnosis. Anemia, if present is usually mild, though may be more severe in select cases due to pulmonary hemorrhage or autoimmune hemolytic anemia in SLE; peripheral smear shows normocytic normochromic red cells. Non-specific markers of inflammation, including CRP and ESR, are elevated.

Serology

Serological investigations assist in evaluation of the cause and monitoring disease activity (Table 20.3, Fig. 20.1). Low levels of total hemolytic complement (CH50) and complement component 3 (C3) are seen in postinfectious GN and SLE. Patients with lupus may additionally show reduced levels of C1q and C4 due to activation of the classic complement pathway. Complement-mediated MPGN is usually associated with low C3; however, other complement factors may be affected depending on the site of dysregulation in the alternate pathway. Positive antistreptolysin O titers and antideoxyribonuclease B suggests streptococcal

Table 20.3 Diagnostic evaluation of patients with rapidly progressive glomerulonephritis

Complete blood counts; peripheral smear for type of anemia; reticulocyte count; erythrocyte sedimentation rate, coagulation profile
Blood levels of urea, creatinine, electrolytes, calcium, phosphate, uric acid
Urinalysis: proteinuria; microscopy for erythrocytes and leukocytes, casts
Complement levels (C3, C4, CH50); additional evaluation may be required based on biopsy findings Antistreptolysin O, antinuclear antibody, anti-double stranded DNA antibodies; anti-extractable nuclear antibodies Antinuclear cytoplasmic antibodies (ANCA): Indirect immunofluorescence, ELISA
Renal biopsy (light microscopy, immunofluorescence, electron microscopy)
Required in specific instances
Anti-GBM IgG antibodies
Blood levels of cryoglobulin, hepatitis B and C serology
Chest: radiograph, CT (patients with Goodpasture syndrome and vasculitides)
Sinuses: radiograph, CT (patients with granulomatosis with polyangiitis)

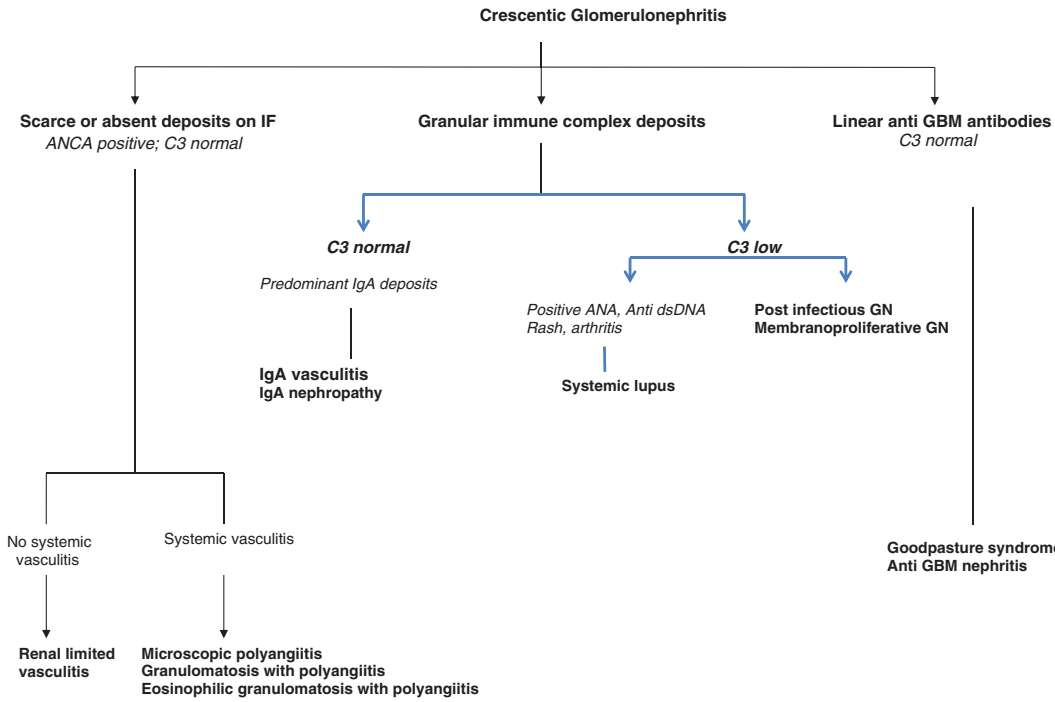

Fig. 20.1 Diagnostic evaluation of crescentic glomerulonephritis, based on renal histology and serological findings

infection in the past 3 months. Patients with SLE show antinuclear (ANA) and anti-double stranded DNA autoantibodies.

Elevated levels of ANCA suggest an underlying vasculitis, and are present in most patients with pauci-immune crescentic GN. Most ANCA have specificity for MPO or PR3. ANCA should be screened by indirect IF and positive tests confirmed by PR3-ELISA and MPO-ELISA. In patients with pauci-immune crescentic GN, negative results from IF should be tested by ELISA because 5% serum samples are positive only by the latter. GPA is associated with PR3 ANCA, which produces a cytoplasmic staining pattern on IF (c-ANCA). Renal limited vasculitis and drug induced pauci-immune crescentic GN are typically associated with MPO ANCA that shows perinuclear staining on IF (p-ANCA). Patients with MPA have equal distribution of MPO ANCA/p-ANCA and PR3 ANCA/c-ANCA. Approximately 10% of patients with GPA or MPA have negative assays for ANCA. P-ANCA autoantibodies are also found in 20–30% patients with anti-GBM GN, and occasionally in idiopathic immune complex RPGN, inflammatory bowel disease, rheumatoid arthritis and SLE [27].

Apart from diagnosis, ANCA titers have also been used for monitoring activity of systemic vasculitis. Persistent or reappearing ANCA positivity in patients in remission may be associated with disease relapse in AAV. The risk of relapse in patients who show persistently negative ANCA titers is low. An isolated rise in ANCA titers should not be used for modifying treatment in patients with systemic vasculitis [28]. The persistence or recurrence of ANCA positivity, or an increase in ANCA titers are only modestly predictive of future disease relapse and should not be used to guide treatment decisions. Such patients should be closely followed and diagnostic efforts intensified to detect and treat relapses.

Patients with AAV occasionally show autoantibodies to human lysosome-associated membrane protein-2 (hLAMP-2) [29]. These antibodies were also seen in a few patients with pauci-immune focal necrotizing crescentic GN who were negative for ANCA [30]. The hLAMP-2 autoantibodies became undetectable following initiation of immunosuppressive treatment and were detected during clinical relapse.

High titers of anti-GBM IgG antibodies, demonstrated by IF or ELISA, are seen in anti-GBM nephritis or Goodpasture syndrome and correlate with disease activity. About 5% of ANCA positive samples are also anti-GBM positive and approximately 20–30% of anti-GBM positive samples are ANCA positive. Serology for ANCA is therefore recommended in all patients with either anti-GBM antibodies in blood or linear IgG deposition along the GBM. The initial clinical outcome for these patients is similar to that of anti-GBM disease, though relapses may occur as in systemic vasculitis [1].

Renal Histology

Light Microscopy

Renal histological findings in various forms of crescentic GN are similar. A glomerular crescent is an accumulation of two or more layers of cells that partially or completely fill Bowman space. The crescent size varies from circumferential to segmental depending on the plane of the tissue section and the underlying disease. Crescents in anti-GBM nephritis or AAV are usually circumferential, while they are often segmental in immune complex GN. Interstitial changes range from acute inflammatory infiltrate to chronic interstitial scarring and tubular atrophy. Once the glomerular capillary loop is compressed by the crescent, tubules that derive their blood flow from that efferent arteriole show ischemic changes.

Crescents may be completely cellular or show variable scarring and fibrosis. Cellular crescents are characterized by proliferation of macrophages, epithelial cells and neutrophils (Fig. 20.2). Fibrocellular crescents show an admixture of collagen fibers and membrane proteins amongst the cells (Figs. 20.3 and 20.4). In fibrous crescents, the cells are replaced by collagen. Renal biopsies from patients with vasculitis show crescents in various stages of progression, indicating episodic inflammation. Early lesions have segmental fibrinoid necrosis with or without an

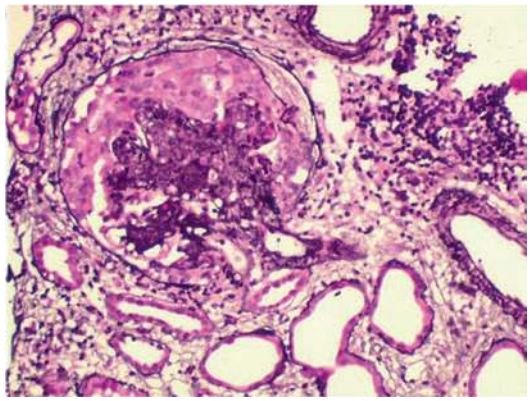

Fig. 20.2 Cellular crescent compressing the glomerular tuft. Silver methenamine stained, original magnification ×800

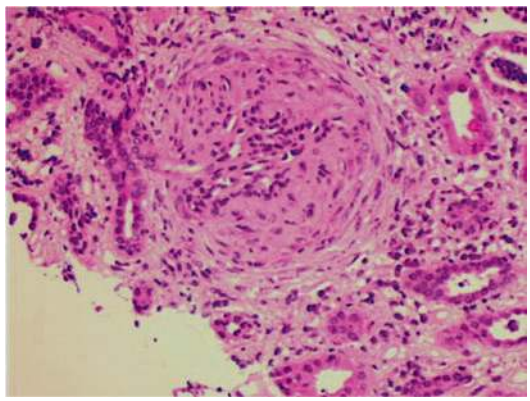

Fig. 20.3 Fibrocellular crescent with compression of glomerular tuft and partial sclerosis. There is chronic interstitial inflammation, tubular atrophy and interstitial fibrosis in surrounding area. Hematoxylin and eosin–stained, original magnification ×800

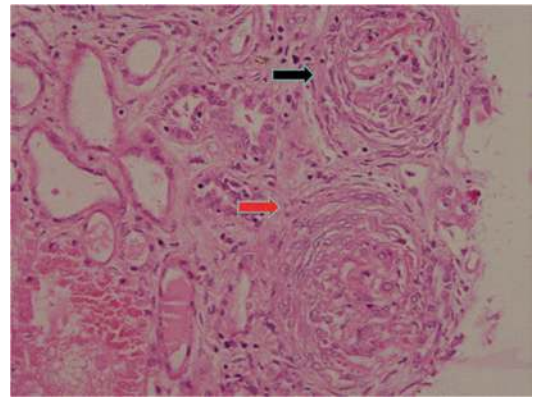

Fig. 20.4 Glomeruli showing cellular (red arrow) and fibrocellular crescents (black arrow) causing compression of underlying glomerular tuft. Note the disruption of Bowman capsule. Hematoxylin and eosin–stained, original magnification ×200

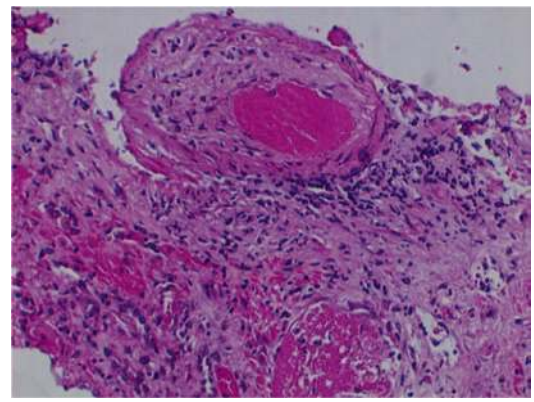

Fig. 20.5 A patient with pauci-immune crescentic glomerulonephritis. A small artery shows features of active vasculitis; its wall shows neutrophil infiltration, fibrin deposition and lumen occluded by a thrombus. The perivascular area shows interstitial hemorrhage and inflammation. Hematoxylin and eosin–stained, original magnification ×600

adjacent small crescent. Severe acute lesions show focal or diffuse necrosis in association with circumferential crescents. Features of small vessel vasculitis, affecting interlobular arteries (Fig. 20.5) and rarely angiitis involving the vasa recta might be seen.

ANCA associated GN is classified based on light microscopy findings [31]. Biopsies are categorized as focal, crescentic and sclerotic based on the predominance of normal glomeruli, cellular crescents, and globally sclerotic glomeruli, respectively. A fourth category represents a mixed or heterogeneous phenotype. Although the classification system is believed to have prognostic value for 1- and 5-year renal outcomes, and may guide therapy, it needs to be validated in children.

Immunohistology and Electron Microscopy

The presence, location and nature of immune deposits on IF and electron microscopy can help

determine the underlying cause of crescentic GN. The crescents stain strongly for fibrin on IF [32]. Mesangial deposits of IgA are found in IgA nephropathy and IgA vasculitis. While IgA is dominant or co-dominant in IgA nephropathy, deposits of IgG, IgM, fibrinogen, and C3 may also be seen in the glomeruli. Postinfectious GN has granular, subepithelial deposits of IgG and C3. Full house capillary wall and mesangial deposits of granular IgG, IgA, IgM, C3, C4 and C1q are seen in SLE. Immune complex-mediated MPGN is characterized by the immunoglobulin and complement deposits, while C3 glomerulopathy is characterized by glomerular complement deposits in the absence of significant immunoglobulins. Electron microscopy usually shows subendothelial and mesangial deposits in C3 glomerulopathy and MPGN.

Anti-GBM disease is characterized by linear staining of the GBM with IgG (rarely IgM and IgA) and C3 (Fig. 20.6).

The third group of crescentic GN has few or no immune deposits by IF or electron microscopy. The majority of these patients are ANCA positive, though some may have ANCA-negative, pauci-immune crescentic GN.

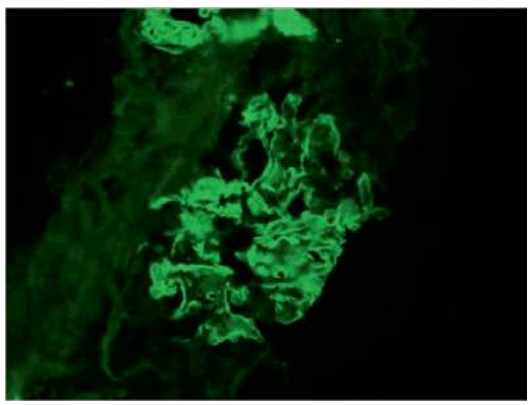

Fig. 20.6 Immunofluorescence microscopy (original magnification ×1200) in a patient with anti-glomerular basement membrane antibody-mediated crescentic glomerulonephritis showing linear deposition of IgG on the capillary wall

Evaluation and Diagnosis

It is necessary to make an accurate and rapid diagnosis in RPGN as treatment strategies vary and delay in instituting treatment increases the risk of irreversible damage. All patients should undergo a kidney biopsy promptly. While the majority have crescentic GN, the detection of thrombotic microangiopathy (affecting interlobular arteries and arterioles) or diffuse proliferative GN is not unusual. The diagnosis of the etiology of crescentic GN depends on integration of clinical data and findings on serology and renal histology (Table 20.3, Fig. 20.1).

Treatment

The heterogeneity and unsatisfactory outcome of RPGN has led to institution of multiple treatments. Evidence-based data is limited and treatment guidelines for children are based on data from case series and prospective studies in adults.

Empiric therapy with daily intravenous (IV) pulses of methylprednisolone (15–20 mg/kg, maximum 1 g) for 3 days should be initiated in patients with severe disease, particularly if kidney biopsy is likely to be delayed. This empiric initial therapy will not alter the histologic abnormalities on a kidney biopsy that is performed within a few days. Plasmapheresis can be considered in the empiric initial phase, especially if the patient presents with hemoptysis.

More specific therapy is started once the diagnosis is established. Treatment of RPGN typically comprises two phases: *induction* of remission and *maintenance* (Table 20.4). The first phase aims at control of inflammation and the associated immune response. Once remission is induced, the maintenance phase attempts to prevent further renal damage and relapses. All patients with RPGN, irrespective of the underlying diagnosis, will benefit from supportive man-

Table 20.4 Treatment of crescentic glomerulonephritis

Induction
Methylprednisolone 10–30 mg/kg (maximum 1 g) IV daily for three doses
Prednisolone 1.5–2 mg/kg/day oral for 4 weeks; taper to 0.5 mg/kg/day for 3 months; then, 0.5 mg/kg on alternate days for 3 months
[a]Cyclophosphamide 500–750 mg/m^2 IV every 3–4 weeks for six doses
[b]Plasma exchange (1–1.5 volume) on alternate days for 2 weeks
[c]Rituximab: 750 mg/m^2 (maximum 1000 mg), every 2 weeks for two doses
Maintenance
Azathioprine 1.5–2 mg/kg/day for 12–18 months
Alternate day low-dose prednisolone
Consider mycophenolate mofetil (1000–1200 mg/m^2/day) if disease activity is not controlled with azathioprine or patient does not tolerate azathioprine
Rituximab can be used for maintenance, given every 6 months or when B lymphocytes reconstitute
Agents for refractory disease
Intravenous immunoglobulin, TNF-α antibody (infliximab), rituximab

[a]The dose of cyclophosphamide is increased to 750 mg/m^2 if there is no leukopenia before the next dose. Dose reduction is necessary in patients showing impaired renal function. Alternatively, cyclophosphamide is given orally at a dose of 2 mg/kg/day for 12 weeks

[b]Plasma exchange can be initiated early, especially if patient is dialysis-dependent at presentation or if the biopsy shows severe histological changes (>50% crescents). It is useful in anti-GBM nephritis and may be useful in ANCA-associated vasculitis. It can be considered in patients with immune complex crescentic GN if there is unsatisfactory renal recovery after steroid pulses

[c]Rituximab may be used as first line in children and adolescents with AAV, particularly PR3-AAV. It may be used as an alternative initial treatment in patients with less severe disease or in whom cyclophosphamide is contraindicated

agement, including maintenance of fluid and electrolyte balance, provision of adequate nutrition, and control of infections and hypertension. Some patients require dialysis at diagnosis or soon thereafter.

Medications

Steroids After the initial IV pulses, steroids are typically given as high-dose oral prednisone (1.5–2 mg/kg daily) for 4 weeks, with tapering to 0.5 mg/kg daily by 3 months and alternate day prednisone for 6–12 months. Based on recent studies, a reduced-dose, tapering regimen of oral glucocorticoids-could be considered for a patient with new onset or relapsing AAV [33].

Cyclophosphamide Historically, cyclophosphamide has been an important part of induction regimens. Most centers prefer the use of IV compared to oral therapy. The European Vasculitis Study Group (EUVAS) compared IV pulse cyclophosphamide (15 mg/kg every 2 weeks for 3 pulses, followed by pulses at 3-week intervals until remission, and then for another 3 months) with daily oral cyclophosphamide (2 mg/kg/day) for induction of remission [34]. They showed that the time to remission and proportion of patients in remission at 9 months was similar in both groups. The cumulative dose of cyclophosphamide in the daily oral group was twice that in the IV group (15.9 g *vs.* 8.2 g; $P < 0.001$), and the latter had a lower rate of leukopenia. A meta-analysis of nonrandomized studies showed that pulse cyclophosphamide was significantly more likely to induce remission and had a lower risk of infection and leukopenia. Pulse cyclophosphamide dosing may, however, be associated with a greater risk of relapses, exposing patients to further immunosuppression [35]. The dose of oral and IV cyclophosphamide is 2 mg/kg/day and 500–750 mg/m^2, respectively. The dose should be adjusted to maintain a nadir leukocyte count of 3000–4000/μL 2 weeks post-treatment. While most of the data on cyclophosphamide is from AAV or lupus nephritis, a similar regimen is recommended for crescentic IgA nephropathy or IgA vasculitis.

Rituximab B cell depletion with rituximab has similar efficacy as cyclophosphamide in many studies. It has been used successfully in patients with refractory lupus nephritis and AAV [36]. The Rituximab in ANCA-Associated Vasculitis (RAVE) trial compare rituximab with standard therapy for inducing remission in patients with AAV [37]. Patients received either rituximab (375 mg/m^2/week for 4 weeks) or cyclophosphamide (2 mg/kg/day). Both groups received 1–3

pulses of methylprednisolone (1000 mg each), followed by tapering dose of prednisone. At 6 months, 64% in the rituximab group achieved remission vs. 53% in the control group. Of the subgroup that had relapsing disease, rituximab was superior to cyclophosphamide in inducing remission (67% versus 42%) at 6 months. Thus, therapy with rituximab was not inferior to treatment with cyclophosphamide for induction of remission. Patients from the RAVE study who achieved complete remission were followed through month 18 [38]. During follow-up, those treated with rituximab received no further therapy, while those treated with cyclophosphamide were converted to azathioprine. At 18 months, the proportion of patients remaining in complete remission was similar (39% rituximab vs. 33% cyclophosphamide). Interestingly, a post hoc analysis of the RAVE trial found a higher remission rate for the PR3-AAV subgroup at 6 months treated with rituximab [39]. A similar association was not seen with MPO-AAV.

The RITUXVAS study randomized patients with AAV to receive a standard steroid regimen plus either rituximab (375 mg/m^2/week for 4 weeks) with two IV cyclophosphamide pulses, or IV cyclophosphamide for 3–6 months followed by azathioprine [40]. There was no significant difference in the rate of remission, severe adverse events, and death in the two groups.

Mycophenolate mofetil (MMF) MMF has been used in observational studies as part of induction therapy for vasculitis, though it may have a greater role in the maintenance phase. Results from the EUVAS MYCYC trial showed that MMF was non-inferior to cyclophosphamide for remission induction in AAV, but resulted in a higher relapse rate [41]. MMF has also been used as an alternative to azathioprine for maintenance therapy in patients with AAV. However, the IMPROVE trial (International Mycophenolate Mofetil Protocol to Reduce Outbreaks of Vasculitides) showed that relapses were significantly more common in patients receiving MMF compared to azathioprine, with no difference in severe adverse events [42]. The Kidney Disease Improving Global Outcomes (KDIGO) Clinical Practice Guideline for Glomerulonephritis recommends using azathioprine as the first choice for maintenance therapy in ANCA vasculitis and considering MMF as an alternative in patients who are allergic to or intolerant of azathioprine [43].

Intravenous Immunoglobulin (IVIG) A number of studies have examined the efficacy of IVIG in subjects with AAV and RPGN, and reported benefit lasting for up to 3 months [44]. The exact mechanism of action is unclear with evidence for both non-specific anti-inflammatory and anti-cytokine effects and specific correction of immunoregulatory defects. In a study of AAV patients with persistent disease activity, 14/17 patients in the IVIG group had reduction in disease activity, compared to 6/17 in the placebo group [45]. The indications for initial or adjunctive treatment with IVIG is not defined.

Methotrexate The NORAM study compared the effectiveness of orally administered methotrexate and cyclophosphamide in adult patients with early systemic vasculitis and mild renal involvement [46]. Induction of remission was similar at 6 months, but relapses were more frequent after treatment withdrawal in methotrexate treated patients. Methotrexate is not recommended for patients with moderate to severe renal dysfunction.

Azathioprine Most patients with AAV need long-term maintenance immunosuppression due to the risk of relapses. Extended treatment with cyclophosphamide has been used in adults but has significant risks and is not the preferred approach for children. While azathioprine is not effective at inducing remission, it is useful for long-term prevention of relapses. The timing of the switch from cyclophosphamide to azathioprine was clarified by the CYCAZAREM trial, which compared switching from cyclophosphamide to maintenance azathioprine at 3 versus 12 months [47]. At 18 months, the two groups had similar remission rates, renal function and patient survival. There is uncertainty regarding the appropriate duration of maintenance treatment, with most patients with pauci-immune crescentic GN treated for 2 or more years.

Newer Agents

T lymphocyte depletion with Campath 1-H (alemtuzumab), an anti-CD52 monoclonal antibody, has been tried with variable success in patients with GPA and other vasculitides [48]. In a retrospective cohort study, 85% of patients with refractory AAV achieved remission after alemtuzumab, but the majority relapsed after a median of 9 months [45].

A multicenter, double-blind, placebo-controlled study evaluated belimumab for preventing relapses in patients with AAV [49]. Belimumab is a monoclonal antibody that prevents the survival of B lymphocytes by blocking the binding of soluble human B lymphocyte stimulator protein to receptors on B lymphocytes. Patients were randomized to receive azathioprine, low-dose oral steroids and either IV belimumab or placebo for maintenance of remission. The addition of belimumab did not reduce the risk of relapse.

Avacopan, which targets the complement system by blocking the C5a receptor, has been investigated as an approach to reduce corticosteroid exposure in AAV. A phase II trial showed that avacopan was well-tolerated and safe in the short-term and may be able to replace high-dose corticosteroids [50]. This was followed by a phase III randomized, controlled trial comparing avacopan with a tapering schedule of prednisone in patients with AAV concurrently treated with cyclophosphamide (followed by azathioprine) or rituximab [51]. Avacopan was noninferior to prednisone taper for the end-point of remission at week 26 and was superior to prednisone taper for sustained remission at week 52. The number of steroid-related adverse events was higher in the prednisone group than in the avacopan group.

Plasmapheresis

Plasmapheresis or plasma exchange has been used for the treatment of crescentic GN with variable success. The mechanism of action is not clear, but is believed to involve removal of pathogenic autoantibodies, complement proteins, coagulation factors and cytokines. Plasma exchange has been shown in controlled trials in adults to have therapeutic benefit in anti-GBM disease, with clearance of auto-antibodies, lower serum creatinine and improved patient and renal survival [52]. However, adults who were anuric with severe azotemia, dialysis dependent or those who had more than 85% crescents on renal biopsy had minimal benefit. The role of intensive plasma exchange versus IV methylprednisolone, in addition to oral steroids and cyclophosphamide, was examined by the MEPEX trial in patients with AAV and serum creatinine >500 μmol/L (5.65 mg/dL) at presentation [53]. Patients receiving plasma exchange were more likely to be off dialysis at 3-months (69% *vs.* 49%) and had a lower risk of ESKD at 12 months, but there were limited benefits on long-term renal function or survival. Another study showed that plasma exchange improved medium-term renal survival, even when initiated in patients with serum creatinine levels >250 μmol/L (2.85 mg/dL) [54].

More recently the PEXIVAS trial looked at initial treatment with plasma exchange vs. no plasma exchange (with either cyclophosphamide or rituximab administered to all patients) and two different regimens of oral steroids in patients with severe AAV [33]. The study failed to demonstrate that plasma exchange delayed the time to ESKD or death.

Retrospective data in children with RPGN show benefits of plasma exchange if commenced within 1 month of disease onset [55]. Anecdotal reports confirm the efficacy of plasmapheresis in patients with RPGN due to lupus, IgA vasculitis and severe proliferative GN, and in life-threatening pulmonary hemorrhage. Prospective studies in patients with pauci-immune crescentic GN suggested that kidney outcomes were better with plasma exchange and immunosuppression vs. immunosuppression alone [56]. However, a meta-analysis of renal vasculitis or idiopathic RPGN concluded that adjunctive plasma exchange did not improve renal and patient survival [57].

According to the KDIGO Guidelines, the routine use of plasma exchange is not recommended for patients presenting with a GFR <50 mL/min/1.73 m^2, but it can be considered in those with more severe AKI or in those with alveolar hemorrhage and hypoxemia. The dose recommended for adults is 60 mL/kg volume replacement [43]. For AAV, seven treatments over 14 days are prescribed and for patients with anti-GBM antibodies, daily exchanges are performed for 14 days or until anti-GBM antibodies are undetectable.

In our practice, we consider plasma exchange for patients with severe AKI or alveolar hemorrhage with hypoxia, and use 1–1.5 volume exchange per the schedule discussed above.

Immune Complex Crescentic GN

Therapy for immune complex crescentic GN depends on the underlying disease. The treatment of IgA nephropathy and lupus nephritis presenting with RPGN is discussed in their respective chapters. Patients with idiopathic immune complex crescentic GN should be treated similarly to those with pauci-immune crescentic GN.

Postinfectious RPGN

Poststreptococcal GN presenting with extensive crescents is rare and the benefits of intensive immunosuppressive therapy are unclear, since most patients recover spontaneously. Nevertheless, immunosuppressive therapy with corticosteroids and alkylating agents has been used in patients with renal failure and extensive glomerular crescents [58]. Despite the lack of evidence-based data, we usually treat patients with postinfectious RPGN and crescents involving 50% or more glomeruli with 3–6 IV pulses of methylprednisolone, followed by tapering doses of oral steroids for 6 months. Eradication of the infection and/or removal of infected prostheses are necessary for resolution of immune complex GN associated with active infections.

Pauci-immune Crescentic GN

Standard induction therapy is IV pulses of methylprednisolone daily for 3 days followed by oral prednisone and cyclophosphamide or rituximab. For cyclophosphamide, the choice between oral (2 mg/kg/day for 3 months) and IV (15 mg/kg every 2 weeks for three doses and then every 3 weeks for 3–6 months or 500–750 mg/m^2 every 2 weeks for 3–6 months) depends on institutional practice. While both oral and IV regimens are effective, IV is preferred due to the lower cumulative dose and lower risk of toxicity. After approximately 3–6 months, cyclophosphamide is replaced by a medication with a lower risk of toxicity. Rituximab, for induction is usually dosed at 750 mg/m^2 (maximum dose 1000 mg), with two 2 doses 14 days apart. Therapeutic plasma exchange can be added to the induction regimen for children who are dialysis dependent, those with pulmonary hemorrhage and hypoxia or not responding satisfactorily to induction treatment [33, 53].

Therapy during the maintenance phase is tapering doses of oral prednisolone and azathioprine, usually for 18–24 months. For most patients, a reduced-dose glucocorticoid tapering regimen can be used, particularly after studies like PEXIVAS showed that patients on the reduced-dose tapering regimen had similar rates of remission and fewer adverse effects compared with those on standard-dosing regimens [33].

A longer duration of therapy (3 years or more) may be needed in patients showing relapses, elevated ANCA titers and those with PR3-ANCA [2]. Approximately one-third of patients have one or more relapses, requiring reinstitution of induction therapy. Since intensive immunosuppression is associated with increased risk of infection, the use of prophylactic antimicrobials against *Pneumocystis carinii* and *Candida* may be required during induction.

For AAV, the KDIGO guidelines recommend using rituximab for children and adolescents, and for those with relapsing disease or PR3-AAV. They also suggest that for those with severe GN and creatinine >4 mg/dL, a combination of two pulses of cyclophosphamide with rituximab can be used for induction.

Anti-GBM Crescentic GN

Prompt institution of plasma exchange is necessary in these patients. Double volume exchange is done daily, and subsequently on alternate days until anti-GBM antibodies are no longer detectable (usually 2–3 weeks) [1, 59]. The patients are also treated with IV methylprednisolone (three doses of 20 mg/kg on alternate days) followed by high-dose oral prednisolone. Co-administration of cyclophosphamide (typically PO, 2 mg/kg daily for 3 months; IV may also be used) is effective in suppressing further antibody production. Pulmonary hemorrhage responds to plasma exchange and IV steroids. As anti-GBM disease does not usually have a relapsing course, long-term maintenance therapy is not required and steroids can be tapered over the next 6–9 months. Patients treated early in the course of their illness do satisfactorily. In patients who develop ESKD, transplantation should be deferred until anti-GBM antibodies are undetectable for 12 months, at which point disease recurrence is unlikely.

A proportion of patients with anti-GBM nephritis also show positive ANCA, most often p-ANCA. While the precise significance of the dual positivity is unclear, their outcome is similar to isolated anti-GBM disease. In view of a higher risk of relapses, these patients require a longer course of maintenance immunosuppressive therapy (as for ANCA-associated GN).

Outcome

The outcome for patients has improved in the last decades, such that almost 60–70% patients have normal long-term renal function. Patients with poststreptococcal crescentic GN have a better prognosis, with most showing spontaneous improvement. The prognosis is better in patients with poststreptococcal crescentic GN with subepithelial, rather than subendothelial or intramembranous deposits. Outcomes in patients with pauci-immune crescentic GN, MPGN and idiopathic RPGN are less favorable than IgA vasculitis or SLE. ESKD may occur on the long-term in up to 25% patients with AAV.

The outcome is determined by the severity of renal failure at presentation and promptness of intervention, renal histology and underlying diagnosis. Studies have shown that the use of plasma exchange, high percentage of normal glomeruli, and absence of glomerulosclerosis, tubular atrophy and arteriosclerosis, were associated with better renal recovery [60]. The potential for recovery correlates with the relative proportion of cellular or fibrous components in the crescents, and the extent of tubulointerstitial scarring and fibrosis.

Post-transplant Recurrence

Based on experience in adult patients, we suggest that patients with ANCA positive vasculitis should have sustained remission for 1 year before considering a transplant [61]. A positive ANCA titer at the time of transplantation does not increase the risk of allograft recurrence. Conditions associated with higher risk of histological recurrence include MPGN, IgA nephropathy, IgA vasculitis and lupus. Graft loss due recurrence is uncommon and occur in <5% of cases.

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Part V

Complement Disorders

The Role of Complement in Kidney Disease

21

Michael Kirschfink and Christoph Licht

The Emerging Concept of Complement-Mediated Diseases

As a key mediator of inflammation complement also significantly contributes to tissue damage in various clinical disorders [1]. Clinical and experimental evidence underlines the prominent role of complement in the pathogenesis of numerous inflammatory diseases including immune complex and autoimmune disorders, such as systemic lupus erythematosus and autoimmune arthritis [2–4]. Complement deficiencies represent approximately 4–5% of all primary immunodeficiencies, in part closely connected with renal disorders [5].

In clinical practice, overactivation of the complement system is the cause of several inflammatory diseases and life-threatening conditions, such as adult respiratory distress syndrome (ARDS) [6], the systemic inflammatory response syndrome (SIRS), sepsis [7], and multi-organ failure after severe trauma, burns or infections [8]. Complement has also been implicated in neurodegenerative disorders, such as Alzheimer's disease, multiple sclerosis, and Guillain-Barré syndrome. In recent years complement activation has also been recognized as a major effector mechanism of ischemia/reperfusion injury [9, 10].

Over the past two decades our understanding of the role of complement in renal disorders has considerably advanced and genomic studies have revealed multiple strong associations of genetic variants of complement proteins with kidney disease [11, 12].

The inflammatory response induced by artificial surfaces in hemodialysis and other extracorporeal circuits may lead to organ dysfunction. Here, complement activation has been associated with transient neutropenia, pulmonary vascular leukostasis and occasionally even with anaphylactic shock of variable severity [13, 14].

The Complement System

With more than 50 proteins acting as components within the activation cascade and as regulators or receptors on multiple cells, complement is a vital part of the body's innate immune system which provides a highly effective means for the destruction of invading microorganisms and elimination of immune complexes [15, 16]. In addition, complement also modulates the adaptive immune response through modification of

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T- and B-cell function employing specific receptors on various immune cells [17]. Moreover, a normally functioning complement system also participates in hematopoiesis, reproduction, lipid metabolism and tissue regeneration [18]. Essential intracellular immune modulatory functions of the complement system have recently been discovered promoting the survival and activation of T-lymphocytes [19].

Most complement proteins are secreted by the liver and contribute to the acute phase response. However, other tissues like the kidney also produce complement proteins to a significant amount [20]. Complement genes are distributed across different chromosomes, with 19 genes comprising three significant complement gene clusters in the human genome [21].

Complement can be activated via three pathways (Fig. 21.1), the classical, the alternative, and the lectin pathway, all of which merge in the activation of complement C3 and subsequently lead to the formation of the cytolytic membrane attack complex (MAC), C5b-9 [22, 23]. Following complement activation, the biologically active peptides (anaphylatoxins) C3a and C5a are released and elicit a number of proinflammatory effects, such as chemotaxis of leukocytes, degranulation of phagocytic cells, mast cells and basophils, smooth muscle contraction, and increase of vascular permeabiltity [24, 25]. C3a activates mesangial cells leading to proliferation and secretion of extracellular matrix [26]. It also induces in tubular epithelial cells the production of proinflammatory cytokines [27]. Upon cell activation by these complement split products the inflammatory response is further amplified by subsequent generation of toxic oxygen radicals and the induction of synthesis and release of arachidonic acid metabolites and cytokines. Consequently, an (over-)activated complement system represents a considerable risk of harming the host by directly and indirectly mediating

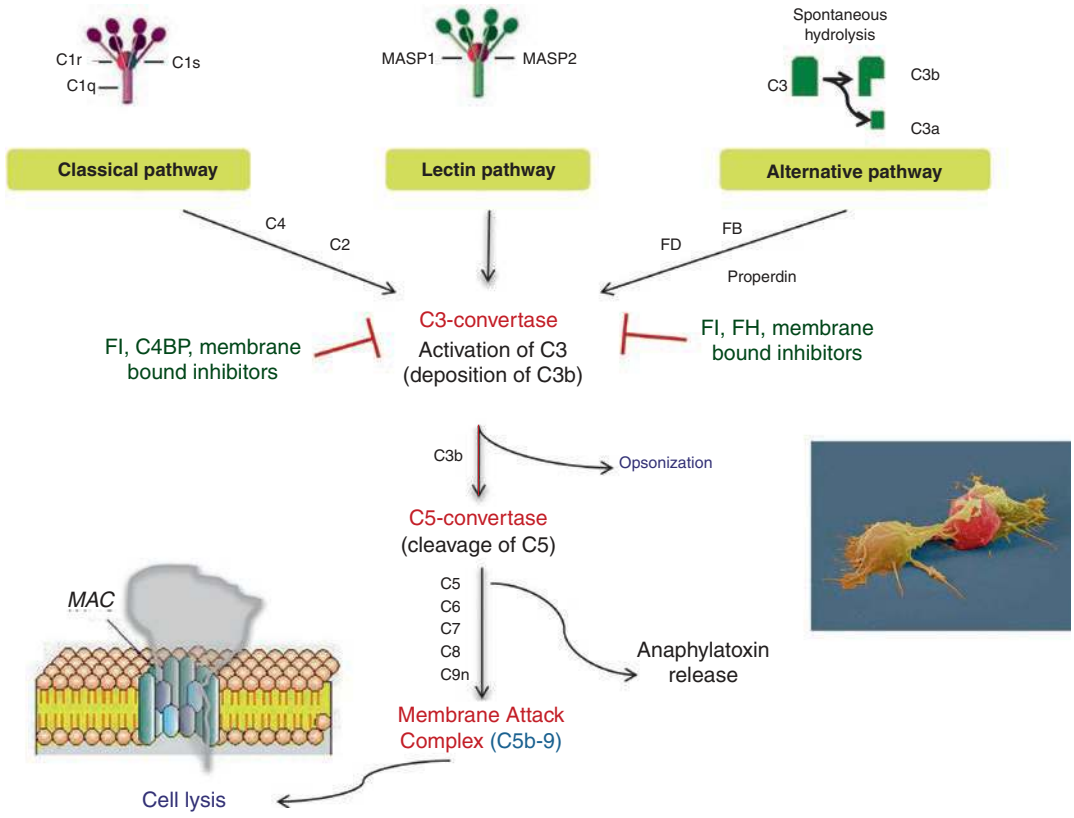

Fig. 21.1 A schematic overview of complement activation via the classical, lectin and alternative pathways

inflammatory tissue destruction [1]. Multiple interactions exist between the complement, coagulation and fibrinolytic systems altogether being of utmost importance in the pathogenesis of thrombotic microangiopathy (TMA) [28].

Under physiological conditions, activation of complement is effectively controlled by the coordinated action of soluble as well as membrane-associated regulatory proteins [29]. Soluble complement regulators, such as C1 inhibitor, C4b-binding protein (C4bp), Factors H (FH) and I (FI), clusterin and S-protein (vitronectin) restrict the action of complement in body fluids at multiple sites of the cascade (Fig. 21.1). In addition, each individual host cell is protected against the attack of homologous complement by surface proteins, such as the complement receptor 1 (CR1/CD35), the membrane cofactor protein (MCP/CD46), the glycosylphosphatidylinositol (GPI)-anchored proteins, decay-accelerating factor (DAF/CD55), and protectin (CD59) [29, 30].

Diagnosing Complement-Mediated Nephropathies

Detecting Complement Activation

In recent years, great progress has been made in complement analysis to better define disease severity, evolution and response to therapy (Tables 21.1 and 21.2) [31]. However, a comprehensive analysis going beyond C3 and C4 is still performed only in specialized laboratories (http://www.ecomplement.org/list-of-diagnostic-labs.html). The diagnostic work-up of a patient with a suspected complement-associated disease should start with the assessment of the total activity of the classical (CH50) and alternative (AH50) pathway. For rapid deficiency analysis, an ELISA has been developed that examines all three activation pathways in parallel [32]. These global tests provide information about the integrity of the entire complement cascade. A missing or

Table 21.1 Biochemical complement analysis

Functional assays	*Total complement activity (screening for complement deficiency)* • CH50 and AH50 hemolytic assays for CP and AP activity • Enzyme immunoassays (ELISA) for specific evaluation of CP, LP and AP activity using C5b-9 as readout
	Functional activity of single components • Hemolytic assays for single components (e.g. C3) using corresponding deficient sera as test system • ELISA for MBL/MASP functional activity using deposition of C4 as readout • C1 inhibitor assay (chromogenic assay or EIA) for diagnosis of HAE and acquired angioedema
Proteins	*Concentration of single components by immunoprecipitation (RID), nephelometry, ELISA* • C3 and C4 to detect "hypocomplementemia" • Follow-up of a low activity detected in total complement activity screening (any component) • C5-C9, Properdin, MBL at recurrent neisserial infections • C1 inhibitor for diagnosis of HAE and acquired angioedema
Activation products	*Concentrations of split products or protein-protein complexes by ELISA, preferentially based on antibodies to neoepitopes expressed selectively on the activation products* • Split products from components after proteolytic cleavage (e.g. C3a, C3d, C4a, C4d, C5a, Ba, Bb) • Complexes between the activated component and its inhibitor (e.g. C1rs-C1 inhibitor) • Macromolecular complexes (e.g. the AP convertase C3bBbP and the terminal sC5b-9 complex)
Autoantibodies	*Assessment of autoantibody concentrations by ELISA or functional assay* • Anti-C1q—SLE; anti-C1 inhibitor—angioedema; anti-FH—aHUS; C3 NeF/C5 NeF—MPGN, DDD/C3G
Surface proteins	*Flowcytometric quantification* • DAF/CD55 and CD59 for diagnosis of PNH

Table 21.2 Recommended complement analysis in nephropathies

Disease	Analysis
Systemic Lupus Erythematosus	CH50, C1q, C4 (C4A/B), C3, C3d or SC5b-9, anti-C1q autoantibodies
Atypical Hemolytic Uremic Syndrome	CH50, APH50, C3, C3a/C3d, SC5b-9, CFH, CFI, CFB Anti-CFH autoantibodies *C3, CFB, CFH, CFHRs, CFI, MCP/CD46, THBD/CD141 (molecular analysis)*
C3 Glomerulopathy (DDD, C3GN)	CH50, APH50, C3, C3a/C3d, SC5b-9, C3 NeF, CFH Anti-CFH autoantibodies *C3, CFB, CFH, CFHRs, CFI, MCP/CD46, THBD/CD141 (molecular analysis)*

greatly reduced activity in either test indicates primary complement deficiency affecting the classical or the alternative or both pathways but may also be due to secondary deficiency caused by increased consumption. Age- and sex-related diffrerences in the complement system should be taken into account in data interpretation [33].

Analysis of individual components and regulators provide insight in which portion of the complement cascade either a lack of function or an (over-)activation occurs. Recently, an algorithm to differentiate primary from secondary aHUS has been proposed based on the assumption that primary aHUS is caused by permanent (genetic or autoimmune) complement dysregulation as opposed to transient complement dysregulation in secondary aHUS. In brief, eculizumab treatment is proposed in TMA patients with evidence for complement activation aimimg to normalize terminal complement activity (MAC/C5b-9) in a first step. In a second step, proximal complement activity (C3 consumption; FH and FI levels) should be evaluated and eculizumab treatment should be continued in patients with proximal complement dysregulation only (primary aHUS), but not in patients with normal proximal complement activation (secondary aHUS) [34].

Analysis of the plasma concentrations of individual complement components such as C3 and C4 is still performed in many clinical laboratories. These tests, however, detect both native and activated, i.e., already "consumed" complement proteins and are strongly influenced by fluctuations in protein synthesis, in particular during the early acute-phase reaction. Modern complement analysis focuses on the quantification of complement-derived split products (e.g., C3a, C3d, C5a or Bb) and/or protein-protein complexes (e.g., sC5b-9), thereby providing comprehensive insight into the actual activation state of the complement system. The choice of the appropriate parameters allows to determine exactly which pathway is activated [35, 36]. The soluble activation product of the terminal complement cascade, sC5b-9, has received considerable attention, as unrestricted progression to its final steps has been linked to specific pathology, i.e. aHUS [37], and has recently been suggested as severity criteria in transplantation-associated thrombotic microangiopathy [38]. The therapeutic efficacy of C5 antibodies in treating aHUS patients is reflected by sC5b-9 suppression [39]. More recently, endothelial cell based ex vivo assays detecting C3 and/or C5b-9 deposition on cultured human microcvascular endothelial cells (HMEC) are offered to detect complement dysregulation in individual patients on target tissue rather than in the circulating blood [40, 41]. While not routinely available, yet, such tests add valuable insights to the complement diagnostic repertoire and have also proven to being successful in determining treatment duration [42].

Of note, complement dysregulation can be primary due to genetic (mutations in complement components and/or regulators) or autoimmune (autoantibodies) alterations, or secondary in context of underlying conditions such as infections, drugs, or mechanical stress (i.e., hypertension) of endothelail cells [43].

A recommended algorithm of complement analysis for both primary and secondary nephropathies is shown in Fig. 21.2.

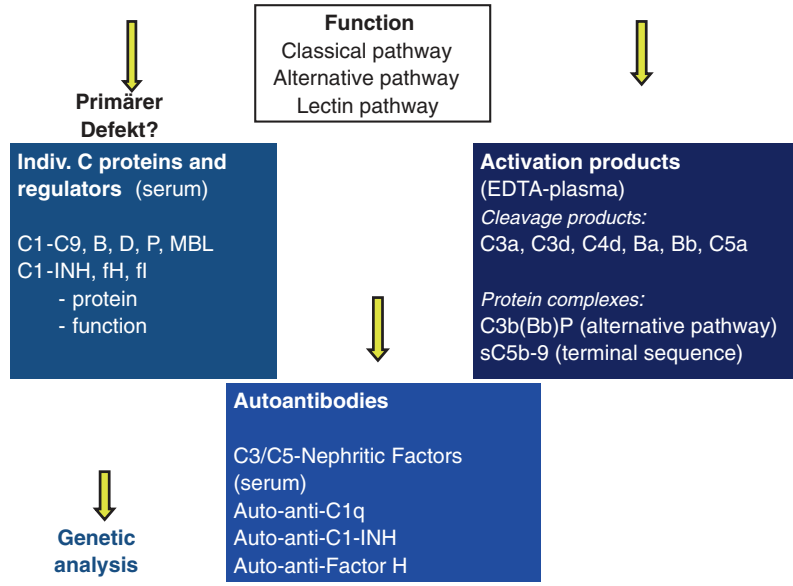

Fig. 21.2 Algorithm of complement analysis

Detecting Autoantibodies

Similar to *loss* or *gain of function* mutations in complement regulators or activating components, overactivation of complement can also be caused by autoantibodies. Autoantibodies to FH (DEAP HUS) [44], FB (DDD) [45], C1q (SLE) [46], or to C1 inhibitor (hereditary angioedema) [47] can be detected by ELISA with the respective purified complement proteins immobilized on a microtiter plate. Results from serial dilutions of patient sera or plasma should be interpreted in comparison with data from large control panels.

Nephritic factors comprise a heterogeneous group of autoantibodies against neoepitopes generated in the C3 and C5 convertases of the complement system, causing its dysregulation [48]. C3 nephritic factor (C3NeF), found in all types of MPGN [49], but especially in dense deposit disease (DDD), can be measured in a decay assay as C3NeF stabilizes the alternative pathway C3 convertase, C3bBb. In this semi-quantitative screening assay C3NeF stabilizes the C3 convertase on sheep erythrocytes, thereby causing increased complement activation and eventually hemolysis [50]. Fluid-phase conversion of C3 upon mixture of normal serum and C3Nef containing patient serum can also be visualized as emerging protein bands of C3b and C3c at lower molecular weight using an immunofixation assay [51]. Recently an ELISA for C3NeF has been reported [36]. Both C3 and C5 nephritic factors correlate with C3 consumption, while only C5 nephritic factors correlated with sC5b-9 levels [52].

Collecting Blood and Urine Samples

Proper collection of (blood and urine) samples for diagnostic analysis is essential [53]. Sample collection should aim at freezing the complement activation status of the patient at the time of blood draw. Without inhibition, physiological and pathological complement activation would continue, thereby obscuring the actual complement activation status and preventing meaningful data interpretation. Therefore, EDTA at $\geq$10 mM final concentration is used as standard anticoagulant since it blocks the *in vitro* activation of the complement system by way of its Mg^{2+} and Ca^{2+} complexing properties. Heparin and citrate are less useful [54]. Centrifuged plasma should be kept

on ice or in a refrigerator if analyzed on the same day; for later processing, the sample should be aliquoted, frozen and stored at −70 °C (−20 °C for short-term [days] storage). Repeated freezing and thawing of aliquots should be avoided because of the risk of *in vitro* activation (above). If needed, frozen samples should be shipped on dry ice.

In urine, the measurement of activated complement components (such as C3) or degradation products (such as C3a or sC5b-9) can be affected by high amounts of urea and urine proteases, so that the immediate addition of protease inhibitors at sampling is required. However, as oberved by us (Kirschfink, unpublished) and shown by van der Pol et al. [55], appearance of complement activation products in proteinuria may also be the consequence of extrarenal (artificial) rather than intrarenal complement activation.

Immunohistological Diagnosis

The diagnosis of many autoimmune (or immune complex diseases) is based on the detection of immunoglobulins and complement deposition in various tissues. For immunohistochemical diagnosis, antibodies against C1q, C3b, C4b, C4d, and C5b-9 are suitable and are available for frozen and in part also for paraffin fixed tissue. Positive staining identifies a direct impact of complement in the disease process, indicates disease activity and allows for the differentiation of the complement activation pathways involved. The presence of C3b suggests ongoing inflammation, while C3d deposits in the absence of C3b point to a non-active disease process. While its specificity is debatable, for long time C4d has been accepted as biomarker of humoral renal graft rejection [56, 57]. However, more recent studies question the clinical significance of C4d in antibody-mediated rejection [58], whereas it appears as if combined C4d and C3d deposition in IgAN is associated with faster disease progression [59].

Targeting Complement in the Treatment of Kidney Disease

The unraveling of a key role for complement dysregulation in an increasing spectrum of kidney diseases provides an unprecedented treatment approach to diseases, previously only poorly managed and often progressing to ESRD and/or death [60]. Furthermore, despite significant progress in biocompatibility of hemodialysis membranes complement activation during extracorporeal treatment still remains a relevant issue [61].

The overarching principle is the restitution of proper complement control. This may be achieved by replacement of missing or defective complement factors or removal of inhibiting autoantibodies via plasma exchange (PLEX)/plasma infusion (PI). Further strategies include the replacement or supplementation of endogenous soluble complement inhibitors (such as C1-inhibitor, FH, recombinant soluble complement receptor 1 [rsCR1]), the administration of antibodies to block key proteins of the cascade reaction (e.g., C5) or to neutralize complement-derived anaphylatoxins, especially C5a (reviewed in [62–66]).

The use of C1INH (Berinert®) in patients receiving deceased donor kidney transplants with high risk for delayed graft function (DGF) may show significant improvement in outcomes post transplant [67].

Both, eculizumab and ravulizumab prevent the release of the highly potent inflammatory anaphylatoxin C5a and the assembly of the membrane attack complex C5b-9 with the advantage of leaving the activation phase of complement up to the generation of the C3 opsonins C3b and iC3b intact. Despite excellent results of treatment with eculizumab and ravulizumab in cinical trials, their is still a great uncertainty with respect the optimal treatment strategy, especially on the decision how to proceed when a patient is stable and in remission [68]. The potential risk of aHUS recurrence after discontinuation of eculizumab treatment

raises the question how long eculizumab should be administered [69]. Risk factors for aHUS recurrence after eculizumab discontinuation include a positive family history of aHUS, presence of pathogenic genetic variants, and extra-renal manifestations of aHUS prior to eculizumab treatment [70]. In a recent survey, Fakhouri et al. confirmed the central role of complement mutations for aHUS recurrence but also concluded that a strategy of eculizumab discontinuation guided by the genetic diagnosis was in most patients reasonable and safe [71]. Collection of outcome data in patients with aHUS, either receiving eculizumab or other treatments, will certainly help to optimize therapy. While the treatment of aHUS with eculizumab has been highly successful in most cases [72–74], its use for other complement-mediated renal diseases is still a matter of ongoing clinical research [75, 76]. The off-label use of eculizumab in patients with C3 glomerulopathy (C3G) including dense deposit disease (DDD) and C3 glomerulonephritis (C3GN) has yielded mixed responses thus far, without uniformly countering key pathological markers (that is, proteinuria), which has prompted the evaluation of alternative therapeutics interferring with the complement cascade more proximally.

Eculizumab is currently being used in renal transplantation and has been evaluated in several clinical trials to minimize the consequences of ischemia-reoxygenation injury (IRI), prevent or treat relapsing or de novo aHUS, and to prevent and cure humoral rejection in patients at high immunological risk (i.e., DSA; ABO-incompatibility) [77, 78]. However, the C5 inhibitor appears less effective in preventing delayed graft function [78].

A second anti-C5 monoclonal antibody with increased half-life, named ravulizumab, has recently been approved by the US Food and Drug Administration for paroxysmal nocturnal haemoglobinuria (PNH) and aHUS in adults and children from 1 months of age [79]. With an extended plasma residence it only requires administration every 8 weeks instead of biweekly, an important step in improving patient management. The long-lasting anti-C5 antibody Ravulizumab has successfully been used in adult and pediatric aHUS [79, 80], and is currently being tested in clinical trials for lupus nephritis and IgA nephropathy.

Besides monitoring of eculizumab-treated patients on the basis of traditional parameters such as lactate dehydrogenase (LDH), hemoglobin (Hb), haptoglobin, platelets and serum creatinine, additional analyses of complement activation including CH50 and AH50 (reflect inhibitory efficacy), sC5b-9, C3 and C3d (indicating potential ongoing *in vivo* activation) provide valuable informations on therapeutic efficacy and may allow—together with drug monitoring—for the re-evaluation of the use and dosage of eculizumab in the heterogeneous group of complement-mediated renal diseases [68, 81].

In certain indications, broader inhibition of complement at the level of C3 may warrant investigation, leveraging clinical benefits over existing therapies, and C3-targeted therapeutics are now being evaluated in long-awaited phase II/III trials [65, 82, 83]. Specifically, alternative C3 convertase (C3bBb) inhibitors at the level of FB or C3 have successfully been tested in paroxysmal nocturnal hemoglobinuria (PNH), and are currently clinically trialed in C3 glomerulopathy (C3G).

Ongoing clinical trials are examining the efficacy of specific blockade in C3G with narsoplimab, sut-imlimab, and danicopan, all of which inhibit complement activation more proximally. Other renal diseases, such as IgA nephropathy, lupus nephritis, and membranous nephropathy are also under study with complement inhibiton. (Gavrillaki and Brodski, 2020) While broader intervention at the level of C3 or the overactive AP seems mechanistically more justified, targeted C5aR1 inhibition using the orally available drug candidate avacopan has shown early clinical promise in C3G and ANCA vasculits [84]. Moreover, an anti-MASP2 mAb is currently being evaluated in clinical trials for various nephropathies, such as IgA nephropathy, aHUS and Lupus Nephritis. Inhibitors targeting the lectin pathway, of FB, FD and C3 are currently at varying stages of clinical development [65, 85].

Summary and Perspectives

The complement system, a complex network of proteins and critical part of the innate immune response significantly contributes to the pathogenesis of inflammatory kidney diseases. A thorough understanding of the basic disease mechanism and careful follow-up are needed for optimal therapy. Comprehensive modern serological and molecular complement analysis is indispensable for correct differential diagnosis of the renal disorders.

Selective complement targeting to inhibit cascade activation can halt or even reverse renal disease. However, to delineate which pathway(s), and at what level, intervention could be effective still requires further translational and clinical research to open new avenues for successful treatment strategies for renal disease.

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Atypical Hemolytic Uremic Syndrome

22

Michal Malina, Veronique Fremeaux-Bacchi, and Sally Johnson

Introduction

The hemolytic uremic syndrome (HUS) is a thrombotic microangiopathy (TMA) characterized by the triad of thrombocytopenia, nonimmune microangiopathic hemolytic anemia, and acute kidney injury [1]. The most frequent form of HUS in children is secondary to Shiga toxin (Stx)—producing *Escherichia coli* (STEC) and the term atypical HUS (aHUS) was initially used to designate any HUS not caused by STEC. It is now clear that within the umbrella of aHUS are a number of specific causes of HUS—for example *Streptococcus pneumoniae* infection, cobalamin C defect, Diacylglycerol kinase ε (DGKε) defect and various underlying conditions.

Atypical HUS without coexisting disease or specific infection is mostly a disease of complement alternative pathway (AP) overactivation, due to hereditary mutations in complement genes or acquired autoantibodies against complement factor H (FH). The clinical characteristics of patients, patient outcome and genotype-phenotype correlations were described [2–7]. Therefore, the term aHUS is today preferentially used to designate HUS without coexisting disease or specific infection [5, 6, 8–11]. Plasma exchange (PE) was the mainstay of treatment for aHUS until 2009, with considerable morbidity in children [12, 13]. Since 2009, terminal complement blockade therapy by eculizumab has dramatically changed the hitherto dismal outcome of the disease [14, 15]. The aims of this chapter are to summarize the previous era of treatment, to review new knowledge in the domain of atypical HUS and to scan the horizon for future developments in the management of atypical HUS.

Definition of Atypical HUS

Atypical HUS is one of a number of causes of TMA—life or organ threatening diseases characterized by microthrombi in small blood vessels which can be classified according to etiology and/or physiopathology [16–19] (Fig. 22.1). The two most important TMAs to exclude when suspecting aHUS are thrombotic thrombocytopenic purpura (TTP) and shigatoxin associated HUS (STEC HUS). The latter is the most common TMA affecting the kidneys in children. It is caused by

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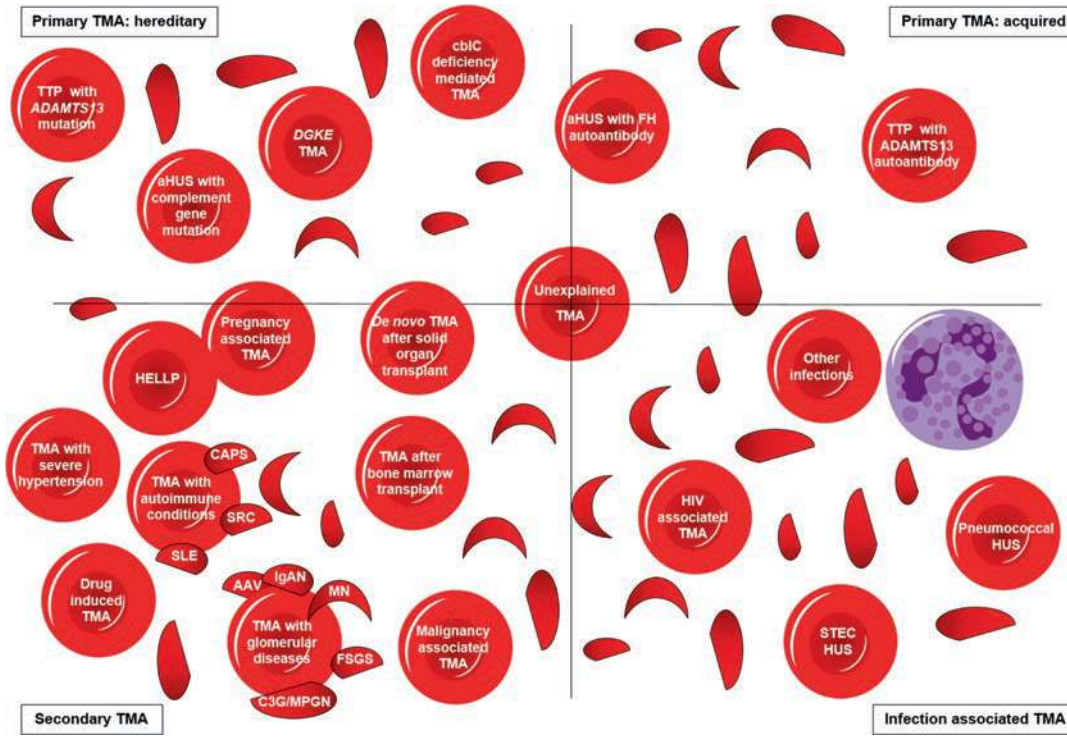

Fig. 22.1 Thrombotic microangiopathies are classified into: Inherited or acquired primary; secondary; or infection associated TMAs. Current classifications define primary TMAs as hereditary (mutations in ADAMTS13, MMACHC (cb1c deficiency), or genes encoding complement proteins) or acquired (autoantibodies to ADAMTS13, or autoantibodies to complement FH, which is associated with homozygous CFHR3/1 deletion). TMA is associated with various infections: in STEC-HUS and pneumococcal HUS, distinct mechanisms result in TMA; in other infections, the processes are not defined and in some cases the infection may trigger manifestation of a primary TMA. Secondary TMAs occur in a spectrum of conditions, and in many cases the pathogenic mechanisms are multifactorial or unknown. The classification presented here is not unequivocal: in some secondary TMAs, for example pregnancy-associated TMA or de novo TMA after transplantation, a significant proportion of individuals will have a genetic predisposition to a primary TMA. *AAV* ANCA-associated vasculitis; *ADAMTS13* a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; *aHUS* atypical hemolytic uremic syndrome; *C3G* C3 glomerulopathy; *CAPS* catastrophic antiphospholipid syndrome; *cblC* cobalamin C type; *DGKE* gene encoding diacylglycerol kinase ε; *FH* factor H; *HELLP* syndrome of hemolysis, elevated liver enzymes, and low platelets; *HUS* hemolytic uremic syndrome; *IgAN* IgA nephropathy; *MN* membranous nephropathy; *MPGN* membranoproliferative glomerulonephritis; *SRC* scleroderma renal crisis; *STEC* shiga toxin–producing Escherichia coli; *TMA* thrombotic microangiopathy; *TTP* thrombotic thrombocytopenic purpura. Reproduced from Brocklebank et al. [19]

intestinal infection by certain strains of E coli carrying a plasmid for producing shigatoxin, particularly serotypes O157:H7, O104:H4 and O26 and in rare cases by Shigella dysenteriae [20, 21]. This type of HUS was previously labeled as typical or D+, however this classification is now obsolete.

TTP is an important cause of TMA that must be ruled out before making a diagnosis of aHUS. It is due to a severe deficiency (<10%) in ADAMTS13 (A Disintegrin And Metalloproteinase with a ThromboSpondin type 1 motif, member 13) activity, either from a congenital absence of functional protein caused by homozygous or compound heterozygous mutations in the *ADAMTS13* gene, or due to anti-ADAMTS13 antibodies [22].

TMA can also occur secondary to a coexisting disease or condition, such as malignancy or autoimmune disease. This is more common in adults than children, with the exception of post-hematopoietic stem cell transplant (HSCT) TMA.

As the classification of TMA has evolved with increasing understanding [23], there is general agreement that the term aHUS defines patients with HUS without a coexisting disease or specific infection [5, 6, 8–11]. This chapter is focused on aHUS according to this definition.

Incidence and Prevalence of Atypical HUS

Atypical HUS, defined as indicated above, is an ultra-rare disease. In the United States, aHUS is considered to have an annual incidence rate of two new pediatric cases per million total population [24]]. An incidence of approximately 0.11 new pediatric cases per million total population per year was also observed between July 2009 and December 2010 in an exhaustive cohort of children with aHUS from France, the United Kingdom, Spain, Netherlands and Canada [13]. A recent systematic review has reported an overview of global incidence and prevalence of aHUS [25]. Eight studies were reviewed from Europe, Australia, and New Zealand [5, 26–32]. In Europe the reported incidence (all ages) ranged between 0.23 and 1.9 per million annually [5, 32]. In Australia a pediatric study reported a calculated incidence of 0.44 per million annually [28]. Studies reporting incidence for individuals under 20 years of age ranged between 0.26 and 0.75 per million annually [27, 32]. A systematic review by Yan reported that in individuals under 20 years of age, the prevalence of aHUS ranged between 2.21 and 9.4 per million people [25].

The Alternative Pathway of Complement

The alternative pathway (AP) of the complement system plays a predominant, though not exclusive role in aHUS (Fig. 22.2) [33–46]. The complement system is composed of plasma proteins that react with one another to opsonize microbes and induce a series of inflammatory responses that help the immune cells to fight infection. There is mounting evidence that complement participates not only in the defense against pathogens, but also in host homeostasis [47–52]. The complement cascade can be activated by three different pathways. While the activation of the classical and the lectin pathways occurs after binding to immune complexes or microorganisms respectively, the AP is continuously activated and generates C3b which binds indiscriminately to pathogens and host cells. On a foreign surface, C3b binds factor B (FB), which is then cleaved by Factor D to form the C3 convertase C3bBb. The C3 convertase, which is stabilized by its binding to properdin, induces exponential cleavage of C3b and the generation of C3bBbC3b complexes with C5 convertase activity. The C5 convertase cleaves C5 to generate C5a—the most potent anaphylatoxin—and C5b which initiates the formation of the membrane attack complex (MAC or C5b-9), able to lyse pathogens [52] (Fig. 22.2). The CAP amplification loop is normally strictly controlled at the surface of the host quiescent endothelium, which is protected from the local formation of the C3 convertase by complement regulatory proteins. These include regulators in serum, such as FH and Factor I (FI), as well as membrane bound CD46 (membrane cofactor protein (MCP), which cooperate locally to inactivate C3b. FH is the most important protein for the regulation of the CAP and consists of 20 short consensus repeats (SCRs) and contain two C3b-binding sites (Fig. 22.3). MCP is a widely expressed transmembrane glycoprotein that binds C3b and inhibits complement activation on host cells. The serine protease FI cleaves C3b in the presence of various cofactors including FH, complement receptor 1 (CR1, CD35) and MCP. Coagulation regulator thrombomodulin (THBD) enhances FI-mediated inactivation of C3b in the presence of FH [47, 52].

Over the past 20 years, genetic discoveries have substantially improved our understanding of the mechanisms responsible for aHUS and driven development of novel therapeutic strategies) [33–46] (Fig. 22.4). In a large genetic screen of 794 aHUS patients, rare variants in one the 5 genes (*CFH*, *C3*, *CFI*, *CFB*, or *CD46*) that encode proteins involved in the regulation of the alternative pathway of complement were identified in 41% of patients and combinations of mutations were noted

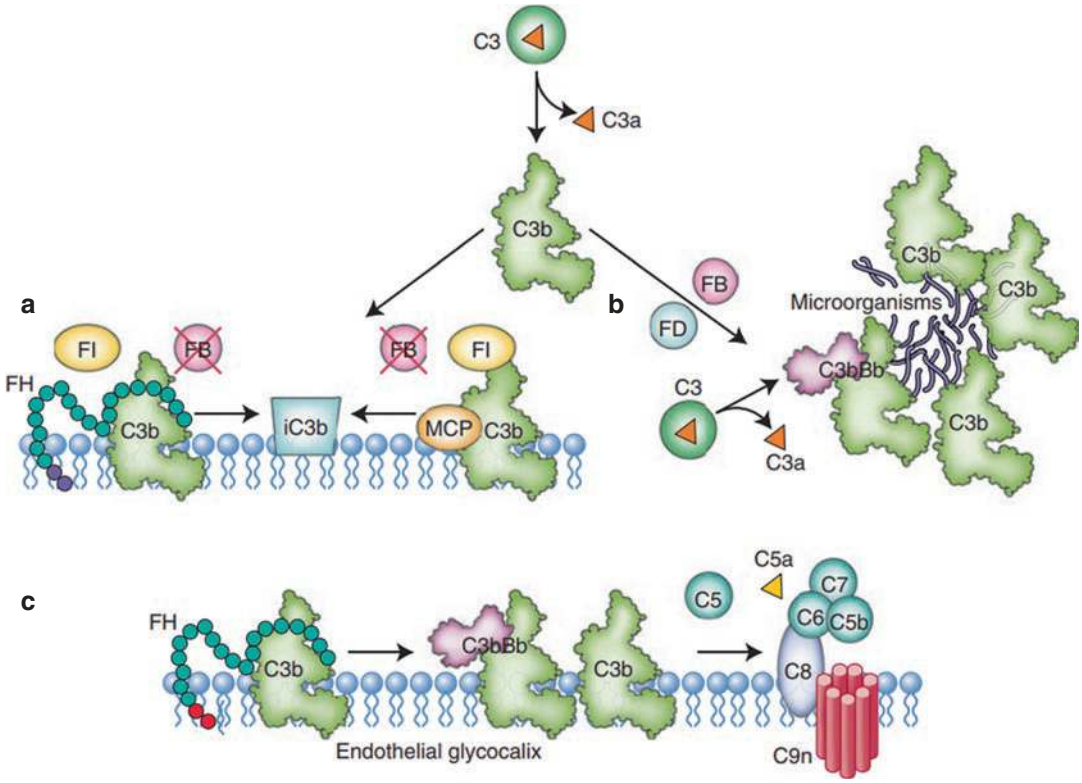

Fig. 22.2 Complement activation and its regulation. aHUS is the prototype of a disease resulting from inefficient protection of endothelial cells against complement activation. (**a**) Protection of cells surface. The AP is permanently active, with a continuous formation of small amounts of the C3 convertase C3bBb at the cell surface. To prevent unopposed complement activation resulting in cell damage, the complement system is tighly regulated. The glycocalyx is a multifunctional thick carbohydrate layer containing glycoaminoglycans (GAG) (heparin sulphate, sialic acid, polyanions) that covers all endothelial cells, in particular the glomerular endothelium in the kidney. FH binds to GAG and C3b. MCP is constitutionally anchored to endothelial membrane. Under normal conditions, the C3 convertase formation is stopped by the interaction of FH or MCP with C3b, which makes further binding of FB to C3b impossible. C3b is then cleaved by FI to iC3b, which cannot bind FB. (**b**) Activation of complement and covalent attachment of C3b to the microbial surfaces. The major function of complement is to act as a defense mechanism against microbes. Very small amounts of C3b are normally present in plasma due to low levels of spontaneous C3 cleavage but C3b can bind to bacteria. Once C3b is covalently bound to the surface of microorganisms, FB binds to it and becomes susceptible to cleavage by Factor D (FD). The resulting C3bBb complex is a C3 convertase that will continue to generate more C3b, thus amplifying C3b production. C3b attaches to bacterial surfaces for opsonization by phagocytes and simultaneous activation of the cytolytic terminal complement cascade. (**c**) In the case of aHUS, AP activation is uncontrolled and C3 convertase C3bBb and C5 convertase C3bC3bBb are formed. During complement activation, C5 is split into C5a and C5b. C5b together with complement proteins C6, C7, C8 and C9 form the C5b9 complex in sublytic quantities that activate endothelial cells to produce prothrombotic factors. *AP* alternative pathway, *C3bBb* C3 convertase; *C3bC3bBb* C5 convertase; *FB* complement factor B; *FD* complement factor D; *FH* complement factor H; *FI* complement factor I; *MCP* membrane cofactor protein (CD46)

in 3% of patients [53]. Predisposition to aHUS is inherited in an autosomal recessive or autosomal dominant manner with incomplete penetrance.

An updated database describing all rare variants identified in aHUS is available at https://www.complement-db.org [54, 55] and these genetic abnormalities are described in more detail in specific sections of this chapter. In addition, acquired autoantibodies to the FH protein (anti-FH) have been demonstrated in patients with aHUS, also described in more detail below.

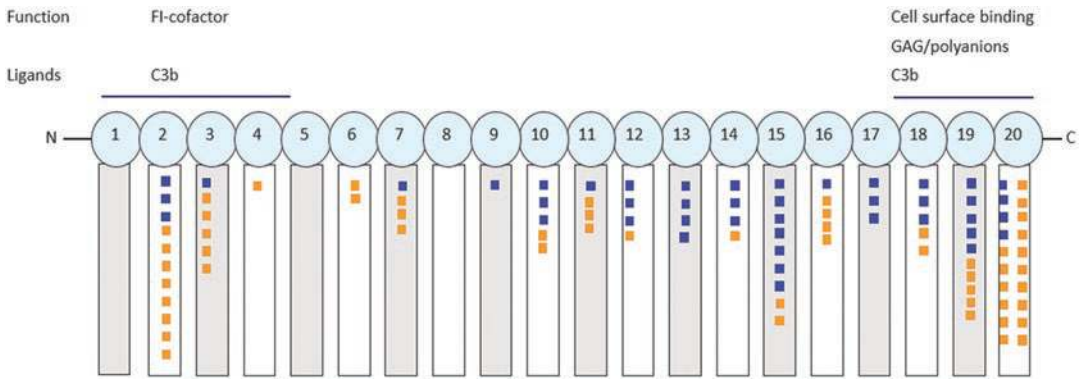

Fig. 22.3 Complement factor H. FH is a plasma protein consisting in 20 domains called short consensus repeats (SCRs) (*numbered circles*). FH has two C3b binding sites: one is localized within the N-terminal SCR 1–4, implicated in the cleavage of C3b by FI and the other in the C-terminal SCR 19–20, implicated in cell surface binding. FH regulates the formation, stability and decay of the C3 convertase C3bBb. *94* rare variants in *CFH* reported in the aHUS database https://www.complement-db.org are shown within columns. Blue squares indicate frameshift, deletion, nonsense and conserved cysteine affected variants (prediction of quantitative FH deficiency), orange squares indicate missense variants (with or without demonstrating functional consequences). 20% of all variants are located in the C-terminus SCR 20 and are mostly associated with normal FH plasma level. *FH* complement factor H; *FI* complement factor I; *GAG* glycoaminoglycans; *SCR* short consensus repeat

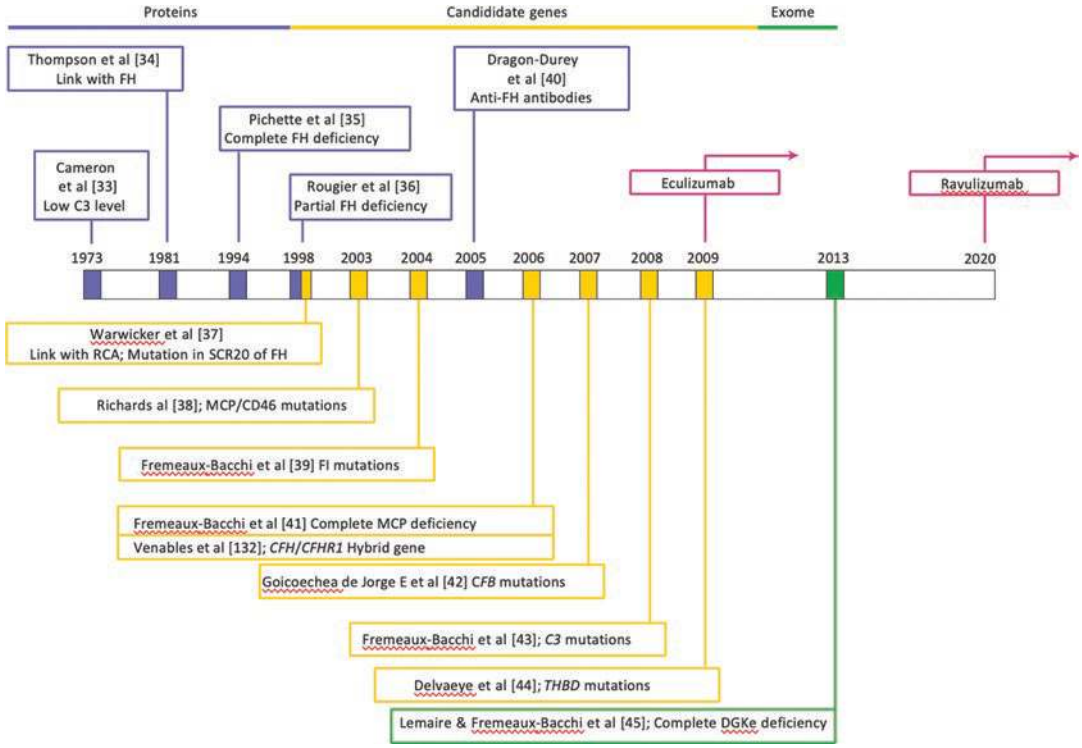

Fig. 22.4 Discoveries that allowed a better understanding of the pathophysiology of aHUS during the last decades. This led to the approval of eculizumab for the treatment of patients with aHUS, to control the overactivation of complement. *FB* complement factor B; *FH* complement factor H; *FI* complement factor I; *DGKE* diacylglycerol kinase ε; *MCP* membrane cofactor protein (CD46); *THBD* thrombomodulin; RCA regulators of complement activation; *CFHR* complement factor H related protein

Clinical Presentation

The majority of children with aHUS present with the complete triad of HUS; microangiopathic hemolytic anemia (with hemoglobin <10 g/dL, presence of schistocytes, high lactate dehydrogenase (LDH), decreased haptoglobin levels) thrombocytopenia with platelet count <150 × 10^9/L and acute kidney injury (serum creatinine above the upper limit of normal). Approximately 60% of them require dialysis at the first episode. Severe hypertension is common. However, the complete triad may be missing at admission and a gradual onset is possible. Particularly, platelet count may be >150 × 10^9/L (approximately 15% of patients) and hemoglobin may be >10 g/dL (approximately 5% of patients) [5]. Children may also have normal serum creatinine at presentation (approximately 15%) [56] and/or present with proteinuria/nephrotic syndrome/hematuria/hypertension as the only kidney manifestations. Thus, any association of two components of the triad with the third one missing can be a manifestation of HUS. While kidney biopsy is not required to establish the diagnosis when full-blown HUS is present, it is useful when hematology criteria are missing or incomplete and any time the diagnosis of HUS is uncertain, to document that the underlying histological lesion is TMA.

Age and Gender

In a cohort of French children with aHUS (66.2% of whom had a proven genetic or acquired complement abnormality), the mean age at onset was 1.5 years (0 to <15 years). 56% (50/89) of children had onset between birth and 2 years of age (28% between birth and 6 months, 28% between 6 months and 2 years) [5], similar to the proportion of 22% (10/45) of children having onset between 1 month and 1 year in another series [4] and 36.3% (53/146) less than 2 years and 19.8% (29/146) less than 1 year in another [57]. Atypical HUS in children is as frequent in females as in males (female-to-male ratio 0.9), in contrast with the female preponderance when the disease presents in adulthood (female-to-male ratio 3) [4, 5]. In a large series from the Global aHUS Registry, 387/851 (45%) of patients with aHUS presented before the age of 18 years (mean 3.8 years) and 166/387 (43%) of those with pediatric onset were female.

Age at onset in children varies according to the underlying genetic or acquired abnormality (more information about specific genetic/acquired abnormalities is given below). Onset between birth and 1 year of age has been reported in the majority of aHUS patients (37/50) reported to date with *DGKE* mutation [45, 46, 58–61] and all children with homozygous *CFH* mutation. It is also frequent in children with heterozygous *CFH* or *CFI* mutation-associated HUS. Conversely, *MCP* mutation-associated HUS in children exceptionally starts before the age of 1 year but most often between age 2 and 12 years. Anti factor H autoantibody associated HUS (anti-FH HUS) is also mostly a disease of late childhood and adolescence (onset between 5 and 12 years, mean age 7.6–9 years in five series including a total of over 500 patients with this form of aHUS [62–67]. *C3* or *CFB* mutation-associated HUS and aHUS without complement mutation or anti-FH antibodies appears to start at any age [2, 5, 25].

Family History

As indicated above, despite aHUS being a genetic disease, a family history of HUS is present in only 20–30% of patients [2, 4, 5] due to incomplete penetrance. The diagnosis of HUS may be unknown in the family and questioning should ask about cases of acute or chronic kidney failure, thrombocytopenia, anemia, hypertension, dialysis and graft failure in the pedigree as well as about consanguinity, which is significant for homozygous mutations in *CFH*, *MCP* and *DGKE*. No familial case of anti-FH antibody-associated HUS has been reported [68].

Triggering Events

Atypical HUS episodes in children are frequently triggered by intercurrent infections, whatever the genetic background. Specific reported infections include varicella [69], influenza [70, 71], Bordetella pertussis [72] and recently SarsCov2 virus [73].

Diarrhea precedes the onset of aHUS in at least one third of children and upper respiratory tract infections in at least 10% [4, 5]. This frequency of diarrhea at onset of aHUS explains why the former "post-diarrheal" or "non post-diarrheal" (or D+/D-) criterion to differentiate STEC-HUS from aHUS was frequently misleading. It is, however, often unclear whether gastrointestinal symptoms in aHUS are linked to an infectious trigger or whether they are manifestations of intestinal TMA. Rare patients (approximately 1%) have been reported in whom the first episode of aHUS was caused by STEC gastroenteritis, with the diagnosis of aHUS being retained because the patient had subsequent relapses and a familial history of aHUS (one patient with *MCP* mutation) [5], a severe course possibly favored by the genetic complement abnormality (one patient with *CFH* mutation) [74] or recurrence after kidney transplantation (two patients with *CFI* or *MCP* mutation—the latter also in the mother who donated the kidney) [75] . In a cohort of 75 patients with proven STEC HUS, four patients (5%) were found to have pathogenic variants in complement genes, including one patient with severe outcome. In aHUS secondary to anti-FH antibodies, a gastrointestinal prodrome (such as diarrhea, vomiting and/or abdominal pain) has been reported in 27.7% [65, 76, 77]. This type of aHUS is more common in the Asian subcontinent where it comprises 56% of cases compared with 10–25% of European cohorts [66, 77]. A recent study looking for gastrointestinal pathogens in aHUS secondary to anti-FH antibodies showed that twice as many patients had evidence of gastrointestinal pathogens compared with those without anti-FH (62.5% compared with 31.5%) including Clostridium difficile, Giardia intestinalis, Salmonella, Shigella, Rotavirus, Norovirus and Entamoeba histolytica. No stool was positive for Shigatoxin [78]. However, STEC has been reported as the trigger for HUS in a couple of patients with anti-FH HUS [62, 65]. Interestingly, the association of homozygous *MCP* mutation with common variable immunodeficiency has been reported [41]. Therefore, patients with homozygous *MCP* mutation should be investigated for immunodeficiency that may require immunoglobulin therapy to prevent infections—and thus decrease the frequency of HUS relapses triggered by infections.

Lastly, pregnancy is the trigger for aHUS in 20% of adult women [5] and 86% of women with pregnancy—associated HUS (mostly in the postpartum period) have a complement mutation [79]. For this reason, pregnancy-associated HUS is now classified as aHUS.

Histology of Atypical HUS

The underlying histological lesion of aHUS is TMA involving afferent arterioles and glomerular capillaries. Characteristic features during the acute phase are platelet and fibrin thrombi within glomerular capillaries and the thickening of glomerular capillary walls related to endothelial cell swelling and detachment and the accumulation of flocculent material (proteins and cellular debris) between the endothelial cells and the basement membrane, with double contour appearance. Mesangiolysis (fluffy mesangial expansion) is also common. Bloodless and ischemic glomeruli related to the narrowing or occlusion of the capillary and arteriolar lumen can be observed. Arterial changes range from endothelial swelling and intramural fibrin to fibrinoid necrosis with occlusive thrombi and fragmented red blood cells. Immunofluorescence studies for immunoglobulin G or C3 deposits are generally negative. C5b-9 staining has been reported in microangiopathy attributed to complement abnormalities and other causes, however its presence is not reliable.

Chronic lesions are characterized by mesangial sclerosis, thickening of capillary walls with sparse or diffuse double contours, ischemic changes of glomeruli and mucoid intimal hyperplasia and narrowing of the arterial lumen (onion-skinning). The time course for histological resolution of TMA is unknown and therefore it is difficult to know if presence of chronic features points to an ongoing active TMA process or to a chronic sequel [80, 81].

There is a general consensus that it is not possible to determine the etiology of TMA from histological morphology [82].

Manifestations Outside the Kidneys

Although the TMA process predominates in the kidney vasculature, other organs may be involved. The most frequent manifestation outside the kidneys during acute episodes of aHUS is brain involvement, reported in 15–20% of children with aHUS [5, 83–86]. Symptoms can be seizures, altered mental status, altered consciousness, visual problems (diplopia, sudden visual loss), paresis and coma. Computed tomography scan is useful to rule out cerebral bleeding. Magnetic resonance imaging (MRI) shows hyperdensities of variable severity and extension. Focal cerebral infarction is possible. The prognostic significance of MRI abnormalities is generally uncertain. The frequency of cardiac involvement is poorly documented, but life threatening ischemic myocardiopathy may occur, which makes sequential troponin level assay, electrocardiography and echocardiography advisable during acute episodes [86–89] . Peripheral acute ischemia leading to gangrene of fingers/hands and toes/feet [90], skin necrosis [91–93] or retinal ischemia with sudden visual loss [94] have been reported in a few patients. Manifestations outside the kidneys may also include pancreatitis (increase in pancreatic enzymes with or without clinical/radiologic signs) and/or hepatitis (increase of hepatic enzymes) (5–10% of patients) and, exceptionally, intra-alveolar hemorrhage, severe gastrointestinal manifestations including intestinal perforation or life-threatening multiorgan failure (2–3% of patients [5, 91]. Severe gastrointestinal symptoms (abdominal pain, vomiting, diarrhea, biochemical pancreatitis and hepatitis), myocardial and neurological manifestations appear to be particularly frequent in patients with anti-FH antibodies [62, 65, 77, 84, 90].

Four children with aHUS have been reported who developed cerebral ischemic events due to stenosis of cerebral arteries after several years on dialysis [95–98]. One of them also had stenosis of coronary, pulmonary and digestive arteries [96]. These observations have suggested that local complement activation during acute episodes and/or subclinically in the long term, may lead to such macrovascular complications, independently or as aggravating factors of the vascular consequences of long-term dialysis. Prospective studies are required to document whether aHUS patients have an increased risk of cardio-or cerebro-vascular events and of arterial disease due to the local complement activation [99].

Experience of eculizumab to treat manifestations of aHUS outside the kidneys is limited to case reports. However, eculizumab was impressively effective in two children with acute distal ischemia [90] or skin necrosis and intestinal perforation [91], respectively. Eculizumab may rescue central nervous system manifestations, as suggested by nine case reports, including four in children [85, 86, 88, 100, 101]. Eculizumab also appeared life-saving in four children with myocardial involvement [86–89]. Lastly, two children who had developed cerebral artery stenosis stopped having ischemic events under eculizumab therapy, with non-progression of arterial stenosis documented in one [95, 97].

Making the Diagnosis of Atypical Hemolytic Uremic Syndrome

When the features of HUS are present (as summarized in Table 22.1), careful assessment is required to exclude possible causes before a provisional diagnosis of aHUS can be reached [102, 103] (Fig. 22.5 and Table 22.2).

Table 22.1 Investigations to support the presence of a thrombotic microangiopathy

Test to confirm a TMA	Result in aHUS	Comment
Haemoglobin	Low	
Platelet count	Low	
Blood film	Fragmented red blood cells	
Direct antiglobulin test	Negative	May be positive in pneumococcal HUS
Reticulocyte count	High	Low suggests either bone marrow problem or ESKD

Table 22.1 (continued)

Test to confirm a TMA	Result in aHUS	Comment
Lactate dehydrogenase	High	
Creatinine	High	Previous measurements are helpful to exclude CKD
Urinalysis	Blood, protein	
Kidney ultrasound scan	Normal sized or large kidneys, often echobright	Small kidneys suggest ESKD
Plasma C3	Low or normal	Not sensitive or specific for aHUS

ESKD end stage kidney disease; *CKD* chronic kidney disease

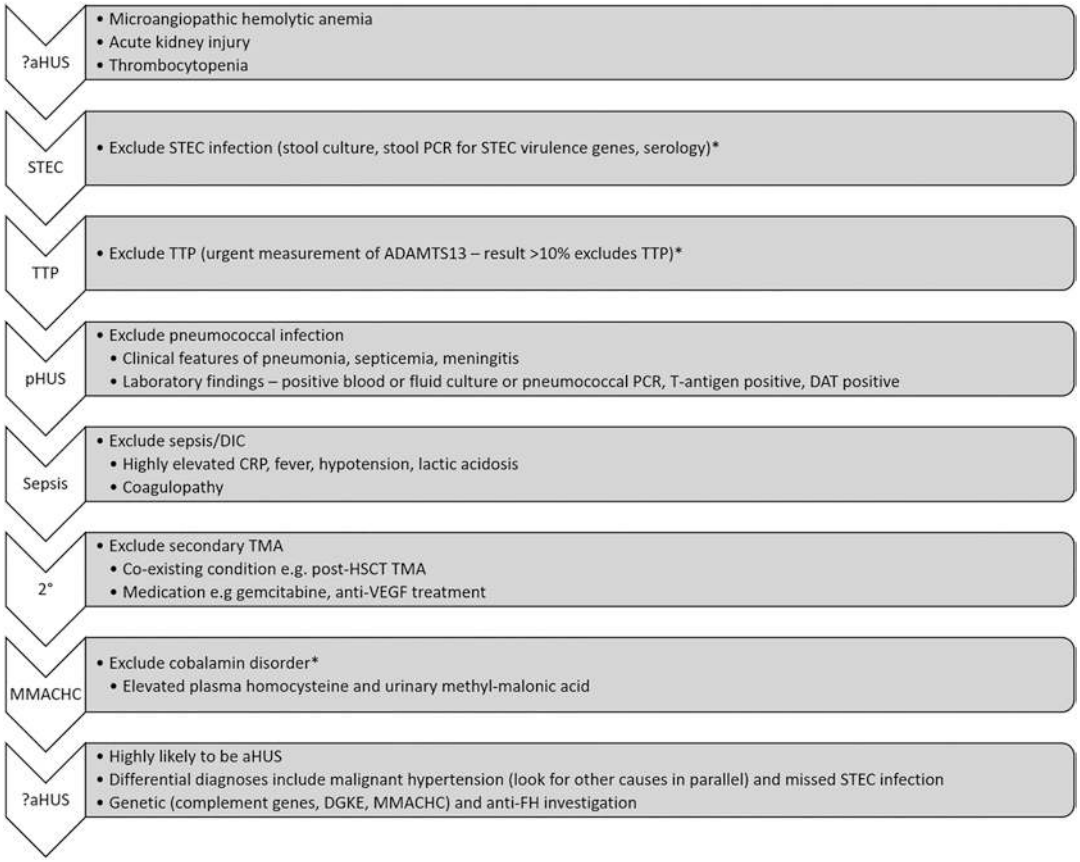

Fig. 22.5 Suggested approach to making the diagnosis of atypical HUS. When the clinical triad of microangiopathic hemolytic anemia, acute kidney injury and thrombocytopenia are present without clinical evidence of STEC infection, further evidence should be sought for STEC and pneumococcal infection. TTP should be excluded, along with secondary causes of TMA. The triad of HUS may indicate sepsis, which should be sought and excluded. Evidence for cobalamin disorder should also be sought. *these investigations may take some time to return and treatment with anti-C5 therapy should not be delayed if results are not available and the diagnosis of aHUS is strongly suspected. In practice, if an alternative diagnosis is secured after commencement of anti-C5 therapy, it can be discontinued. *STEC* shiga-toxin producing Escherichia coli; *PCR* polymerase chain reaction; *TTP* thrombotic thrombocytopenic purpura; *ADAMTS13* a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; *pHUS* pneumococcal haemolytic uremic syndrome; *DAT* direct antigen test; *TMA* thrombotic microangiopathy; *HSCT* hematopoietic stem cell transplant; *VEGF* vascular endothelial growth factor; *DIC* disseminated intravascular coagulation; *CRP* C-reactive protein; *DGKE* Diacylglycerol Kinase Epsilon; *MMACHC* methylmalonic aciduria and homocystinuria type C, FH factor H

Table 22.2 Investigations to rule out an alternative diagnosis

Tests for differential diagnoses	Result	Differential diagnosis
ADAMTS13 activity	<5%	TTP
Stool culture for E. coli O157	Positive	STEC HUS
Stool PCR for STEC virulence genes	Positive	STEC HUS
Serology for STEC	Positive	STEC HUS
T-antigen	Positive	Pneumococcal HUS
Pneumococcal PCR (blood/fluid)	Positive	Pneumococcal PCR
Coagulation	Prolonged PT/PTT and low fibrinogen	Sepsis/DIC
C-reactive protein	High	Sepsis/infection[a]
Plasma homocysteine	High	Cobalamin C disorder
Urinary MMA	High	Cobalamin C disorder
ECG/Echocardiogram	Evidence of left ventricular hypertrophy	Malignant hypertension
Anti-double stranded DNA antibodies	Positive	Systemic lupus erythematosus
Renal transplant—donor-specific antibodies	Positive	Antibody mediated rejection
Renal transplant—C4d staining	Positive	Antibody mediated rejection
Anti-phospholipid antibody	Positive	Anti-phospholipid antibody syndrome
Serum/urine electrophoresis[b]	Paraprotein	Plasma cell dyscrasia
Serum free light chains[b]	Positive	Plasma cell dyscrasia

ADAMTS13 a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; *STEC* shiga toxin producing E. coli; *PT* prothrombin time; *PTT* partial thromboplastin time; *DIC* disseminated intravascular coagulation; *MMA* methylmalonic acid

[a] Infection can be a trigger for an episode of aHUS in a susceptible individual

[b] Unlikely in children

Since the most common cause of HUS in children is STEC infection and because STEC infections may be asymptomatic, thorough microbiological assessment is required. This is reviewed in Chap. 24, but in brief requires stool culture (which commonly only detects E. coli serotype O157:H7) and stool polymerase chain reaction (PCR) for STEC virulence genes. Serological assessment may also be helpful. Medical history and physical examination usually eliminate HUS secondary to a coexisting condition—mostly HSCT- and generally suffice for the diagnosis of *Streptococcus pneumoniae*-HUS, that occurs mostly in children less than 2 years of age presenting with symptoms of invasive infection (pneumonia, meningitis, bacteremia) (see Chap. 2). Malignant hypertension can be difficult to distinguish from aHUS, and careful assessment for alternative causes of hypertension may help in this differential diagnosis. TTP must be excluded by urgent assessment of ADAMTS13 activity, since there is treatment dichotomy depending on the result. Evidence for a cobalamin C defect (raised plasma homocysteine and raised urinary methylmalonic acid) must also be sought since alternative treatment will be required [20, 102, 104–114].

Biological Assays to Support the Clinical Diagnosis of Atypical HUS

STEC infection should be ruled out as soon as possible when aHUS is suspected. Stools should be collected at admission or rectal swab performed if no stools are available, allowing stool culture and fecal PCR or immunologic assay for Stx (see Chap. 26). Negative results, mostly due to delayed stool collection or prior administration of antibiotics, are observed in at least 30% of cases classified clinically as STEC-HUS [21, 106]: in such cases, the clinical diagnosis should prevail. Congenital TTP requires urgent plasma infusion (PI) and acquired TTP requires urgent plasma exchange (PE) plus corticosteroids and rituximab. Blood samples collected before PE/PI

are required for ADAMTS13 activity assays, which most commonly rely on the cleavage by plasma ADAMTS13 of the von Willebrand Factor (VWF) peptide containing the cleaving site of VWF (Fret-VWF 73). Results can be available within a few hours [115, 116]. A limitation is the interference of hyperbilirubinemia [117]. Results of commercial kits show reasonable though not full agreement (80–90% concordance) with Fret-VWF [116, 118, 119].

Lastly, assays to detect a cobalamin defect should be part of the initial biological sampling in any child suspected to have aHUS. Cobalamin defect—associated HUS, which can be rescued by hydroxocobalamine treatment, may occur in neonatal forms presenting with neurological, cardiac or multivisceral involvement, but at least as frequently in late-onset forms presenting with predominant or isolated HUS during childhood or early adulthood [110, 111, 120–122].

Complement Investigations in Patients Suspected to Have aHUS

All patients suspected of having aHUS should have blood sampling before PE/PI for measurement of C3, C4, FH, FI and FB plasma levels and screening for anti-FH antibodies. A recent publication from seven European laboratories reports the standardization of the enzyme linked immunosorbent assay technique for anti-FH antibodies [123], which could be developed in other countries. MCP surface expression on polymorphonuclear or mononuclear leucocytes is also required.

As indicated above, decreased C3 levels are observed in only 30% of children with aHUS [2, 4–6]. Therefore, a normal C3 level does not rule out the diagnosis of aHUS. Normal C4 concentration associated with decreased C3 level confirms activation of the AP as would a decreased FB concentration. As the C3 level is normal in patients with isolated MCP mutation and decreased in patients with high titer anti-FH antibodies, aHUS in a pre-adolescent or adolescent child—the age of onset in these two subgroups of complement-dependent HUS—is most likely MCP mutation-associated-HUS if C3 level is normal or anti-FH antibody-associated HUS if C3 level is low.

As indicated above, decreased FH or FI plasma levels are observed in approximately 50% and 30% of patients with mutations in *CFH* or *CFI* genes, respectively [5, 6]. Therefore, a normal FH or FI plasma level does not exclude a mutation in the corresponding gene.

Recent data suggest that levels of C5a and soluble C5b-9 (sC5b-9) are elevated during acute episodes of aHUS and may be biological markers to differentiate aHUS from TTP [124]. Increased C5a and sC5b-9 plasma levels have been confirmed in approximately half of aHUS patients during acute phases of the disease and also during remission [125]. However, a normalization of complement activation products levels after remission, including sC5b-9, has been reported by another group [126]. This, and the fact that sC5b-9 may be elevated in conditions which are not aHUS [127], means that the usefulness of these markers for routine clinical care remains to be determined.

Genetic Screening in Patients with Atypical HUS

Genetic screening results are not required for urgent therapeutic decisions but are necessary to establish whether the disease is complement-dependent or not, prognosis, risk of relapses and of progression to kidney failure, genetic counselling, decisions for complement blockade treatment duration and for kidney transplantation. The six complement genes identified as susceptibility factors for aHUS (*CFH*, *CFI*, *MCP*, *C3*, *CFB* and *THBD*) should be analysed by direct sequencing or next generation sequencing analysis. Multiplex ligation dependent probe amplification (MLPA) is required to detect hybrid *CFH* genes (5% of patients) and copy number variations in *CFH* and CFH Related (CFHR) protein genes. Because of the frequency of combined mutations indicated above, all six genes should be screened for mutations in all patients. Screening for *DGKE* mutations should be per-

formed in children with onset of aHUS before the age of 1–2 years and maybe in older children if further reports indicate *DGKE* mutation-associated HUS may occur later in life. Sequence variants in complement genes have been identified in 5 of 13 patients with anti-FH antibody—associated HUS in one series [128], but none of 26 patients in another series [62]. Genetic analyses even when anti-FH antibodies are present may be justified. If the patient has anti-FH antibodies and a mutation, treatment should be decided according to the antibody titer and the functional consequences of the mutation [103]. Next-generation sequencing analysis allows the simultaneous study of all potentially relevant genes and should reduce the turnaround time for results and the cost of genetic analysis. Exome sequencing, which was successfully used to identify *DGKE* mutations [45], is still limited to research laboratories.

Rare Coding Variants in the CFH Gene

The role of *CFH* in aHUS was first suggested more than 40 years ago (Fig. 22.4). A decrease of plasma C3 level was first reported in 1973 in five patients with severe HUS [33] and low FH plasma levels were first reported in 1981 in a 8-month-old boy with HUS [34]. However, it is only in 1998 that Warwicker et al., by linkage analysis, could establish the link between aHUS and the Regulators of Complement Activation (RCA) region in chromosome 1q32, and the presence of mutations in *CFH*, mainly in the SCR 20, despite normal plasma levels of FH and C3 [37] .

During the last 15 years, at least 90 different rare variants of *CFH* with minor allele frequencies (MAF) <0.1% including missense or nonsense variants, short deletions or insertions, located everywhere in the gene, have been identified and referenced in the FH aHUS mutation database. The type I mutations, which induce a quantitative deficiency of the FH protein (low FH plasma levels), are located everywhere in the gene. By contrast, the mutations which induce a decreased ability of FH to bind to endothelial cells-bound C3b while plasma levels of FH are normal (namely type II mutations), are mostly located in SCR 20 (Fig. 22.3). More than 90% of reported mutations have been heterozygous and plasma C3 levels are decreased in approximately 50% of patients [2, 5, 45, 58, 65]. Less than 20 children (2–4% of reported children with aHUS), mostly from consanguineous families, carried a *CFH* homozygous variant or two heterozygous variants leading to complete FH deficiency, with permanently very low C3 levels. *CFH* pathogenic variants are the most common among aHUS patients, accounting for 20–30% of all aHUS cases in registries from the United States and Europe [1, 3–5] (Table 22.3).

The *CFH* gene is in close proximity to genes encoding for the five *CFHR* proteins that are thought to have arisen from several large genomic duplications. All *CFHRs* share a high degree of homology, which makes the region particularly prone to genomic rearrangement. The C-terminal SCR domains of CFHR1 proteins show a high level of amino acid sequence identity to the C-terminal recognition region of FH, representing the central combined cell surface anchoring- and C3b recognition region of FH. Using MLPA, genetic rearrangements between CFHR1 and FH, which result in a hybrid CFH/CFHR1 gene leading to the formation of hybrid CFH/CFHR1 protein have been reported in several unrelated aHUS patients from distinct geographic origins [129–132]. Two types of factor H/CFHR1 hybrid proteins have been described. One hybrid protein comprises the first 18 SCRs of FH linked to the C-terminal two SCRs of CFHR1. The second fusion protein has the first 19 SCRs of FH linked to SCR5 of CFHR1. Both hybrid factor H/CFHR1 proteins differ from their native C-terminal FH domain 20 by two amino acids only, at positions S1191L and V1197A [132]. They lack proper FH cell binding and protection from complement and are directly implicated in the disease pathogenesis [132].

Conversely, two types of hybrid CFHR1/CFH genes that encode a fusion protein with the first three short consensus repeats (SCRs) of FHR1 and the last two SCRs of FH or with the first four SCRs of FHR1 and the terminal SCR20 of FH have been identified in aHUS patients. Functional

Table 22.3 Frequency of complement and DGKE abnormalities in children and adults with atypical hemolytic uremic syndrome in four cohorts from Europe and the USA

	[2]			[5][a]			[4]	[3]
	Total	Children	Adults	Total	Children	Adults	Children	Children + adults
Number of patients	256	152	104	214	89	125	45	I44
CFH mutation, %	25.3	15.6	25	37.5	11.3	32	11	27
Homozygous	4.2	–	–	1.8	4.4	0	–	
Heterozygous	21.1	–	–	25.7	16.8	32	–	
MCP mutation, %	7	9.2	3.8	9.3	13.5	6.4	9	5
Homozygous	–	–	–	2.8	5.6	0.8	–	–
Heterozygous	–	–	–	6.5	7.8	5.6	–	–
CFI mutation, %	3.9	2.6	5.7	8.4	6.7	9.6	7	8
C3 mutation, %	4.6	3.9	5.7	8.4	7.8	8.8	9	2
CFB mutation, %	0.4	–	–	1.4	1	2.4	4	4
Anti-CFH antibodies, %	3.1	3.9	1.9	6.5	11	3.2	13	–
THBD mutation, %	5	7.8	0.9	0	0	0	0	3
Combined mutations, %	3	–	–	4.2	3.4	4.8	4	5.5
Complement-mediated HIS, %	52.3	53	43	65.7	64.7	67.2	55	46
DGKE mutation, %	–	–	–	3.2	7.9	0	–	–
No identified abnormality, %	47.7	47	57	31.1	27.4	32.8	45	54

CFB complement factor B; *CFH* complement factor H; *CFI* complement factor I; *DGKE* diacylglycerol kinase ε; *MCP* membrane cofactor protein (CD46); *THBD* thrombomodulin
% percentage of patients; – not documented
[a]DGKE mutations were identified in seven children who were previously within the group with no complement mutation identified [5]

studies revealed that the hybrid protein causes complement dysregulation at the cell surface by acting as a competitive antagonist of FH.

Rare Coding Variants in the CFI Gene

To date more than 100 *CFI* distinct rare variants have been published, located everywhere in the gene. All but one variants are heterozygous [4]. *CFI* pathogenic variants induce a default of secretion of the mutant protein (de Jong) and less frequently disrupt its cofactor activity, with altered degradation of C3b in the fluid phase and on surfaces. However, 40% of *CFI* mutations have no identified functional consequences and their link with the disease remains unclear. Plasma C3 levels are below the normal range in approximately 50% of patients with *CFI* variants and FI levels are slightly decreased in 30% [5]. *CFI* rare variants account for 4–8% of aHUS cases [5].

Rare Coding Variants in the MCP Gene

More than 100 distinct rare variants in *MCP* gene have been reported in cohorts of patients with aHUS. Fifty percent of variants identified in MCP gene are splice site nonsense or frameshift variants. In the French aHUS cohort, one third of *MCP* mutations are homozygous and two-thirds are heterozygous [5]. Over 80% of the pathogenic variants induce a reduction in MCP expression on granulocytes. Plasma C3 level is normal in patients with isolated *MCP* mutations [5].

Rare Coding Variants in the C3 Gene

Screening the French aHUS cohort for mutations in the C3 gene led to the discovery of heterozygous pathogenic variants including a recurrent C3 variant (p.R139W) in aHUS patients. Functional studies showed that the nucleotide change induces either a defect in the ability of C3 to bind the regulatory proteins MCP and FH (indirect gain of function mutation) or an increase in the capacity of C3 to bind FB (direct gain of function mutation). In both cases, the genetic change induces enhanced C3bBb convertase formation and complement activation on cell surfaces [133]. There are now at least 90 distinct C3 mutations reported in hundreds of aHUS patients,

however few functional studies have been reported. It is estimated that ~2–10% of incident aHUS patients will carry a C3 pathogenic mutation. The majority of these patients have persistently low plasma C3 levels [43].

Rare Coding Variants in the CFB Gene

Very few pathogenic variants of *CFB* with functional consequences have been identified. Therefore, *CFB* mutations account for only 1–2% of aHUS patients (Table 22.3). Functional analyses demonstrated that aHUS-associated *CFB* mutations are exclusively gain-of-function mutations that result in enhanced formation of the C3bBb convertase [134, 135]. *CFB*-mutated patients exhibit a permanent activation of the alternative pathway with very low C3.

Out of 9 *CFB* rare variants characterized using functional *in vitro* assays, only 5 revealed a gain-of-function phenotype; the other variants are classified of undetermined significance [134].

Combined Complement Gene Mutations

Only 8–10% of patients with mutations in *CFH*, *C3*, or *CFB* had combined mutations, whereas approximately 25% of patients with mutations in *MCP* or *CFI* had combined mutations [53].

Mode of Inheritance and Penetrance

Twenty to 30% of patients have a familial history of aHUS. More frequently the disease is sporadic with only one case per family. However, *de novo* mutations are exceptional [135]. Among pedigrees with familial aHUS, transmission of the disease is autosomal recessive in cases with homozygous or compound heterozygous mutations in *CFH* or *MCP*. Transmission is autosomal dominant in cases with a heterozygous mutation. Disease penetrance in family members who carry the heterozygous mutation has been evaluated to be approximately 50%, as only half of these subjects develop the disease. This may be an overestimate, due to the issue of reporting bias, since pedigrees with more that one affected individual are more likely to be studied than those with just one patient. The identified mutation therefore appears to be a risk factor for the disease rather than its direct and unique cause and aHUS has to be regarded as a complex polygenic disease which results from a combination of genetic risk factors. Homozygous haplotypes (defined by five frequent genetic variants transmitted in block) in CFH (at risk CFH tgtgt), MCP at risk (MCP ggaac) [136] and CFHR1*B allele [137] have been demonstrated to be more frequent in patients with aHUS than in controls. In addition, precipitating events or triggers appear required for the disease to manifest in patients genetically at risk.

Complement Alternative Pathway: From Gene Change to TMA Lesion

Mutations in the genes *CFH*, *MCP* and *CFI* impair the mechanisms that regulate AP activation and gain of function variants in C3 and CFB increase AP activation. Whatever the pathogenic variants identified, endothelial cells are no longer protected from complement activation [138, 139]. The increased production of MAC at the endothelial cell surface induces alterations of these cells, which become procoagulant by producing high molecular weight multimers of von Willebrand Factor, thus triggering the formation of thrombi [140–142]. In addition, complement activation at the surface of platelets triggers platelet activation and aggregation and this contributes to the formation of thrombi within the microcirculation [143]. This physiopathological model is corroborated by transgenic animal models. Mice which express a *FH* variant lacking the C-terminal 16–20 domain responsible for the interaction of FH with C3b and the endothelium develop HUS similar to the human disease [144].

Variants of Unknown Significance in Complement Genes: Disease Relevant or Benign?

Over the coming decade, the challenge will be to optimize and to implement at scale, strategies that use human genetics to further the understanding of disease, and to maximize the clinical benefit of those discoveries. The modern genetic screening test to identify genetic abnormality in aHUS patients includes next generation sequenc-

ing (NGS) with at least a panel of 5 genes (*CFH*, *CFI*, *MCP*, *C3* and *CFB*), Sanger sequencing and MLPA with an interpretation of the clinical consequences.

Not all detected complement gene variants have clinically relevant consequences. The standards and guidelines published in 2015 by the American College of Medical Genetics (ACMG) lay out an extensive framework of evidence for interpretation of sequence variants, including guidance for using population data and computational and predictive tools. The variants are classified along a gradient ranging from those that almost certainly have a pathogenic role to those that are very likely benign. However functional characterization of aHUS associated *FH* variants reveals limitations of routinely used variant classification methods. Access to resources that catalogue genetic variation across populations (such as gnomAD) has enabled the confident exclusion of genetic variants too common in population-level data to be plausible causes of rare diseases. As a general rule, variants with a MAF <0.1% might be considered relevant for the pathogenesis of aHUS or other complement-mediated disorders.

In 2021 this rule cannot be applied for variants in the complement genes. Genetic data are now available for >140,000 individuals from diverse populations in the Genome Aggregation Database (gnomAD). These data indicate that rare variants in the five complement genes with MAFs of <0.1% are present in 3.7% of healthy individuals and pathogenic variants can be found in 1% of samples of DNA from healthy blood donors. Only 9 out the 15 genetic changes in *CFB* identified in patients with aHUS led to functional activity changes compared to the wild-type protein [134]. Furthermore, only 29 of 79 rare variants in the *CFH* gene with a MAF <0.1% that have been identified in patients with aHUS are classified as pathogenic based upon the demonstration that they impair CFH regulatory activity [145]. The classification of complement gene variants relies on tools that help predict the potential pathogenicity of a variant [146].

In clinical practice, analysis of functional alterations in complement proteins takes into account the level of expression of the encoded protein (in plasma for CFH and CFI and at the granulocyte surface for CD46), the impact of the variant on the function of the encoded protein (assessed using *in vitro* assays and prediction of the pathogenicity of a variant based on functional domains) and *in silico* analyses. Establishing a causal relationship is difficult with the lack of experimental data. According to ACMG guidelines, more frequently the variants have only moderate evidence for pathogenicity and the variant will be classified as a variant of undetermined significance VUS). The current classification of complement gene variants is not optimal and the clinical relevance of individual variants should therefore be regularly re-evaluated.

In summary, not all detected gene variants have clinically relevant consequences. In practice, where a VUS is found in a complement gene of a patient with aHUS, it is important that other causes of a TMA are still excluded, rather than attributing causality. In addition, it is important not to screen family members for the presence of a VUS, since this could attribute risk where none exists or conversely, falsely reassure when risk still exists.

Genetic Abnormalities in Genes Not Related to Complement

Diacylglycerol Kinase ε Mutations

Diacylglycerol kinase ε (DGKε) is an intracellular lipid kinase highly expressed in glomerular capillaries, podocytes and platelets of healthy mice and humans. DGKs are enzymes that phosphorylate diacylglycerol molecules to phosphatidic acid. Using exome sequencing, deficiency in DGKε was established as a novel cause of pediatric onset aHUS in 2013 [45]. Subsequent to the first publication of 13 aHUS children from 9 kindreds, 6 new cases from 4 kindreds have been identified [58, 59], followed by a third cohort with clinical information on 15 patients based on data from the UK National Renal Complement

Therapeutics Centre including patients from UK, United Arab Emirates and New Zealand [46]. This cohort also established the presumed prevalence and incidence of DGKε-aHUS in the UK population at 0.009 per million per year, when the incidence rate of complement-mediated aHUS was 0.47 per million per year [46]. The phenotypic spectrum and outcome of *DGKE* disease was reviewed by Azukaitis et al. in a global cohort of 44 (including 10 previously unpublished) cases [60].

Transmission of *DGKε* mutations follows an autosomal recessive pattern and all patients reported to date carry homozygous or compound heterozygous nonsense, splice site or frameshift mutations. A likely explanation for the pathogenesis of *DGKε* mutations is that the loss of DGKε enhances protein kinase C activation in endothelial cells, platelets and podocytes, which may result in upregulation of prothrombic factors and platelet activation and altered podocyte function [45, 147]. However, the pathophysiological mechanisms of DGKε nephropathy have not yet been fully understood.

The aHUS relapses are clustered in early life and appear to be less prevalent later. In addition to presentation with aHUS, patients carrying *DGKε* mutations can also present with proteinuria without aHUS or steroid resistant nephrotic syndrome with MPGN pattern on biopsy. The symptoms can overlap in individuals, when in early life patients present with nephrotic range proteinuria and relapsing course of aHUS progressing further to chronic kidney disease (CKD) later in life with proteinuria, microscopic hematuria and hypertension. Neither the UK nor the global cohort found predictors that increase the risk of reaching end stage kidney disease or evidence of a significant role for complement activation on progression and relapses of *DGKε*-aHUS [46, 60]. These resolve regardless of therapy including complement blockade by eculizumab. Moreover, there were aHUS episodes or relapses during treatment with complement blocking therapy. Therefore, complement blocking therapy is probably not beneficial in this specific cohort of aHUS and patients should be managed supportively and in those already on eculizumab, withdrawal should be considered. DGKE nephropathy appears to take a slowly progressive course; only 20% of patients reach ESRD within 10 years of diagnosis [60]. There are no reports of DGKE nephropathy recurrence after transplantation (6 transplant cases reported as of October 2019) [46, 60]; therefore, individuals who progress to end-stage kidney disease (ESKD) should undergo kidney transplantation without the need for preemptive eculizumab [46, 60].

Cobalamin C Metabolism Defect Related HUS

A defect in the remethylation pathway caused by cobalamin C deficiency can lead to a clinical presentation very similar to HUS. It can present fulminantly in the neonatal period or later in life. The triad of HUS is also accompanied by other metabolic symptoms like delayed development and growth, seizures, hypo or hypertension and leucopenia. The inheritance is autosomal recessive, and it is usually caused by a defect in the *MMCHC* gene.

The major markers are elevated homocysteine and methylmalonic acid levels in plasma. Levels of homocysteine over 50 μM/L with normal levels of vitamin B12 and folate are pathognomonic. TMA in cobalamin C deficiency is believed to be caused by the endothelial toxicity of high plasma homocysteine levels.

Treatment is by loading dose of intramuscular vitamin B12 (hydroxycobalamin) followed by lifelong supplementation [110, 121]. Although rare, Cblc deficiency should not be missed since the prognosis of undiagnosed patients is dismal, it can easily be treated once detected. Hence, plasma homocysteine should be included in the routine diagnostic panel of aHUS.

Rare Variants in Genes with Debatable Clinical Relevance

Genetic defects in *THBD*, which encodes thrombomodulin, a protein that interconnects the coagulation cascade and complement system, have been suggested to contribute to the pathogenesis of aHUS. Few mutations in *THBD* affecting the

functions of the protein have been identified to date, with a frequency varying from 0 to 5% of all aHUS cases [2, 3, 44] (Table 22.3). Burden or aggregate association tests, in which all rare variants affecting the same gene are combined into one test, are used to increase the statistical power for rare variant association. Although rare variants in *THBD* have been reported in 41 patients with aHUS, their frequency is not significantly higher in these patients than in controls the general population [148]. Therefore, the link of *THBD* with aHUS remains debatable.

The potential clinical relevance of rare variants in genes such as *PLG* (which encodes plasminogen) [149], *INF2* (which encodes inverted formin 2) [150], *VTN* (which encodes vitronectin) [148] and *CLU* (which encodes clusterin) [151] identified in patients presented with some features of HUS warrant further assessment.

Acquired Complement Abnormalities in Atypical HUS

Anti-factor H Autoantibodies

Anti-factor H (anti-FH) autoantibodies are identified in 5–11% in European aHUS cohorts and in about 20% in Asian aHUS cohorts [65, 76, 79]. Interestingly, they are identified in more than 56% cases from India [80]. A Czech cohort showed a rather outlying large proportion of anti FH antibodies in comparison to other European cohorts of aHUS at 61% which could be due to small sample size and sampling method [67].

Anti-factor H autoantibodies associated HUS (anti-FH HUS) usually manifests later than genetic types of aHUS caused by factor H mutations, usually between 5 and 15 years of age [77]. An international aHUS registry reported a median of age of 13.1 (6.1–31.3) years at presentation [81] for this group of patients. However, the youngest reported aHUS patient with anti-FH antibodies identified was younger than 1 year and the oldest reported patient was over 75 years old. Most of the published series show a slightly higher prevalence of anti-FH HUS in males.

Anti-FH antibodies bind mostly to SCR 19 and 20 of FH but also to other epitopes of FH and thus inhibit the majority of regulatory functions of FH at cell surfaces [153]. Plasma C3 level is decreased in 40–60% of patients with anti-FH antibodies during the acute phase, while FH levels are decreased in only approximately 20% of patients [62]. C3 levels are significantly lower in patients with very high anti-FH antibody titer.

Ninety percent of patients with anti-FH antibodies have a complete deficiency of *CFHR1* and *CFHR3* due to a homozygous deletion of *CFHR1-R3*, a polymorphism carried by 2–9% of European, 16% of African and ≤2% of Chinese healthy controls [128, 154]. The reason why individuals with *CFHR1-R3* deletion develop anti-FH antibodies is uncertain. The current theory linking the deletion in CFHR1 with the generation of antibodies is based upon the interaction of the FH protein that is used by pathogens for immune evasion. In individuals with *CFHR1 gene* deletion, CFHR1 protein is recognized as foreign by their immune system. When a CFHR1-deficient individual is infected by an organism that can bind CFHR1, CFHR3 and FH proteins, the FH protein is changed by the infectious organism to resemble CFHR1 and host immunity mounts a response, leading to production of anti-FH inhibiting FH, thus leading to endothelial dysfunction and symptoms of aHUS. This is corroborated by structural differences found between CFHR1 and FH [155].

Fujisawa et al. described three aHUS patients where anti-FH antibodies affected platelets directly [82]. Washed platelets aggregated more when in contact with plasma from these patients compared to plasma from healthy controls or from aHUS patients with complement genetic variants.

Anti-factor I Antibodies

Two cases of anti-FI antibody-associated HUS have been reported to date [156]. The clinical relevance of these antibodies is difficult to establish, also given their extreme rarity.

Outcome of Atypical HUS Prior to the Availability of C5 Inhibition Therapy

In the era before eculizumab became available the death rate in children with aHUS was 8% [5], 9% [4] and 14% [2] in three pediatric series at average follow-up times of 3.8, 7.5 and 3 years, respectively. Most deaths occurred in children less than 1 year of age and at first episode or during the first year after onset. Approximately 20% of children progressed to ESKD or died at first episode or within <1 month after onset, 30% within 1 year and 40% at 5 years follow-up. The most severe outcome was in children with *CFH* mutations, of whom one third progressed to ESKD or died at first episode, half at 1 year and two thirds at 5 years follow-up. The prognosis of *CFI* and *C3* mutation-associated HUS was hardly less severe than that of *CFH* mutation-associated HUS. *MCP* mutation-associated HUS in children had the best prognosis, with an ESKD risk of 25% at median follow-up of 18 years. aHUS in children with no complement mutation identified also had a relatively favorable outcome [5].

Lastly, the outcome of anti-FH antibodies associated HUS was poor when treatment was limited to PE, including death in 10% of patients, CKD in 40% and ESKD in one third at mean follow-up 39 months [62, 152]. Early treatment with a combination of PE, corticosteroids and immunosuppressants allowed a more favorable outcome, similar to that of *MCP* mutation—associated HUS [5, 64].

Less than 10% of children with *DGKE*-aHUS progress to ESKD in the first year after onset, but patients with this form of aHUS develop proteinuria and nephrotic syndrome, severe hypertension and progress to CKD stages 4–5 (eGFR 15–29 mL/min/1.73 m^2 or ESKD) between the age of 20 and 25 years [45, 46, 60].

In the pre-eculizumab era, several series suggested that approximately half of children with aHUS experienced relapses [4, 5]. Among children who had not died or reached ESKD at first episode or at 1 year follow-up, 25% had relapses during the first year and 47% after the first year. However, a high relapse rate after the first year was mostly in patients with *DGKE* (83% during the first year, 50% up to 5 years) or *MCP* mutations (25% during the first year, 92% after the first year), while relapse rate after the first year was 20–30% in other genetic subgroups [5, 45, 46, 60]. Despite this risk of relapses in the long term, *MCP* mutation-associated HUS has the best prognosis in children, as indicated above. Last, anti-FH HUS had a relapsing course in two third of patients when untreated or treated only with PE/PI [62, 65], which was reduced to approximately 10% by early treatment combining PE + immunosuppressants + corticosteroids [5, 64].

Treatment of Atypical HUS Prior to the Availability of C5 Inhibition Therapy

Plasma Therapy

PE (or PI when PE was not possible) was first line treatment for aHUS until recent year [12] Approximately 15 case reports, mostly in children with *CFH* mutation, showed that early, intensive plasmatherapy, followed by maintenance PE/PI, could prevent relapses and preserve kidney function at follow-up up to 6 years [8, 9, 157]. However, although plasmatherapy was associated with complete or partial remission (hematologic remission with kidney sequels) in approximately 80% of aHUS episodes in children, half of them had died or reached ESKD at 3 years follow-up [2]. In addition, plasmatherapy carried significant morbidity. An audit of complications in children receiving PE for aHUS revealed 31% developed catheter-related complications (including infection, thrombosis and hemorrhage) and 11% developed plasma hypersensitivity [13]. The benefit of PE/PI is uncertain in *DGKE* mutation-associated HUS, as proteinuria, the main marker of a progressing course in *DGKE* mutation–associated HUS, persisted in 9 of the 12 patients who received plasmatherapy [45, 46, 60].

Kidney Transplantation

The risk of post-transplant recurrence of aHUS was 60% in the pre-complement blockade era [2, 104]. Forty percent of recurrences occurred during the first month after transplantation and 70% during the first year. Graft survival was 30% at 5 years follow-up in patients with recurrence, compared to 68% in those without recurrence [104]. Eighty percent of patients who had lost a prior graft from recurrence had recurrence after retransplantation. The main independent risk factor for recurrence was the presence of a complement mutation. The highest risk (approximately 80%) was in patients with *CFH* and *C3* or *CFB* gain of function mutation, the risk in patients with *CFI* mutation was approximately 50% and patients with no complement mutation identified had the lowest risk (approximately 20%) [104]. The risk of post-transplant recurrence in patients with *MCP* mutation has been shown to be low (<10%) if the mutation is isolated (the graft brings the non-mutated MCP protein), while it is approximately 30% if the *MCP* mutation is associated with a mutation in *CFH*, *CFI* or *C3* [53]. No post-transplant recurrence was observed in four patients with *DGKE* mutations [45, 46, 60]. The risk is low in anti-FH HUS if the antibody titer is low at the time of transplantation, while substantial if elevated [62, 65, 66] [128, 158]. One patient with *THBD* mutation has been reported to have had post-transplant recurrence [159].

This shows that genetic screening is necessary before listing a patient for kidney transplantation to predict the risk of post-transplant recurrence and guide decisions for the choice of the donor and the prevention of recurrence.

PE/PI for post-transplant recurrence generally failed to avoid graft loss [2, 104]. Therefore, prophylactic PE/PI was recommended [160]. The efficacy of this strategy is poorly documented. However, graft survival rate free of recurrence was significantly higher in nine patients who received prophylactic PE/PI than in 62 patients without prophylactic PE/PI [104]. Interestingly, calcineurin inhibitors (CNI) did not significantly increase the risk of recurrence in a recent series, while mTOR inhibitor use was an independent significant risk factor for recurrence, possibly related to the anti-VEGF (vascular endothelium growth factor) action of these drugs [104]. The current consensus is that aHUS is not per se a contraindication to CNI. Strict monitoring of blood levels and overdosage avoidance is recommended, while CNI-free mTOR based immunosuppressive regimens should be avoided [161].

Treatment Recommendations

During the initial manifestation of HUS, unless it is a relapse in a patient already know to carry a risk variant in genes associated to aHUS, diagnosis is challenging, and aHUS cannot be excluded purely on clinical grounds or complement markers. Children with suspected aHUS should ideally be transferred to a children's kidney center capable of kidney replacement therapy and intensive care. In contrast to adults, and because the incidence of acquired TTP in children is very low, immediate PE whilst awaiting ADAMTS13 results is not routinely recommended. Children in whom complement driven aHUS is strongly suspected or proven should receive eculizumab (see below) as first line treatment, to avoid PE and the complications of central catheters [103]. Confirmation of a complement mutation is not required for the decision of treatment initiation in such cases. As treatment delay may affect recovery of kidney function, eculizumab treatment should be initiated as soon as possible, ideally within 24–48 h of onset or admission. In addition to targeted treatment with eculizumab, symptomatic care is based on general recommendations for AKI [162] and on consensus from observational studies. The cornerstone is appropriate fluid management, kidney replacement therapy in patients with high urea or unsafe electrolyte profile and stopping of nephrotoxic drugs. Red blood cell transfusions should be given to patients who have symptoms of severe anemia or when hemoglobin falls rapidly. Platelet transfusions are not advised unless patient has a life threating bleeding or requires invasive procedure like placement of vascular catheter for adequate dialysis.

Eculizumab

Eculizumab, a monoclonal humanized anti-C5 antibody, prevents C5 cleavage and the formation of C5a and C5b-9, thus blocking the C5a pro-inflammatory and the C5b-9 pro-thrombotic consequences of complement activation. It has been the accepted treatment of paroxysmal nocturnal hemoglobinuria (PNH), another complement dependent disease, for more than 15 years [163].

The rationale for treatment with complement C5 blockade was first proposed based on first two aHUS patients treated successfully with eculizumab in 2009 [164, 165], followed by plethora of successful cases [15, 85, 87, 89–91, 166–170].

Four observational prospective single-arm non randomized multinational trials [14, 171, 172] demonstrated the efficacy of eculizumab to stop the TMA process, allowing sustained remission of aHUS with improved or preserved kidney function in the majority of patients. One of these trials [172] specifically studied eculizumab in children with aHUS. Figure 22.6 shows the remarkable efficacy of eculizumab in these patients. Data from the trials also suggested that an early switch from PE/PI to eculizumab or the use of eculizumab as first line therapy gave patients the best chance of full recovery of kidney function. Treatment was well tolerated, with no increase of adverse events over time. However, two of the 100 patients who entered these trials developed meningococcal meningitis. Eculizumab is administered by intravenous infusion according to the weight-directed dose and schedule shown in Table 22.4.

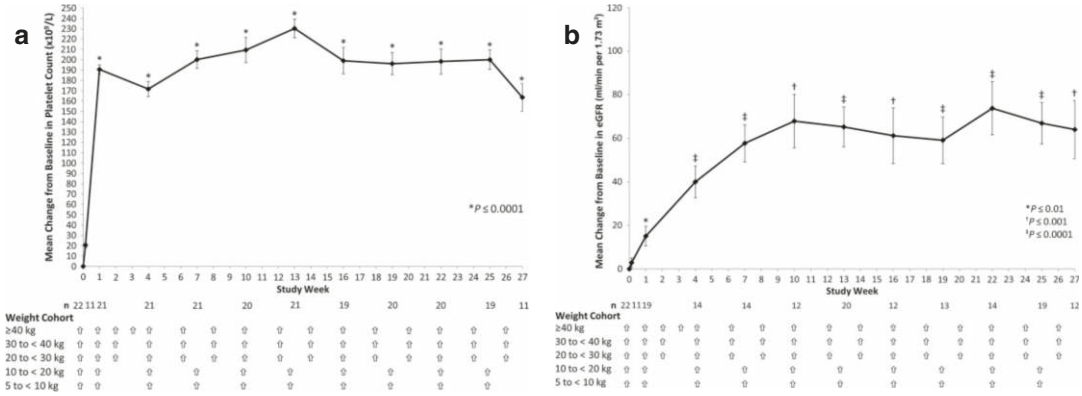

Fig. 22.6 (**a**) Improvement in platelet count over 27 weeks of eculizumab treatment in 22 children with aHUS. N values <5 were not included. Bars represent standard error of the mean (SEM). (**b**) Improvement in estimated glomerular filtration rate (eGFR) over 27 weeks of eculizumab treatment. N values <5 were not included. Bars represent SEM. Arrows denote administration of eculizumab

Table 22.4 Dosing of eculizumab based on European Medicines Agency: Summary of product characteristics Soliris, https://www.ema.europa.eu/en/documents/product-information/soliris-epar-product-information_en.pdf, February 2022

		Initial dose	Maintenance dose
Adult dosing schedule (intravenous infusion)		900 mg every week for first 4 weeks	1200 mg at week 5 then 1200 mg every 14 days
Paediatric dosing schedule according to body weight (intravenous infusion)	>40 kg	Dose as per adult schedule	Dose as per adult schedule
	30–40 kg	600 mg every week for first 2 weeks	900 mg at week 3 then 900 mg every 14 days
	20–30 kg	600 mg every week for first 2 weeks	600 mg at week 3 then 600 mg every 14 days
	10–20 kg	600 mg first week only	300 mg at week 2 then 300 mg every 14 days
	5–10 kg	300 mg first week only	300 mg at week 2 then 300 mg every 21 days

Eculizumab is currently approved by both the Food and Drug Administration and the European Medicines Agency for the treatment of atypical HUS. The cost of the drug and the presumption that patients should receive lifelong treatment played a major role in approving the cover of the costs by healthcare systems. Access and funding are achievable in USA, Australia, United Kingdom and in most states of the European Union, with exception of Bulgaria and Romania. Other countries with availability include Israel and Japan. African and Asian countries, including China and India, have no access to the drug. The availability generally corresponds to the countries' economic position (apart from New Zealand where access is very limited).

Ravulizumab

A second generation complement inhibitor, ravulizumab, has recently been developed by targeted re-engineering of eculizumab. Two structural changes were incorporated, aimed at extending the terminal half-life. The first change enhanced the dissociation rate of the mAb:C5 complex at pH 6.0, eliminating the target mediated antibody clearance. The second change enhanced the affinity of the antibody to human neonatal Fc receptor [173].

Ravulizumab has not been directly compared to eculizumab in a clinical trial. However, efficacy and safety were confirmed in a phase 3 single-arm trial in adult patients (n = 56) with aHUS naïve to complement inhibitor treatment [174]. Complete TMA response, defined as normalization of platelet count and LDH and ≥25% improvement in serum creatinine, was achieved in 53.6% of patients in the 26 week study period. This lower response rate than reported within the eculizumab trials may have been due to broader eligibility criteria for recruitment (only 20.5% had genetic complement mutations or anti-FH identified compared with 76% in the equivalent eculizumab trial). There were no severe treatment-related events reported. Four deaths were reported, none of which were considered treatment-related by the study investigator [175].

Two trials tested the efficacy and safety of ravulizumab in children under 18 years of age with aHUS. Fourteen of 18 complement-inhibitor naïve patients with aHUS (78%), achieved complete TMA response with ravulizumab. Ten aHUS patients who were already receiving eculizumab, switched to ravulizumab for a period of 26 weeks without significant safety issues and showed unchanged kidney function and hematological remission of aHUS even after extended observation of one year. No unexpected adverse events, deaths, or meningococcal infections occurred. There were not enough data to demonstrate the effectiveness of ravulizumab in children weighing less than 10 kg [176, 177].

Taken together, these trials indicate that ravulizumab, is effective for the long-term treatment of patients with aHUS. Current evidence suggests an acceptable safety profile, although longer-term surveillance will be required. The main risk of ravulizumab treatment is similar to that of eculizumab arising from the same principle of C5 blockade. Therefore, all patients must strictly adhere to the same prevention protocols against meningococcal infection as with eculizumab. Dose and schedule information for ravulizumab are shown in Table 22.5.

There are now two licensed treatments for patients with aHUS. Clinicians and patients now have a choice between short-acting and long-acting C5 inhibition. Since the diagnosis of aHUS is complex and not all patients initiated on C5 inhibition will continue with long-term therapy, it may be an option to commence initial treatment with eculizumab, with a switch to long-acting therapy once a need for long-term treatment is established (Fig. 22.7). This approach would minimise the risk of prolonged C5 blockade for those in whom treatment is discontinued due to an alternative diagnosis (for example, a subsequent positive STEC result). However, commencing with ravulizumab at time of initial presentation is also an option.

A subcutaneous ravulizumab formulation is currently undergoing evaluation in a phase 3 trial in adult patients with PNH [177] and may be a future option also for patients with aHUS.

Table 22.5 Dosing of ravulizumab based on European Medicines Agency: Summary of product characteristics Ultomiris, https://www.ema.europa.eu/en/documents/product-information/ultomiris-epar-product-information_en.pdf, February 2022

		Initial dose	Maintenance dose
Adult dosing schedule (intravenous infusion)	≥40 to <60 kg	2400 mg loading dose followed by maintenance dose in 2 weeks	3000 mg every 8 weeks
	≥60 to <100 kg	2700 mg loading dose followed by maintenance dose in 2 weeks	3300 mg every 8 weeks
	≥100 kg	3000 mg loading dose followed by maintenance dose in 2 weeks	3600 mg every 8 weeks
Paediatric dosing schedule according to body weight (intravenous infusion)	>40 kg	Dose as per adult schedule	Dose as per adult schedule
	30–40 kg	1200 mg loading dose followed by maintenance dose in 2 weeks	2700 mg every 8 weeks
	20–30 kg	900 mg loading dose followed by maintenance dose in 2 weeks	2100 mg every 8 weeks
	10–20 kg	600 mg loading dose followed by maintenance dose in 2 weeks	600 mg every 4 weeks

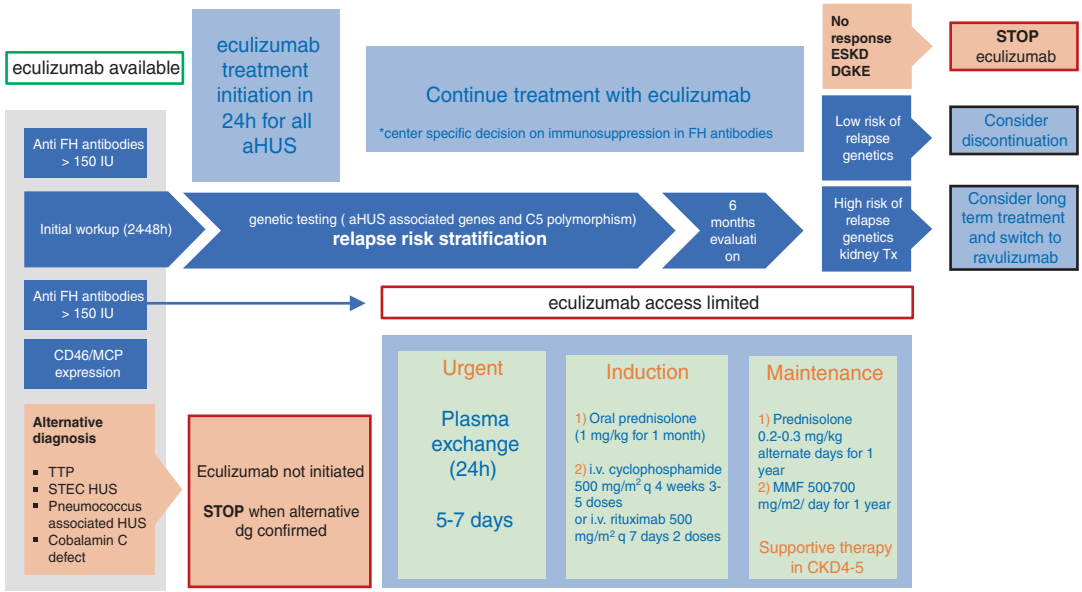

Fig. 22.7 Recommended management approach when atypical HUS is suspected

Risk of Meningococcal Infection on C5 Inhibition Therapy and Its Prevention

The host defense against *Neisseria meningitis* depends on the lytic terminal complement complex C5b-9. The incidence of meningococcal infections in patients with hereditary complete deficiency of terminal complement factors is 0.5% per year, corresponding to a 1000 to 2000-fold relative risk increase compared to the normal population [178]. Patients undergoing eculizumab or ravulizumab treatment have the same risk as these patients. Therefore, prevention of meningococcal infection is crucial, relying on vaccination and antibiotic prophylaxis. Tetravalent conjugated vaccines protect against serogroups A, C, W135 and Y. Polysaccharide

vaccines against serogroup B are also available and recommended for all patients receiving eculizumab.

The frequency of invasive meningococcal infection has been approximately 0.5/100 patient years in patients with PNH treated with eculizumab, despite meningococcal vaccination (not anti-B) [179].

Two of the 100 aHUS patients treated within trial protocols and one among approximately 80 case reports developed invasive meningococcal infection despite being vaccinated [180]. The more recent analysis of eculizumab safety from the Global Atypical HUS registry showed meningococcal infection in two adult cases (0.17 per 100 patients years) and one pediatric patient (0.11 in 100 patient years) out of 1321 patients ever treated with eculizumab. Two patients recovered completely, and one case was fatal [181].

Whilst long-term antibiotic prophylaxis for patients on C5 inhibition is not mandated by the manufacturer, many clinicians add this to their patients' treatment (phenoxymethylpenicillin or erythromycin if penicillin allergic). This is strongly recommended for children and in some countries, this is obligatory [103]. Neither vaccines nor antibiotic prophylaxis guarantee full protection, hence the importance of patient and family education on signs of meningococcal infection and the provision of alert cards to be carried by the patient or their caregiver to present to their health care provider when unwell, in order to minimize delay in recognition.

Treatment of Anti-factor H Antibody Associated HUS

The first large series of patients with anti-FH HUS (treated mostly with PE without immunosuppressants) suggested that many of these patients suffer a relapsing course, leading to end stage kidney disease [62]. A much more favorable outcome, similar to that of *MCP* mutation-associated HUS, has been reported in children with this form of HUS, treated early with PE, immunosuppressants and corticosteroids [5].

The largest experience with the approach to decrease anti-FH antibodies with immunosuppressive treatment comes from India, where 55.8% of their 781 aHUS patients presented with anti-FH antibodies [66].

Combined Plasma exchange (PE) and immunosuppression improved long-term outcomes (HR 0.37; $P = 0.026$); maintenance therapy with corticosteroids and MMF reduced the relapse risk (HR 0.11; $P < 0.001$) [66].

Maintenance treatment with corticosteroids and mycophenolate mofetil (MMF) or azathioprine significantly decreased the risk of relapses, from 87% to 46% at last follow-up [64, 157].

Eculizumab was documented as an effective treatment of HUS symptoms in anti-FH HUS in several cases [88, 182].

In a cohort of 17 patients with anti-CFH antibody associated aHUS, four patients were treated with first-line eculizumab rather than PE. Patients treated with eculizumab achieved remission in 100% of cases, whereas treatment with PE and immunosuppression was associated with a poor rate of renal recovery in 7 of 11 treated. Therefore, treatment with eculizumab did not appear inferior to PE and immunosuppression [183]. This gives patients and clinicians different treatment options with different adverse effect profiles—broad immunosuppression with rituximab or cyclophosphamide, or more targeted treatment with eculizumab [103]. The National Renal Complement Therapeutics Centre in the UK recommends initial treatment with eculizumab for anti-FH HUS based upon a more favorable adverse effect profile .

Global Variations in Atypical HUS Treatment Recommendations

Eculizumab has revolutionized the management of aHUS. The current best recommendations and guidelines are challenging to implement globally, particularly in resource-limited healthcare settings due to the prohibitive cost of eculizumab and its successor ravulizumab. In response to this, the Indian Society of Pediatric Nephrology

have published consensus guidelines for countries lacking access to eculizumab and complex diagnostic facilities [157].

These pragmatic guidelines are mirroring the best recommendations from the pre-eculizumab era [64]. (See also Fig. 22.7).

They recommend treating aHUS where anti-FH antibodies are not suspected with prompt initiation of PE using fresh frozen plasma (The dose is recommended as 1.5 times of the plasma volume or 60–75 mL/kg) and repeated daily until hematological remission is achieved (platelets over 100 × 10^9/L, schistocytes under 2% and LDH in normal range). The tapering follows after 5 days of daily PE or when remission is achieved followed by alternate days PE and 2–3 weeks of twice a week PE.

In case of positive anti-FH antibodies, additional immunosuppression is administered, starting with prednisolone 1 mg/kg daily for 4 weeks followed by alternate day dosing for another 4 weeks and tapering down for 1 year. Cyclophosphamide (500 mg/m^2 q 4 weeks, 3–5 doses) or Rituximab (500 mg/m^2 q 7 day, 2 doses) are given to further decrease the production of antibodies. Mycophenolate mofetil (500–750 mg/m2/day) or azathioprine (1–2 mg/kg/day) are used as additional long-term immunosuppression [157].

C5 Inhibition Therapy Monitoring

Data from the prospective studies show that complete complement blockade is obtained within 1 h after the first dose of eculizumab and is maintained long-term in patients receiving the recommended treatment schedule [184]. The role of assessment of complement blockade during routine use of eculizumab is uncertain. If there are features of inadequate disease control, this should be assessed. Incomplete blockade may be due to insufficient dose (especially in children with a weight just below a weight threshold requiring a higher dose) or heavy proteinuria with nephrotic syndrome (leakage of the drug in the urine). A genetic cause might also have to be considered in aHUS patients of Japanese or Asian origin with poor response to eculizumab and/or complement non-blockade, such as the C5 variant which impairs the binding of eculizumab to C5 [185]. For the long term, complement blockade assessment is mostly required in cases of apparent resistance to eculizumab, including relapse of HUS but also isolated abnormalities in platelet count, LDH and/or haptoglobin levels, appearance or increase of proteinuria or serum creatinine, especially if kidney biopsy suggests ongoing TMA.

Currently available assays of complement blockade under eculizumab are a CH50 or other hemolytic-based assays or the Wieslab Complement System [186]. Due to the site of action of eculizumab, low C3 levels as seen in some mutations are not expected to normalize under eculizumab, and this has been observed [125]. Soluble C5b-9 remains detectable or increased in aHUS patients under eculizumab and therefore cannot be recommended to monitor the efficacy of eculizumab [125, 187]. Most aHUS patients treated within the prospective trials who received the protocol schedule had suppression of CH50 activity and eculizumab trough levels ≥150 μg/ml [14]. However, the correlation between drug levels and complement activity in aHUS patients is not fully established and the availability of plasma eculizumab measurement is currently limited. An additional consideration is that hemolytic assay monitoring may be less accurate for ravulizumab than for eculizumab. One recent study showed that full inhibition of CH50 was not achieved despite high levels of ravulizumab present in plasma [188].

Some authors have reported using assays of hemolytic activity as a tool to lengthen the interval between doses, in an attempt to reduce cost and infusion burden [189]. This approach is not recommended by the manufacturer. It has been recommended that increasing the interval between doses should be considered only in patients who maintain CH50 activity <10% despite longer intervals or lower doses [186].

Duration of C5 Inhibition Therapy in Children with aHUS in Their Native Kidneys

Lifelong treatment with inhibition of C5 for patients with aHUS has been the accepted paradigm. However, there is no definitive evidence for persisting use. The recommendation of not stopping C5 blockade is being reconsidered for some patients with aHUS in their native kidneys based on estimating the risk of relapse by genetic testing.

Having a safe strategy for discontinuation of treatment would be beneficial not only for reducing the significant cost of the therapy but also for safety of the patients and the long-term quality of their life.

Even in the pre-eculizumab era, not all patients relapsed after successful PE. A report of 214 patients, of which 146 were treated with PE, showed that 42% of children were relapse free after 5 years of observation [5]. A Dutch cohort reported sustained remission in 25% of patients [4] and even a cohort of patients with aHUS with changes in the *CFH* gene had normalized kidney function in 22.5% [190]. Following several case reports and series of successful eculizumab discontinuation [170, 189, 191–193], more evidence to support the safety of eculizumab withdrawal appeared from larger studies. The report on this topic from the Global aHUS Registry showed that 33/151 (22%) patients experienced TMA recurrence, particularly in those with pathogenic variants in genes associated with aHUS or with a family history of aHUS. Eight percent then progressed to end-stage kidney disease and 5% required subsequent kidney transplant [194]. During an unprecedented event between 2016 and 2019 when distribution of eculizumab supply was disrupted in Brazil, the effect of discontinuation without stratifying patients based on genetic risk was demonstrated. There were 11 relapses in a cohort of 24 patients, of which 8 occurred in patients with a *CFH* gene variant [195].

A prospective, single-arm study conducted in France stopped eculizumab after at least 6 months of treatment in 55 patients of which 19 were children and showed an overall aHUS relapse rate of 23% (n = 13). Twenty-eight patients (51%) had rare variants in complement genes (*MCP* n = 12, *CFH* n = 6, *CFI* n = 6, FH antibodies, n = 4). Six out of 19 children (32%) relapsed [196]. All 13 patients who relapsed were carrying a variant in genes associated with aHUS except one that was subsequently found to have hereditary ADAMTS13 deficiency (and therefore did not have aHUS). C5 blocking therapy was restarted in all 13 patients. Eleven returned to their baseline kidney function and two remained with decreased function, one progressing to ESKD. Therefore, prompt identification of relapse in aHUS patients who stopped C5 blocking therapy seems to be crucial for preventing long term kidney damage [196].

The overall evidence suggests that patients with no mutation in known complement genes who achieve stable remission for at least 6 months are at low risk of relapse after stopping the treatment. Patients with mutations relapse at much higher rate and in this situation C5 blocking therapy must be restarted promptly to prevent loss of kidney function.

Further safety data for the withdrawal of C5 inhibition in patients with aHUS are required, particularly for patients with mutations. An ongoing trial of stopping eculizumab in patients with aHUS in UK (SETS, EudraCT Number**:** 2017-003916-37) [197] aims to corroborate the current evidence for safe stopping. If all evidence supports safe withdrawal of C5 inhibition in patients with aHUS, the challenge for patients and clinicians will be to implement individualized care plans to enable rapid detection of signs of relapse and facilitate immediate access to C5 inhibition when a relapse occurs. Without this in place, there is a risk that aHUS will cause ESKD.

Current evidence suggests that eculizumab can be withdrawn in a significant proportion of patients and pragmatic recommendations can be drawn from the experience and protocols used in the described trials. The prerequisite is that patient has achieved remission of HUS with no signs of TMA, normalization of platelets, LDH and stable kidney function. The duration from initiation of treatment to its discontinuation is not established, yet the arbitrary duration of 6 months

chosen for the trials could be used as base recommendation. Genetic testing and testing for anti-FH antibodies could help stratifying risk of possible relapse. Patients with no mutation and no anti-FH antibodies (or low titers) are at lowest risk of relapse. Patients with variants of unknown significance and those with pathogenic variants in *CFI* and *CD46* are making a middle tier with slight risk of relapse. Patients with anti-FH antibodies in high titres, pathogenic *FH, FB* and *C3* mutations are at highest risk and the discontinuation of treatment can likely lead to relapse. Most of the relapses would occur in the first weeks after discontinuation and patients need to be reviewed frequently, ideally weekly, with testing eGFR and blood count to identify relapse quickly and restart the treatment.

Further lifelong specialist follow-up of patients who discontinued complement blocking treatment, their education and providing them with straight pathway for review and restarting treatment in case of possible relapse is essential part of the process. There is limited data on another try to stop in case of relapse and restart and it is likely these patients will need lifelong treatment, ideally with long-acting C5 complement blocker, ravulizumab.

Specific patient group are the patients after kidney transplant, where approach needs to be individualized, based on weighing the benefits of continuous or restrictive therapy.

Kidney Transplantation for aHUS Patients Today

Complement blockade therapy has transformed the approach to kidney transplantation for aHUS patients and there are international consensus guidelines regarding the use of eculizumab in this situation (KDIGO 2017). The guidelines state:

1. Kidney transplantation should be deferred until at least 6 months after the start of dialysis because limited kidney recovery may occur several months after starting eculizumab
2. The decision to use anti-complement therapy during transplantation should be based upon recurrence risk
   (a) Those with a high risk (50–100%) of recurrence include patients with previous recurrence, pathogenic mutation or gain of function mutation. They should be treated with prophylactic eculizumab to start on the day of transplantation and to continue indefinitely
   (b) Those with moderate risk of recurrence include patients with no mutation identified, isolated CFI mutations, complement gene variants of unknown significance or persistently low titer anti-FH antibodies. They can be treated with prophylactic eculizumab or PE or without preventative strategy (left to the discretion of the clinician).
   (c) Those with low recurrence risk of recurrence (<10%) include isolated *MCP* mutation or persistently negative anti-FH antibodies. These patients do not require prophylactic treatment with PE or eculizumab. NB with subsequent data, DGKE HUS can also be viewed as low recurrence risk [46].
3. Living donor transplantation can be considered, with the decision relying on a careful assessment of the risk of aHUS in the donor after kidney donation. A risk assessment for potential living related donation requires full genetic screening of the recipient and the donor, leading to the following possible outcomes:
   (a) If the mutation found in the recipient is undeniably responsible for the occurrence of HUS (e.g., *CFH* mutation in C terminus SCR 19 or 20) and is not found in the donor, the risk of HUS is low for the donor and living-related donor transplantation can proceed.
   (b) If the donor has the same mutation as the recipient, the risk of HUS is present for the donor and living-related donor transplantation should not proceed.

(c) If the role of the variant found in the recipient is uncertain (not reported in databases, unknown functional consequences) the risk of HUS is intermediate for the donor, who may share with the recipient an unknown risk factor, and living-related donor transplantation is not advised.
(d) If no mutation is identified in the recipient and the donor, the risk to the donor is not quantified. KDIGO advises that if there is no evidence of alternate complement pathway activation in the donor, donation is feasible.

In general, additional endothelial damaging factors should be avoided, such as delayed graft function (prolonged ischemia time, non-heart-beating donor), cytomegalovirus infection, high levels of CNI and the association of CNI + mTOR inhibitors and hypertension.

Prior to the availability of eculizumab, isolated liver transplantation and combined liver-kidney transplantation were recognized treatment approaches for patients with mutations in FH, C3 and FB (all synthesized in the liver). These had relatively high morbidity and mortality (for example, 20% of patients who received combined liver-kidney transplant for aHUS died) [103, 198, 199]. Eculizumab represents a safer treatment option, but liver transplantation may still have a role when eculizumab is not available [161].

Potential Future Therapies

Current therapies are limited to monoclonal antibodies against C5. Numerous drugs that inhibit complement via different targets are undergoing clinical development, some of which may be suitable for use in aHUS. OMS721 (Narsoplimab) is a monoclonal antibody targeting mannan-binding lectin associated serine protease-2, the effector enzyme of the lectin pathway of the complement system and is currently undergoing a phase 3 clinical trial in adult patients with aHUS (NCT03205995. LMP023 (Iptacopan) is an oral FB inhibitor also undergoing a phase 3 clinical trial in adult patients with aHUS (NCT04889430) [102].

Conclusion

The journey to an effective treatment for aHUS is a striking demonstration that understanding the pathophysiology of a disease opens the way to new therapies. The demonstration that aHUS was mostly a disease of complement dysregulation paralleled the development of the anti-C5 monoclonal antibody eculizumab. Prospective trials as well as off-label clinical experience confirmed the efficacy of complement blockade to prevent progression to ESKD in aHUS patients and allowed successful kidney transplantation in those on dialysis. This new era in the field of aHUS emphasizes the need for an etiology/pathophysiology-based classification of the various forms of TMAs. Hopefully, new discoveries will identify the etiology of the 30% of aHUS cases that remain unexplained today, and the aHUS denomination will then disappear.

An important current question is that of the duration of eculizumab treatment, and prospective studies are underway to define whether discontinuation can be considered, in which patients, and when. Should withdrawal be shown as feasible in some patients, this would decrease the burden and risks of continuous treatment for patients, but also the cost for health care systems.

The addition of long acting ravulizumab to the treatment armamentarium for aHUS brings choices for patients and clinicians that may improve quality of life. Over the next decade, it is possible that further agents will be shown to be effective for the treatment of aHUS. Thus, future treatment of aHUS might comprise a decision about specific treatment and mode of delivery, followed by a decision regarding continuation or discontinuation of therapy with appropriate monitoring. Such options were not imaginable by aHUS patients 20 years ago.

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C3 Glomerulopathies

23

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Introduction

Membranoproliferative glomerulonephritis (MPGN) was originally described as a pattern of glomerular injury seen in patients with Bright's Disease [1]. By mid-1970, MPGN had become a disease of children with three primary subtypes sorted by electron microscopy presenting clinically with nephrotic syndrome and hypocomplementemia [2, 3]. But over the next 40 years, as it became associated with an ever increasing variety of diseases, each with different clinical presentations and etiologies, MPGN has returned to being a pattern that only vaguely points the way to a clinicopathologic diagnosis [4].

Due to the significantly increased understanding of the role of complement in glomerular diseases, two distinct entities have arisen from the MPGN morass, i.e. C3 Glomerulopathy (C3G) and Immune-complex mediated MPGN (Table 23.1). Glomerular diseases associated with abnormalities of the alternative pathway of complement are grouped under the term C3G [5]. There are two major subtypes, C3 glomerulonephritis (C3GN) and dense deposit disease (DDD) sorted by changes seen on electron microscopy. C3G is caused by various genetic abnormalities with or without the development of antibodies against different components of the complement pathway, so-called nephritic factors. Idiopathic MPGN also has two subtypes but these are separated by immunofluorescence findings on renal biopsy. Immune complex-mediated MPGN has dominant or co-dominant immunoglobulins with C3. There is also an immune negative variant of MPGN. The idiopathic forms of these two variants are extremely rare and a detailed search for an underlying cause is required [6]. However, otherwise idiopathic forms of MPGN may be due to abnormalities in the alternative pathway of complement, that is, C3G with immunoglobulin deposition.

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Table 23.1 Classification of IC-MPGN/C3G

	Characteristic findings in C3G	Characteristic findings in IC-MPGN
Light microscopy	– Membranoproliferative pattern – Mesangial glomerulopathy – Necrotizing and crescentic glomerulonephritis – Acute proliferative and exudative glomerulonephritis	– Membranoproliferative pattern – Mesangial glomerulopathy with endocapillary hypercellularity, mesangial hypercellularity and mesangial matrix expansion
Immunofluorescence microscopy	– Dominant C3 deposition along capillary loops and in mesangial areas – IgG, IgM, IgA, C1q can be present but at much lower intensity	– C3 and IgG staining (similar intensity) along capillary loops and mesangial areas
Electron microscopy	– Dense deposit disease: ribbon-like, extremely dense transformation of glomerular basement membranes – C3GN: subendothelial deposits along glomerular basement membranes and in the mesangial matrix	– Immune-complex type electron dense deposits along the subendothelial spaces and in the mesangium

Table 23.1: Overview of biopsy findings in C3G and IC-MPGN. Both entities are indistinguishable on light microscopy. IF staining will determine whether a biopsy is classified as C3G or IC-MPGN. EM can diagnose cases of DDD. C3GN and IC-MPGN are indistinguishable on EM

Histopathology of C3 Glomerulopathy

Light Microscopy (LM)

Historically, dense deposit disease and the more recently described C3 glomerulonephritis were considered to be a form of membranoproliferative glomerulonephritis (MPGN). This despite the fact that DDD and C3GN have a membranoproliferative pattern in only 25% to 30% of patients at presentation. A mesangial glomerulopathy is most common (40–50%), while necrotizing and crescentic glomerulonephritis (~10%) and acute proliferative and exudative glomerulonephritis (~10%) make up the rest (Fig. 23.1) [3, 7–9]. The name membranoproliferative glomerulonephritis describes the pathologic features found in this pattern of glomerular injury. There is new glomerular basement membrane (GBM) formation producing double contours. The proliferative component includes endocapillary and mesangial hypercellularity as well as the cellular interposition between the GBM double contours. At the time of presentation, biopsies of patients with C3G almost always have a very mild membranoproliferative pattern, particularly as opposed to patients with immune-complex mediated MPGN (see below). Mesangial glomerulopathy is limited to variable mesangial changes without GBM alterations. The changes can be so mild that the biopsy appears almost normal. In the well-developed form, there is diffuse mesangial expansion with mesangial hypercellularity. Crescentic C3G at presentation typically shows extensive cellular crescents with necrotizing areas. Where the glomerular tufts can be seen, there is usually some endocapillary and mesangial hypercellularity with neutrophilic infiltration. However, occasionally the glomerular tuft is bland, though the neutrophilic infiltration is most often still present. Acute proliferative and exudative glomerulonephritis is indistinguishable from an infection-related glomerulonephritis and most likely explains why this variant was not described in the early series [3, 10–12]. There is glomerular enlargement due to diffuse endocapillary hypercellularity with neutrophilic infiltration.

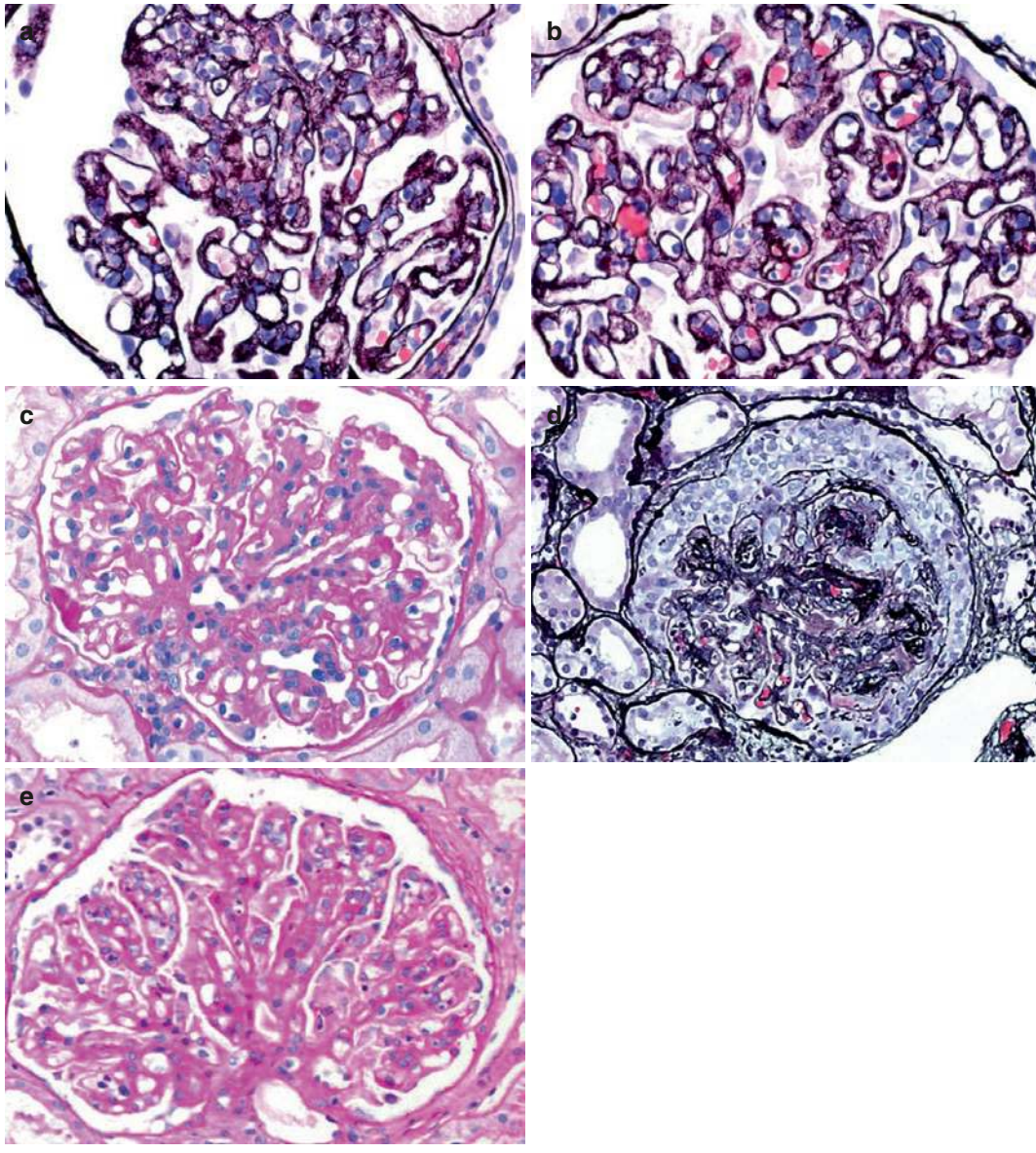

Fig. 23.1 Light microscopy findings in C3G. (**a**, **b**) Mild membranoproliferative pattern with glomerular basement membrane double contours, endocapillary and mesangial hypercellularity with mesangial matrix expansion (Jones silver stain, original magnification 600×). (**c**) Mesangiopathic glomerulopathy with mild mesangial cell and matrix increase (Periodic acid-Schiff reaction (PAS), original magnification 400×). (**d**) circumferential cellular crescent (Jones silver stain, original magnification 400×). (**e**) Acute proliferative and exudative glomerulonephritis (PAS, original magnification 400×)

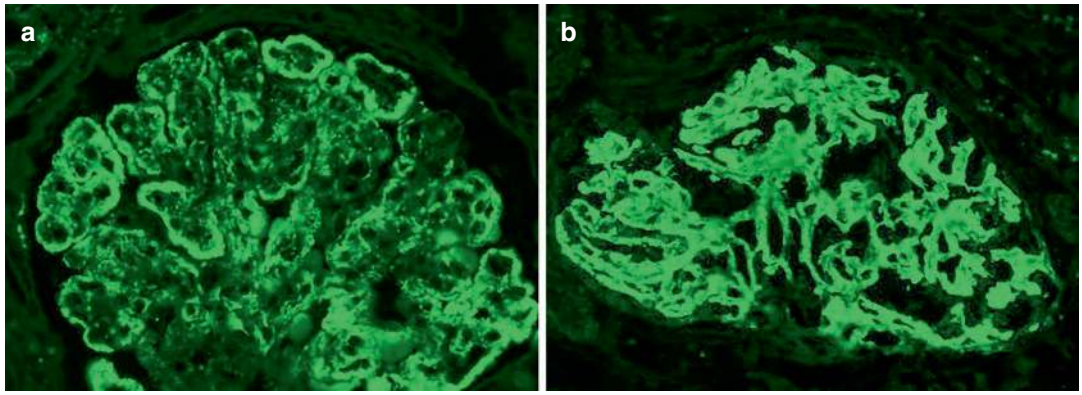

Fig. 23.2 Immunofluorescence microscopy findings in C3G. (**a**, **b**) Intense C3 staining along capillary loops and mesangial areas (fluorescein-conjugated, anti-human anti-C3c, original magnification 400×)

Immunofluorescence Microscopy (IF)

Dominant C3 deposition along capillary loops and in mesangial areas is a required feature for the diagnosis of C3G (Fig. 23.2). IgG, IgM and/or IgA are present in lower intensities in up to 36% of cases [7, 8, 13]. Surprisingly, C1q is seen in up to 30% of biopsies, again at lower intensity [7, 8]. In a large study of C3G, immunoglobulins were present in 46% of patients with C3GN and 36% with DDD [13]. Paraffin IF may reveal a dominant or co-dominant immunoglobulin [14]. The presence of immunoglobulins present on routine IF or on paraffin IF, though often found in C3G, raises the possibility of an infection-related glomerulonephritis or an autoimmune disease. Given that the latter entities are far more common than C3G, they must be ruled out before a diagnosis of C3G is made.

Electron Microscopy (EM)

C3GN is distinguished from DDD on the basis of EM (Fig. 23.3). Dense deposit disease is characterized by extremely electron dense transformation of the glomerular basement membranes with similarly dense spheroids in the mesangium. Large and equally dense sub-epithelial hump-like deposits are present in up to 50% of cases [13]. C3GN most often has electron dense deposits indistinguishable from immune-complex deposits. However, in many cases the deposits have a smudged charcoal gray appearance in the subendothelial space and in mesangial areas. Subepithelial hump-like deposits are commonly seen.

Transition from Acute Proliferative and Exudative Glomerulonephritis to C3G

There is a challenging subset of patients with C3G who initially present with an acute proliferative and exudative glomerulonephritis [15, 16]. Immunofluorescence shows C3 often with IgG in the mesangium and in a granular pattern along the capillary loops. Though this LM pattern and the finding of immunoglobulins on IF are common in C3G, these patients are thought to have an infection-related glomerulonephritis and are treated as such. The diagnosis of an infection associated GN is not surprising given that up to 40% of patients have an infection at the time of diagnosis [13]. Usually there is some renal function improvement, but continued low C3 levels and/or hematuria and proteinuria lead to a repeat biopsy and the diagnosis of C3G is made usually within months but can take several years [16–19].

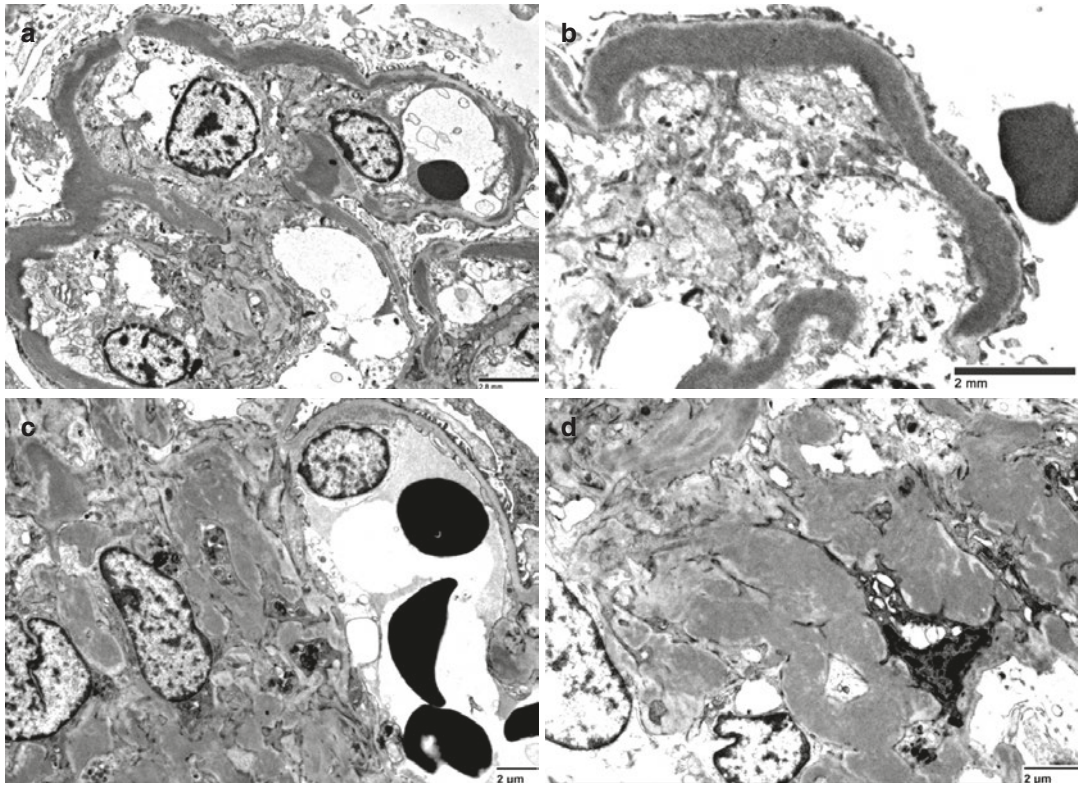

Fig. 23.3 (**a**, **b**) Dense deposit disease. Extremely electron dense transformation of the glomerular basement membranes forming ribbons. Similarly dense spheroids in the mesangium (**a**. unstained, original magnification 8000×; **b**. unstained, original magnification 20,000×). (**c**, **d**) C3GN. Smudgy gray deposits in the mesangium and in subendothelial spaces (**c**. unstained, original magnification 6000×; **d**. unstained, original magnification 8000×)

Histopathology of Immune Complex Mediated Membranoproliferative Glomerulonephritis

By definition, these patients have a membranoproliferative pattern of glomerular disease. There are glomerular basement membrane double contours with cellular interposition accompanied by endocapillary hypercellularity, mesangial hypercellularity and mesangial matrix expansion sometimes forming lobules. IF demonstrates capillary loop and mesangial deposition of C3 and immunoglobulin(s), almost always IgG with or without IgM. EM confirms the LM findings of glomerular basement membrane duplication with cells between the layers and endocapillary hypercellularity. The mesangium is expanded by cell and matrix increase. Immune-complex type electron dense deposits are found along the subendothelial spaces and in the mesangium (Fig. 23.4) [20, 21].

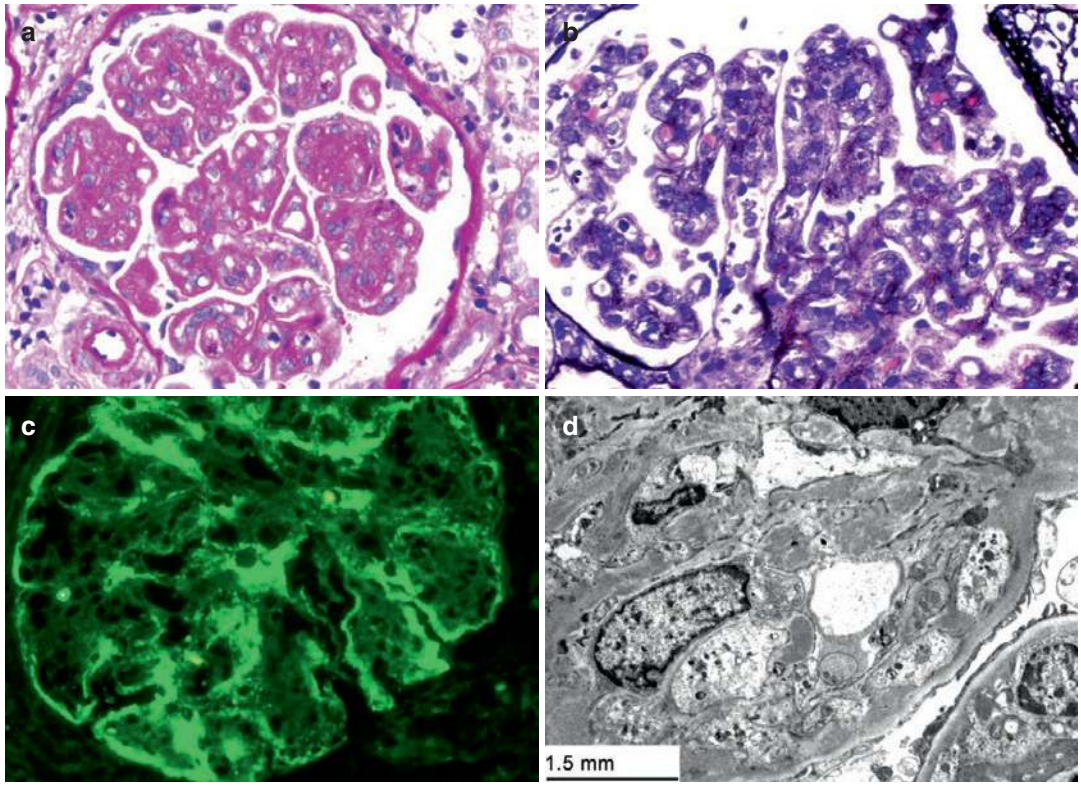

Fig. 23.4 IC-MPGN (**a**) Mesangial cell and matrix increase forming lobules. Endocapillary hypercellularity and thick glomerular basement membranes (PAS, original magnification 400x). (**b**) Endocapillary hypercellularity and mesangial cell and matrix expansion. (Jones silver, original magnification 600x). (**c**) Peripheral granular staining (fluorescein-conjugated, anti-human anti-C3c, original magnification 600x). (**d**) Mesangial and endocapillary hypercellularity. New basement membrane formation with cellular interposition. Electron dense deposits in the mesangium and in subendothelial spaces of the glomerular basement membranes (unstained, original magnification 8,000x)

Classification of C3G

The classification of C3 glomerulopathies (C3G) is based on the pattern and intensity of immunofluorescence seen on renal biopsy (Table 23.1). Prevalent Ig staining prompts the diagnosis of a *secondary* MPGN (see above), and work-up including a panel of auto-antibodies, viral serologies and, exceptionally in pediatric patients, searching for cryoglobulins and monoclonal gammopathies is warranted. However, predominant C3 staining prompts the diagnosis of a primary disease, referred to as C3 glomerulopathy (C3G). Within this category, electron microscopy assessment allows for the differentiation of patients with dense deposit disease (DDD; previously known as MPGN II), in whom dense, sausage-like very intensely osmiophilic deposits are present along the glomerular basement membrane, and patients with predominant mesangial C3 staining and possible presence of subepithelial humps as described in post-infectious mesangial glomerulonephritis. The latter group is diagnosed with MPGN I or C3 glomerulonephritis (C3GN), with MPGN I traditionally being favored in patients with membranoproliferative lesions. Of note, C3G may be familial or sporadic.

Pathogenesis

Much has been learned about C3G and MPGN by studying patients and both spontaneously occurring and genetically engineered animal models (Fig. 23.5). In summary, C3G and IC-MPGN result in principle from dysregulation of the complement alternative pathway in the fluid phase with possible terminal complement pathway (TP) activation, complement (split product) deposition in the kidney and an accompanying inflammatory response. In humans, CAP dysregulation can result from mutations in complement proteins or the presence of autoantibodies.

In animal models, Factor H (FH) deficiency resulted in C3 deposition along the GBM, terminal pathway (TP) activation followed by electron dense transformation of the GBM together with varying degrees of glomerular inflammation and structural damage, hallmark features of MPGN [22, 23]. When C5 activation was prevented by inter-crossing the Cfh−/− strain with C5-deficient mice, the abnormal glomerular C3 deposition remained unchanged but the severity of glomerulonephritis was decreased [23]. However, altering C3 convertase activation by deleting Factor B (FB) or Factor D (FD) prevented MPGN. In Cfh−/− mice that are also deficient in Factor I (FI), activated C3 remains as C3b only. Interestingly, whilst abnormal glomerular C3 developed in both strains, the C3 in the mice with combined deficiency of FH and FI was mesangial in location, whilst in the Cfh−/− strain the C3 was located along the GBM. These data indicate that during uncontrolled C3 activation the nature of the activated C3 fragment may determine the site of its glomerular deposition [24]. What those animals models have shown us is that FH is the critical regulator of glomerular C3 homeostasis, and administration of either mouse or human FH to Cfh−/− mice reduced glomerular C3 staining and increased circulating C3 levels [25]. Mutations in FH are typically associated with atypical hemolytic uremic syndrome (aHUS) indicating that defects in the CAP can give rise to at least two distinct renal phenotypes: thrombotic microangiopathy (TMA) like HUS and glomerulonephritis like C3G. The majority of aHUS-associated CFH mutations does not result in complete deficiency of FH but selectively impairs the surface C3b recognition domains of the protein. In keeping with this, mice expressing a mutant FH molecule that functionally mimicked aHUS-associated mutations known from patients developed TMA not C3G, [23] and this was dependent on C5 [26].

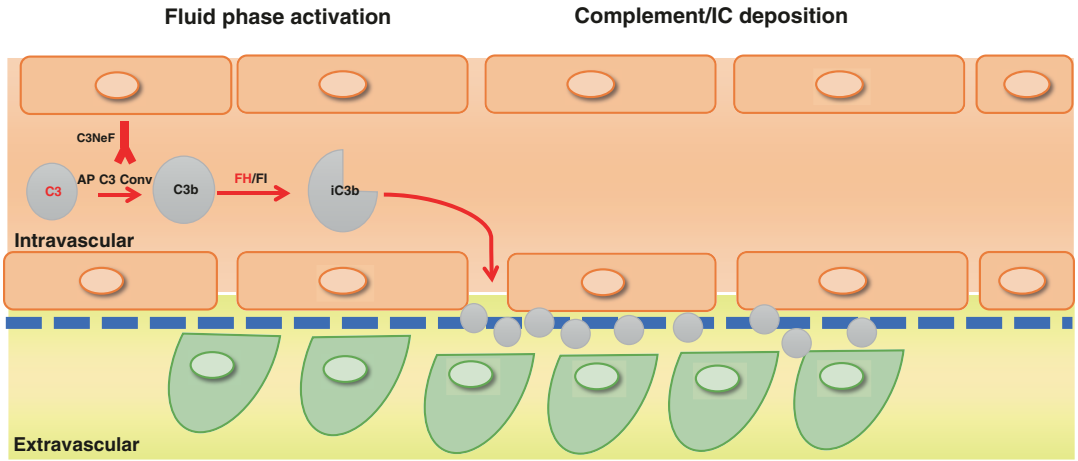

Fig. 23.5 Pathogenetic *concept* of C3G: Genetic or autoimmune factors result in fluid phase dysregulation of the complement alternative pathway at the level of the (alternative) C3 convertase. Excessive C3 activation results—in the presence of proteases (e.g. CFI) and their corresponding cofactors (e.g. CFH)—in the generation of copious amounts of C3 split products which deposit in the glomerular filter triggering local inflammatory responses

On the other hand, complement dysregulation in C3G patients may occur via different scenarios:

- Antibodies stabilizing the CAP C3 convertase and prolonging the natural decay of the CAP C3 convertase, rendering it overactive.
- Mutations or antibodies to FH or FHR that result in the absence or loss of function of FH resulting in loss of control of the CAP C3 convertase and its enhanced activity.
- Mutations in C3 or FB that result in an exceedingly stable C3 convertase with prolonged decay and enhanced function.

In all scenarios, the functional consequence is an enhanced activation rate of C3 mainly in the fluid phase. Autoantibodies associated with C3G (see below) prolong the half-life of the CAP C3 convertase, or the (CAP or CP) C5 convertase and therefore result in TP activation [27, 28].

Autoimmune Forms of C3G

Decreased serum C3 levels were long reported in patients with MPGN, which led to the hypothesis of a circulating factor leading to increased C3 cleavage [29]. When an antibody binding to a neo-epitope of the complement CAP C3 convertase (C3bBb)—delaying its natural decay and thus enhancing its function—was found, it was named C3 Nephritic Factor (C3NeF) [27, 30]. C3Nef is an autoantibody capable of binding the CAP C3 convertase, C3BbB, therefore conferring resistance to its inactivation by regulatory factors such as FH.

More recently, additional autoantibodies or nephritic factors were detected which prolong the half-life of the CAP and CP C3 and C5 convertases:

- *Antibodies that stabilize the CAP and/or CP C3 convertase:* C3NeF [31, 32], C4NeF [33, 34], Anti-Factor B antibodies [35, 36] and Anti-C3b antibodies [35];
- *Antibodies that stabilize the C5 convertase:* C4NeF [34], C5NeF [37];
- *Antibodies impairing CAP (fluid phase) regulation:* Anti-Factor H antibodies (recognizing FH N-terminus) [38–40].

C3NeF is the most prevalent antibody and was detected in 86% of DDD, 46% of C3GN, and up to 50% of patients with IC-MPGN, respectively, and is associated with decreased C3 levels [41, 42]. C3NeF might fluctuate and not correlate with disease activity and might co-exist with complement mutations [43, 44]. Therefore, a positive C3NeF still warrants comprehensive complement diagnostics. Anti-C3b and Bb antibodies, both stabilizing the CAP C3 convertase, have been reported in several patients with C3G and IC-MPGN and are associated with worse outcome if both occur at the same time [36].

Servais et al. identified mutations in complement genes in 18% of C3G patients, while the presence of circulating C3Nef was detected in 59% of cases [45]. More than half of the patients carrying complement gene mutations were also C3NeF positive. C3Nef was more frequent in patients with DDD (86%) and was associated with significantly lower levels of circulating C3. A report of 3 patients with DDD showed a correlation of moderate increases in C3Nef and slight reduction on C3 and disease recurrence post-transplant [46]. In other reports, lower circulating levels of C3 were found in patients with a membranoproliferative pattern of disease, [41] while others have reported that children have significantly lower C3 levels and more frequent C3Nef positivity compared to adults [7].

However, as reviewed by Pickering M et al. [47], C3NeF can be detected in different ways [31, 48]. It is possible that patients may be positive in some but not all available assays. C3NeF and other auto-antibodies found in MPGN were also detected in other kidney diseases, such as systemic lupus erythematosus (SLE) [49], postinfectious GN [50], and meningococcal meningitis [51]. C3Nef levels are occasionally also found in healthy individuals [52], rendering the interpretation of their pathogenic value controversial.

Therefore, the significance of C3Nef in the pathophysiology of C3G and its correlation with

disease course and with response to treatment is still debated and warrants further investigation [47, 53].

Genetic Forms

Early studies found a genetic cause in approximately 18% of patients with IC-MPGN and C3G. By contrast, Bu et al., studying a larger panel of genes by next-generation sequencing (NGS), found a genetic diagnosis in up to 43% [32, 54]. Iatropoulos et al. detected mutation carriers in 17 and 18% of patients with IG-MPNG and C3G, respectively [42]. To date, mutations have been reported in the following complement genes: CFH, CFHR5, CFI, MCP, C3 and CFB and can occur in a heterozygous or homozygous fashion (Table 23.2).

Additional mutations or internal duplications in genes encoding FH related proteins (CFHR), or formation of hybrid genes, have been associated with C3G [55, 56]. In 2009 Gale et al. described 26 individuals of Cypriot ancestry with

Table 23.2 Genetic causes of IC-MPGN and C3G

Gene	Mutation/SNP	Function	Classification	References
CFH	Homo−/compound heterozygous SCRs 1–4 (regulatory domain)	Intact surface binding Reduced C3b binding Loss of FH cofactor & decay accelerating activity	C3G IC-MPGN	Licht et al. [107], Servais et al. [32], Bu et al. [54], Iatropoulos et al. [42]
CFI	Homozygous Heterozygous	Decreased FI mediated C3b degradation	C3G IC-MPGN	Servais et al. [32], Bu et al. [54], Iatropoulos et al. [42], Iatropoulos et al. (2018)
C3	Heterozygous	C3mut—resistant to cleavage by C3bBb C3mut convertase –resistant to FH inactivation C3 binding with FI or FH	C3GN IC-MPGN	Martinez-Barricarte et al. [109], Bu et al. [54], Iatropoulos et al. [42], Iatropoulos et al. (2018)
CFB	Heterozygous/homozygous	Alters C3-FB interaction	C3G IC-MPGN	Iatropoulos et al. [42], Iatropoulos et al. (2018)
Thrombomodulin	Homozygous	Not tested	DDD	Iatropoulos et al. [42], Iatropoulos et al. (2018)
DGKE	Homozygous Heterozygous—unclear impact	Not complement mediated	MPGN	Ozaltin et al. [60], Bu et al. [54]
At risk SNPs (reviewed in Noris and Remuzzi)				
MCP/CD46	Rare SNP	Not tested	C3G IC-MPGN	Servais et al. [32]
CFH	Rare SNP e.g. Y402H (SCR 7)	Not tested	DDD	Abrera-Abeleda et al. [62]
CFHR5	Rare SNP	Not tested	DDD	Abrera-Abeleda et al. [62], Bu et al. [54]
C3	Rare SNP	Not tested	DDD	Abrera-Abeleda et al. [62]

(continued)

Table 23.2 (continued)

Gene	Mutation/SNP	Function	Classification	References
CFHR fusion proteins (reviewed in Smith et al. [83])				
CFHR3–1	CNV *CFHR3–1* hybrid gene	Greater degree of FHR-mediated deregulation	C3GN	Malik et al. [55], Goicoechea de Jorge et al. [59]
CFHR2–5	*CFHR2–5* hybrid gene	Stabilizes C3 convertase, reduced FH-mediated decay	DDD	
CFHR5-CFHR5	CNV Duplication in *CFHR5* exons 2/3	Greater degree of FHR-mediated deregulation	C3G	Gale et al. [57], Goicoechea de Jorge et al. [59]
CFHR1-CFHR1	Internal duplication	Greater degree of FHR-mediated deregulation	C3G	
CFHR5–2	*CFHR5–2* hybrid gene	Greater degree of FHR-mediated deregulation	C3GN	Smith et al. [83]
CFHR1–5	*CFHR1–2* hybrid gene	Greater degree of FHR-mediated deregulation	C3G	

unexplained renal disease and a variation in CFHR5 comprising duplication in its dimerization domain, termed CFHR5 nephropathy [57]. Several more mutations and hybrid variants in CFHR genes have been detected since and are associated with C3G [58]. The role of FHR in C3G was unclear until the report, that FHR1, FHR2 and FHR5 form homo- and heterodimers amongst themselves and with FH. In the dimeric form these proteins are able to compete with FH for C3b binding and protect C3b from inactivation and the complement CAP C3 convertase from decay. This process, termed "deregulation," was increased in patients with CFHR hybrids or CFHR5 mutations [59].

Interestingly, Ozaltin et al. published several patients with MPGN with a homozygous mutation in DGKE, an intracellular lipid kinase that modulates phosphoinositol signaling in the plasma membrane [60]. DGKE mutations can also be found in thrombotic microangiopathy [61]. Bu et al. found heterozygous variants in DGKE in several patients with C3G, as well as in Thrombomodulin, Plasminogen and ADAMTS13. The clinical relevance of these gene variants is still unclear [54].

Risk haplotypes were identified in CFH, C3 and MCP/CD46, with the CFH Y402H haplotype more frequently reported in DDD, and the MCP-652A4G polymorphism in C3G. The presence of two or more complement haplotypes increased the risk of disease [32, 62].

Clinical Presentation

MGPN, C3GN and DDD are rare diseases with an individual annual incidence estimated at 1–2 per million per total population (both pediatric and adult) [63, 64]. C3 glomerulopathies typically present with (possibly nephrotic range) proteinuria and hematuria and hypertension coupled with low circulating C3 levels [65, 66]. However, C3G has a very heterogeneous clinical presentation. This heterogeneity reflects the variety of pathogenetic mechanisms leading to a dysregulated, uncontrolled activation of the CAP.

Presentation at Onset of Disease

Age at onset is very variable, with the earliest reported case being at age 1 [67]. In the most comprehensive cohorts that include adult and pediatric patients, disease onset below age 16 years was described in 59–68% of DDD and 25–31% of C3GN patients [32, 64]. In a pediatric cohort median disease onset was approximately 10 years for both patients with IC-MPGN and C3G [68]. A slight prevalence of males (60%) was reported in some cohorts, [32, 64] but not in the cohort that included pediatric patients only [68].

Clinical symptoms are listed in Table 23.3. Data taken from the 3 cohorts indicate that patients with C3G and IC-MPGN commonly present with microscopic hematuria, significant proteinuria including nephrotic syndrome in up to 40%, hypertension and reduced renal function. Incidence of macrohematuria was reported in 10–23% [7, 64].

Disease onset is often associated with an infection as described in a report of children with DDD, in whom the appearance of renal symptoms was preceded by a respiratory infection in 57% of cases [7]. Recurrent macrohematuria during trivial infectious episodes, a clinical feature typically associated with IgA nephropathy, is not uncommon in C3G [69].

The large variation of age at presentation is probably linked to the fact that in some cases this disease has a subclinical and spontaneously remitting disease course. Hence, microhematuria and low-grade proteinuria can remain undetected for many years, leading to a delayed diagnosis when proteinuria reaches nephrotic range or when kidney failure develops. However, early onset with nephrotic proteinuria and renal failure, though less common, has been reported (patient 9 in [41]).

Worse renal function was found in pediatric patients with C3G and in adult and pediatric patients with C3GN compared to DDD. Low C3 was commonly found in patients with C3G, especially in patients with DDD and IC-MPGN. Over the course of time, C3 can normalize in some patients but might stay low in others, a persistently low C3 was more commonly found in patients with IC-MPGN [68]. C3 levels do not correlate with disease outcome [70].

Family history of glomerulonephritis must be investigated as familial forms have been described in 11% of cases [32] and genetic investigations may be channeled more effectively in these cases.

As the classification system for MPGN has recently evolved, our understanding of the differences in the clinical presentations of MPGN versus C3GN is limited. There are no unique features that clearly distinguish MPGN, DDD and C3GN on clinical grounds. An exception to this rule appears to be CFHR5 associated C3GN, which presents in childhood with persistent microscopic hematuria, synpharyngitic gross hematuria, and a strong family history of ESKD. At present this form of G3GN has been reported primarily in the Cypriot population, but also in two patients with non-Cypriot heritage [57, 71]. Patients with CFHR5 associated C3GN are also reported to have normal serum C3 concentrations.

Table 23.3 Clinical presentation of IC-MPGN and C3G

	Kirpalani (pediatric only)	Medjeral-Thomas	Servais
Number of patients	42 IC-MPGN/ 43 C3G	21 DDD/59 C3GN	29 DDD/56 C3GN
Microscopic hematuria	81/62%	76/65%	19/30%
Proteinuria	–/–	3/3 g/d	5.6/3.6 g/d
Nephrotic syndrome	22/12%	43/44%	38/27%
Hypertension	57/58%	60/39%	21/38%
Creatinine	94/168 µmmol/L	80/124 µmmol/L	–/–
eGFR (ml/min/1.73 m^2)	76/83	–/–	76/66
C3	0.26/0.39 g/L	Low 79/48%	Low 46/46%

Extra-Renal Manifestations

C3G is a complement-mediated disease, secondary to CAP dysregulation. Given that this dysregulation occurs in the fluid phase of blood, extra-renal features of disease are to be expected. In DDD, patients may develop acquired partial lipodystrophy (APL) and ocular lesions similar to soft drusen seen in age-related macular degeneration (AMD) [72].

APL—like DDD and C3GN—is associated with dysregulation of CAP on adipocytes [73] and becomes manifest in the loss of subcutaneous fat tissue, which typically occurs in the upper half of the body (starting from the face and extending to involve the neck, shoulders, arms and thorax) and precedes the onset of renal disease by several years. The median interval between the onset of APL and DDD is about eight years [74]. The majority of APL patients present with low C3 levels and, in addition, are C3NeF positive, which leads to enhanced CAP activation. MPGN was described in about 25% of patients with APL. Patients with combined disease are more likely to present with decreased C3 levels and develop APL earlier in life (about 7.7 years of age) [74].

Patients with DDD can also develop ocular lesions in the form of drusen. Drusen are retinal changes seen as crystalline yellow or white dots, which lie between the retinal pigment epithelium and Bruch's membrane [75]. Drusen can develop in the second decade of life and are responsible for visual disturbances in up to 10% of patients with DDD [72]. The drusen seen in patients with DDD are similar to those seen in age-related macular degeneration (AMD).

Diagnosis

The clinical presentations of MPGN, DDD and C3GN are extremely variable and overlap with many other glomerular diseases, ranging from subtle/subclinical to acute and severe, as exemplified in the following scenarios:

- An incidental finding on routine urinalysis of non-nephrotic proteinuria, often accompanied by microscopic hematuria.
- Macrohematuria concomitant or 2–3 weeks after an intercurrent infectious episode, most frequently of the upper respiratory tract.
- Nephrotic range proteinuria, with or without clinical signs of nephrotic syndrome and renal function impairment.
- Very rarely, an overlap with thrombotic microangiopathy.

Diagnosis of C3G is made with a renal biopsy, which includes light microscopy, immunofluorescence and electron microscopy. Extensive C3 staining allows for the diagnosis of C3GN, predominant IgG staining on the other hand is characteristic for IC-MPGN and electron dense deposits favors the diagnosis of DDD. Despite different features on biopsy, the underlying pathophysiology is similar and requires further workup for complement activation, auto-antibodies and mutations in complement genes.

Differential Diagnosis

The presentation of a nephritic/nephrotic clinical picture is compatible with a variety of diagnoses at disease onset. The clinical picture can be indistinguishable from IgA nephropathy (IgAN), particularly if circulating C3 is normal and macrohematuria in the setting of an infection is observed [69]. Familial disease does not exclude the diagnosis of C3G, particularly but not exclusively in patients of Cypriot descent [55, 57]. Renal biopsy, in particular the immunofluorescence, allows clear discrimination between C3G, in particular the familial forms, and IgAN.

In the presence of reduced levels of circulating C3, particularly in context of an infection during the preceding 2–3 weeks, the diagnosis of acute post-infectious glomerulonephritis (PIGN) is likely, but C3G is also possible though less frequent. In this case, a renal biopsy may not be

definitive as the presence of "humps" in the electron microscopy is common to both, C3G and PIGN [9]. The latter presents with reduced circulating C3 levels, which typically normalize within 12 weeks from disease onset [76]. As C3 levels may be normal also in C3G and given the heterogeneity of the clinical picture of this new disease entity, we suggest that in the case of a clinical picture common to C3G and PIGN, even with normalizing C3 levels and absent proteinuria, patients be advised to perform urinalysis every 3–6 months for 2 years following resolution of the acute clinical picture and to seek medical attention if macrohematuria or significant proteinuria re-appear.

Decreased C3 and C4 are hallmark features of SLE, which can present with proteinuria, microscopic hematuria and hypertension. Low C4 was reported in a small amount of patients with C3G (5–36%) and therefore cannot be used as the differentiating factor [32, 64]. A thorough history and physical examination with attention to extrarenal disease manifestations such as arthritis, rash, neurologic, hepatic, lung or cardiac involvement combined with ANA and anti-dsDNA antibody titers, laboratory evidence of hemolysis, leukopenia, coagulopathy, or hepatitis, strongly support a diagnosis of SLE as opposed to C3G.

Laboratory Evaluation

When the diagnosis of C3G has been made by renal biopsy, analysis of the CAP is warranted:

1. Circulating complement factors including C3, C4, CFH, CFB and markers for complement pathway activation (CH50, APH50, C3d) and terminal complement activation (C5b-9).
2. Circulating autoantibodies such as C3 Nephritic Factor (C3NeF) and anti-FH autoantibodies.
3. Other antibodies that are only measured in specialized research labs can be added in specific cases: anti-CFB antibodies, C4 Nephritic Factor, C5 Nephritic Factor and anti-C3b antibodies.
4. Mutations in genes involved in the regulation of the C3 convertase such as Factor H (FH), Factor I (FI), membrane cofactor protein (MCP/CD46), C3, and Factor B (FB) and screening of the FHR locus with MLPA to detect deletions, duplications or fusion genes. Other genes associated with C3G or IC-MPGN include thrombomodulin and DGKE.
5. When family history is positive for C3G, suggesting a familial form, the FHR locus should also be screened for an internal duplication within the FHR5 gene, described mainly but not solely in individuals of Cypriot origin [57].

In patients with IC-MPGN we also recommend a detailed investigation for secondary causes as listed in Table 23.4.

Table 23.4 Causes of secondary MPGN

Immunoglobulin mediated
Infectious diseases:
Hepatitis B, C, E, EBV, HIV
Endocarditis, shunt nephritis, visceral abscess, empyema
Tuberculosis, leprosy
Brucellosis, Q Fever
Malaria, schistosomiasis
Onchocerca volvulus, Wucheria bancrofti, Loa loa infections
Systemic immune diseases:
Cryoglobulinemia
Systemic lupus erythematosus
IgA vasculitis
Sjögren's syndrome (secondary to cryoglobulinemia)
Hypocomplementemic urticarial vasculitis
Rheumatoid arthritis (Zand et al. [79])
X-linked agammaglobulinemia
Neoplasms/dysproteinemias:
Plasma cell dyscrasia
Monoclonal gammopathy of unknown significance
Fibrillary glomerulopathy
Immunotactoid glomerulonephritis

(continued)

Table 23.4 (continued)

Proliferative glomerulonephritis with monoclonal immunoglobulin deposition (Nasr et al. [7]
Proliferative glomerulonephritis with isolated light chain deposition
Membranoproliferative glomerulonephritis with masked monotypic immunoglobulin deposits
Light-chain and/or heavy-chain deposition disease
Leukemias and lymphomas (with or without cryoglobulinemia)
Bone marrow transplant
Carcinomas, Wilms' tumor, malignant melanoma
Angiofollicular lymph node hyperplasia with or without TAFRO syndrome[a]
Sinus histiocytosis with massive lymphadenopathy
Other:
Alpha-1-antitrypsin deficiency with severe liver disease
Gluten-sensitive enteropathy (celiac disease)
Immunoglobulin negative
Thrombotic microangiopathy including drug induced forms
Sickle-cell disease
Transplant glomerulopathy
POEMS syndrome[b]

[a]TAFRO = Thrombocytopenia, Anasarca, Fever, Renal dysfunction (or Reticulin myelofibrosis) and Organomegaly
[b]POEMS syndrome = Polyneuropathy, Organomegaly, Endocrinopathy, Monoclonal gammopathy and Skin changes

Outcome

Data on long-term outcomes in both native and transplant kidneys based on the new classification is limited and vary dependent on the cohort and disease onset. Medjeral-Thomas, comparing outcomes of DDD and C3GN in a cohort of 80 patients, with 50% having a pediatric onset of disease, reported progression to ESKD in 47% of DDD patients and 23% of C3GN patients with a median follow-up of 28 months. Multivariate analysis suggested crescentic changes, DDD and disease onset over 16 years of age as independent predictors for ESKD [64].

The French cohort of 134 patients reported 10-year renal survival at 63.5%, with no difference between groups (MPGN, DDD, C3GN). Median time from first observation to end-stage renal disease was about 10 years, and in the patients that underwent renal transplant, disease recurrence was observed in over half of the cases, with an additional 17% experiencing thrombotic microangiopathy (TMA) [32, 41].

A large retrospective study comprising 165 children with both IC-MPGN and C3G confirmed that children have a better outcome than adults. After a mean follow-up of 4 years kidney function was preserved, and at 10 years 80% of children analyzed did not meet the composite outcome of eGFR <30 ml/min/1.73 m^2, 50% eGFR reduction or need for kidney replacement therapy [68]. Although hypertension remained prevalent in 42.5% of the cohort at the last follow-up, and the urine protein/creatinine ratio remained elevated (mean 253.8 [range 91.9–415.7] mg/mmol).

Disease recurrence of MPGN and DDD in transplants is common (Table 23.5). Data from the ESPN/ERA-EDTA registry shows an average renal allograft loss of 32.4% at 5-years post-transplant in children [77]. Data suggesting that pediatric patients with DDD are at greater risk of graft loss from disease recurrence has also been reported in the NAPRTCS database [78]. The reason(s) for the increased risk of recurrence in children is not clear. Several limited case series confirm an increased risk of disease recurrence for C3GN and the potential negative impact on allograft survival. Disease recurrence is strongly associated with graft loss [64]. Zand et al. reported outcomes on 21 patients with C3GN, 14 (66.7%) had disease recurrence with median time to graft failure of 77 months (6.4 years) [79]. Concerning risk of relapse following renal transplantation for DDD, in one study the degree of proteinuria was strongly associated with disease recurrence, and the presence of glomerular crescents in biopsies of renal allografts had a significant negative correlation with graft survival [78].

Table 23.5 Disease recurrence post transplantation

	Medjeral-Thomas	Servais
Number of patients	7 C3GN 6 DDD	14 IC-MPGN 10 C3GN 11 DDD
MPGN	–	43%
C3G	57%	60%
DDD	100%	55%

In the familial form of C3G secondary to CFHR5 mutations described in individuals of Cypriot descent [57] prognosis differed significantly between sexes: men were by far more likely to progress to ESKD than women (78% versus 22%).

Treatment

At present, there is no treatment standard or at least a therapeutic agent of proven effectiveness in C3G available. The rarity of this disease, coupled with its protracted and variable natural history, makes clinical trials logistically challenging and the use of different end-points make uniform interpretation of results difficult [80]. However, considering that about 50% of patients proceed to ESKD and may face a high risk of disease recurrence post-renal transplant, concerted efforts to define effective treatment strategies are necessary. Several therapeutic regimens have been employed, utilizing immunosuppressive agents (glucocorticoids, mycophenolate mofetil, calcineurin inhibitors), anti-platelet agents, plasma exchange or infusion and, much more recently, complement blockers [81–83]. Renoprotective agents (such as angiotensin-converting-enzyme inhibitors, ACEi, or angiotensin II receptor antagonists, ARBs) are associated to these treatments almost invariably. As our understanding of the pathophysiology of C3G and of IC-MPGN is rapidly expanding and changing, while reviewing published cases is reasonable, its usefulness in guiding future therapeutic strategies may be limited [47].

The optimal therapeutic strategy should be driven by clinical parameters, such as the degree of proteinuria and impairment in renal function, and also by diagnostic test results (Fig. 23.6). In

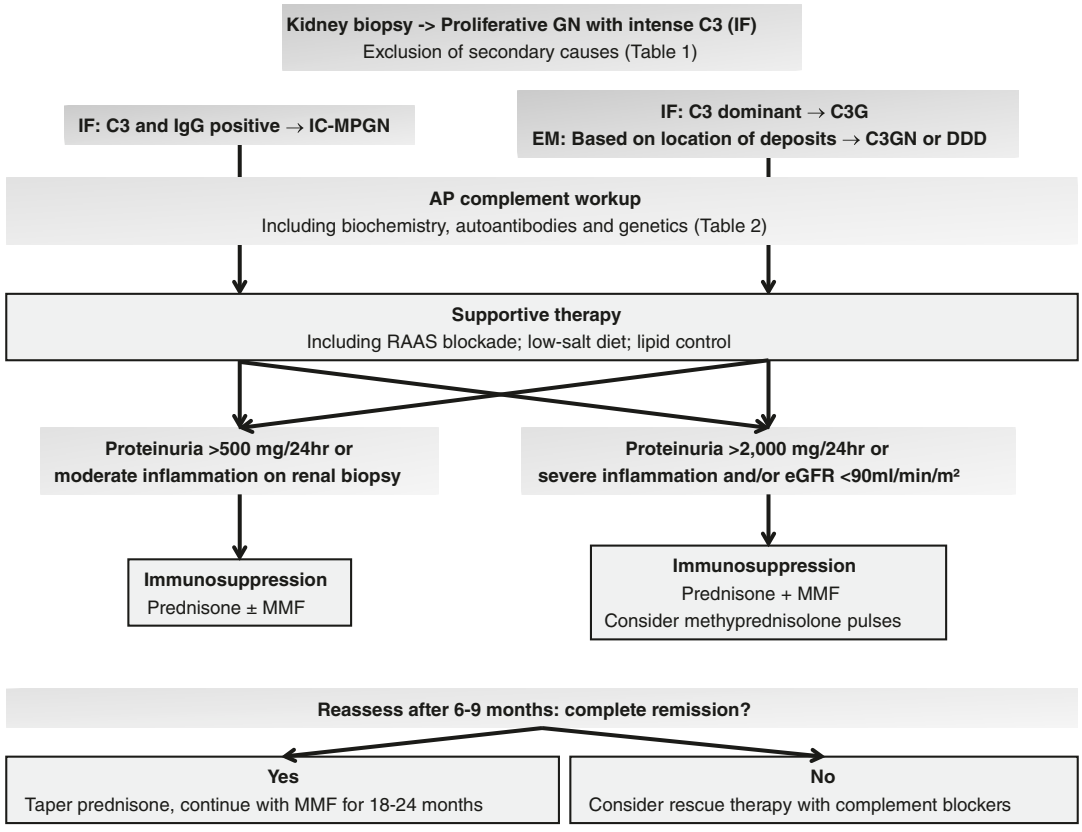

Fig. 23.6 Therapeutic algorithm for IC-MPGN/C3G

the near future, the availability of new therapeutic agents may drastically alter this strategy. Clinicians need to be aware that clinical practices in this field may evolve rapidly.

In the following paragraphs regarding treatment, for all options except eculizumab the literature cited is about different forms of IC-MPGN and C3G. Because evidence is very limited, a consensus of expert opinion currently still provides the most reasonable therapeutic approach to this disease (KDIGO) [84].

Immunosuppressive Agents

There are no published trials on the use of **prednisone** in C3G. Existing literature pertains to primary membranoproliferative glomerulonephritis of all subtypes. In children with all subtypes of primary MPGN, prednisone—specifically, long-term low-dose use of prednisone—was found to have a beneficial effect with respect to the degree of proteinuria and renal survival [85–88]. This observation was confirmed by subsequent studies, in which therapy with prolonged alternate day prednisone delayed deterioration of renal function [87, 89].

However, the response of MPGN patients to glucocorticoids is not homogenous. A MPGN subtype-specific analysis revealed a lack of efficacy in patients with MPGN II/DDD, despite a beneficial effect on all MPGN patients irrespective of the MPGN subtype [87].

Altogether, in all forms of IC-MPGN and C3G, glucocorticoids may be effective, but their nonspecific nature and adverse effects mean that a high price is paid for any beneficial effect on the renal lesion [90]. A reasonable approach based on current knowledge may be that of utilizing 60 mg/m^2 daily for 4 weeks followed by 40 mg/m^2 alternate-day prednisone for other 4 weeks then tapered for a total of 6–9 months in patients with C3G that present with nephrotic-range proteinuria, with or without renal failure, or in patients with persistent proteinuria >500 mg/day despite optimal renoprotective treatment. In children who present intense inflammation in the renal biopsy with marked endo and extracapillary proliferation, or who present an acute deterioration in renal function, intravenous methylprednisolone boluses (e.g., 1000 mg/1.73 m^2 repeated 3 times) may be added at disease presentation. If no significant reduction of proteinuria is observed, steroids should be tapered and discontinued [91]. It is important to recognize that a number of patients with C3G will not respond to this therapeutic approach [47].

Other Immunosuppressive Agents

In idiopathic MPGN patients, **MMF** was administered alone or in combination with corticosteroids, and generated encouraging results [92]. Another report of 13 adult patients with idiopathic MPGN resistant to glucocorticoid treatment (8 weeks at 1 mg/kg/day) showed that adding MMF led to significant reduction of proteinuria and increase of eGFR [93]. MMF in addition to pulse and long-term steroid treatment was also found effective in a pediatric patient [94].

In C3G, recent evidence, albeit retrospective, encourages the use of MMF. Initial findings by Rabasco et al. [95] were recently confirmed and expanded in a study by Caravaca-Fontan et al. [96] which described 97 patients (81 with C3GN, 16 with DDD, 74% adults and 26% children, median age 32 years), 42 of whom received corticosteroids plus MMF. This subgroup showed a significantly higher rate of remission (79%) and a lower likelihood of renal failure (14%) compared to patients receiving other immunosuppressive drugs, eculizumab or renoprotective treatment. The superiority of this therapeutic approach was seen both in patients with genetic complement abnormalities and in patients with autoantibodies to complement components. The only factor significantly associated with a lack of response to this approach was the amount of proteinuria at baseline. Previously, a study performed in 30 young adults [97] with a median age of 25 years showed that this combination therapy induced remission in 67% of cases with a 50% relapse rate upon discontinuation of MMF. In children, retrospective studies describe use of different immunosuppressive agents in small numbers of patients, which makes drawing meaningful con-

clusions difficult. Overall, similar results have been obtained [98–100]. In our experience, MMF is beneficial in patients both with IC-MPGN and with C3G with intense proteinuria. Following tapering and interruption of prednisone in 6–9 months, usually MMF monotherapy is continued for 12–18 months.

Calcineurin inhibitors (i.e. cyclosporine and tacrolimus) are also used in the treatment of MPGN. The efficacy of cyclosporin was recently tested with encouraging results in a trial involving 18 patients with refractory MPGN who also received small doses of prednisolone (0.15 mg/kg/day). Long-term proteinuria reduction with preservation of renal function was observed in 17 of the patients [101]. In two children with idiopathic MPGN with suboptimal response to a prolonged course of steroids, rapid and complete remission of the nephrotic syndrome was achieved after initiation of tacrolimus [102].

Contradictory results are published about the efficacy of cyclosporin A in the treatment of patients with MPGN II/DDD. Kiyomasu et al. report the successful treatment of a patient with MPGN II/DDD resulting in the recovery from nephrotic syndrome using a combination of alternate-day low-dose prednisone and cyclosporin [103]. The numbers of reported children in whom calcineurin inhibitors have been used are too small to draw valid conclusions. This immunosuppressive approach may reasonably be attempted, in the absence of alternatives, when MMF plus corticosteroids have failed to improve proteinuria significantly in a patient with preserved renal function.

The use of **rituximab** has been suggested in the presence of circulating C3 Nephritic Factor (C3Nef), an autoantibody that binds to C3BbB antagonizing its inactivation by circulating regulatory factors. However, to the best of our knowledge, while there are no published results showing this treatment to be effective, there are a few single case reports of its inefficacy [104, 105].

The use of **cyclophosphamide** may theoretically be considered in forms with crescentic glomerulonephritis and rapidly progressive onset, but available data is truly anecdotal.

Taken together, limited uncontrolled data suggest that especially MMF in combination with glucocorticoids may be of use in patients with C3G and intense proteinuria, while at present there is insufficient evidence to support the first-line use of calcineurin inhibitors, cyclophosphamide or rituximab in children with this disease [91].

Plasma Infusion or Plasma Exchange

As reviewed by Smith et al., [53] in FH-deficient mice with C3G, renal C3 deposition and its depletion in plasma are rapidly reversed when (either mouse or human) CFH is administered [25, 106]. These outcomes suggest that in some C3G patients with FH mutations, FH replacement therapy could restore the underlying defect and correct the disease. This has been shown in two siblings with DDD secondary to a functional FH defect in whom chronic plasma infusion prevented disease progression and development of ESKD [107, 108]. Whether administration of exogenous FH to patients without FH mutations would be therapeutically successful is not clear, but this treatment should at least in theory not be effective in patients with a known C3 mutation, in whom a C3 convertase that is resistant to regulation by FH is formed [109].

In all scenarios characterized by deficiency or functional defect of one or more complement components, replacement of this factor/these factors by either plasma infusion or plasma exchange could theoretically be effective [110].

Because of discordant reports in the literature and lack of prolonged efficacy, plasma therapy currently is used very sporadically in children with C3G and IC-MPGN. However, this therapy may be attempted, in the absence of response to immunosuppression, in rapidly progressive forms, particularly if defective FH is found.

Complement Inhibitors

The pathophysiology of C3G suggests therapeutic targeting of CAP dysregulation by complement inhibition. Based on the pathology of disease, anticomplement therapy warrants consideration. This could include (1) C3 convertase inhibition, which may have its greatest utility in

limiting C3 breakdown product deposition on (glomerular) basement membranes; (2) C5 or terminal complement pathway inhibition [47]. Currently, the only commercially available options are the anti-C5 monoclonal antibodies eculizumab and its long-lasting analogue ravulizumab.

Effectiveness of anti-C5 therapy in a mouse model of DDD (*Cfh* deficient mice) [111] provided the rationale and led to the use of a humanized anti-C5 monoclonal antibody (eculizumab) in patients with different forms of C3G [104, 105, 112–115].

Eculizumab is a humanized monoclonal antibody directed against C5, which blocks C5 cleavage, preventing the release of C5a, a potent anaphylatoxin, and C5b, the initial protein of the cytotoxic membrane attack complex (MAC; C5b-9). Its use is well established for aHUS, the classic model of renal disease mediated by the CAP. In C3G, following initial encouraging single case reports and small series, [104, 112, 113, 115] two more recent larger studies have provided disappointing results.

A retrospective French study [116] evaluated 26 patients, 13 of whom pediatric, with a median treatment duration of 14 months. In this study, 6 patients (23%) had a global clinical response, 6 (23%) had a partial clinical response and 14 (54%) no response. The only prospective study that has evaluated eculizumab in IC-MPGN and C3G, the EAGLE study [117] confirmed these negative findings. It evaluated 10 patients, (6 with MPGN, 4 with C3G), all with normal renal function, severe (>3500 mg/24 h) proteinuria and highly elevated sC5b9 (>1000 ng/ml), who were treated with eculizumab for 2 sequential 48-week periods separated by one 12-week washout period. Primary outcome was change in 24-h proteinuria at 24 and 48 weeks. While terminal complement pathway inhibition was achieved in all patients, only 3/10 patients obtained partial remission of proteinuria. During the first treatment period, median proteinuria, albuminemia and lipid profile improved, but these mild benefits were lost during the washout period and never regained in 7/10 patients. More recently, two patients with a mixed aHUS/C3G phenotype benefiting from treatment for eculizumab have been described, suggesting than in patients with an endothelial CAP dysregulation as is present in aHUS, eculizumab is most likely to be beneficial [118].

However, new complement-modulating agents are now in the pipeline. Avacopan, an oral anti-C5aR inhibitor which has proven safe and effective in ANCA-associated vasculitis [119], is currently being investigated in adults and adolescents with C3G and IC-MPGN. As shown in Table 23.6, other agents which target the complement cascade upstream, at the level of the C3 convertase, are also available and studies investigating their safety and efficacy in IC-MPGN and C3G are underway or planned in the near future.

A potential benefit of complement inhibition in the treatment of complement-mediated diseases needs to be balanced against the detrimental effect of complement inhibition in situations when complement activation is required as part of the immune defense of the host, and thorough clinical trials are required before the use of these novel substances in children can be recommended.

Given the heterogeneity of potential and described mechanisms leading to CAP dysregu-

Table 23.6 Complement targeting treatment in IC-MPGN and C3G

Drug	Target	Mechanism	Clinical trial number
ACH0144471	Factor D	Prevents formation of C3 and C5 convertases	NCT03369236, NCT03459443 and NCT03124368
LNP023	Factor B	Prevents formation of C3 and C5 convertases	NCT03832114, NCT03955445
APL2	C3	Prevents formation of C3 and C5 convertases	NCT03453619
AMY101	C3	Prevents formation of C3 and C5 convertases	NCT03316521
OMS721	MASP2	Blocks initiation of lectin pathway	NCT02682407
Avacopan	C5aR1	Blocks anaphylatoxin formation (C3a, C4a and/or C5a)	NCT03301467

lation, it is most likely that no single anti-complement treatment will be universally effective. Rather, patient-tailored therapies chosen on the basis of each patient's specific alteration are the optimal therapeutic strategy. Therefore, upon diagnosis of C3G, extensive and expert assessment of the CAP in each patient is of pivotal importance and this work-up is indispensable in designing future therapeutic trials.

Renoprotective Agents

About 80% of patients are placed on ARBs or ACEi, both first line agents used to improve renal dynamics, decrease proteinuria, control blood pressure and limit glomerular leukocyte infiltration [53]. This approach, coupled with low-sodium diet and lipid-lowering agents when appropriate is recommended for all children with IC-MPGN or C3G. It can be used as exclusive treatment in two cases:

1. Non-nephrotic proteinuria with or without microhematuria and normal renal function/absence of acute renal failure. Close follow-up is needed to assess progression of disease based on renal function, proteinuria, and urine microscopy. Target proteinuria should be below 200 mg/day in children, and additional treatment should be initiated if this target is not reached within a few weeks of optimal dosing of these agents.
2. Histological evidence of advanced chronicity of the renal lesions on the biopsy. Patients with advanced chronic kidney disease (CKD), severe tubulointerstitial fibrosis (TIF), small kidney size, or other findings consistent with chronic disease sequel should—in the absence of systemic disease manifestations—not be treated with immunosuppression [91].

Treatment of Recurrence Post-Renal Transplantation

There is no proven beneficial therapy for recurrent C3G in the renal allograft following transplantation. Therapeutic approaches are similar to those used in primary disease and are therefore not discussed in detail here. Reported treatment of *recurrent idiopathic MPGN* besides conservative medications like RAAS antagonists to control proteinuria and hypertension, includes antiplatelet/anticoagulant agents [120], corticosteroids [121], cyclosporin [122], cyclophosphamide [123], and plasmapheresis [121, 124]. Reported treatment of *recurrent DDD* includes dose reduction, discontinuation or switch (cyclosporin to tacrolimus) of the calcineurin inhibitors used as part of the posttransplant immunosuppression regimen, modification of the prednisone dose (increase; switch from daily to alternate-day), pulse methylprednisolone, or plasmapheresis/plasma exchange [78, 125]. More recently, the use of terminal complement inhibition with eculizumab has been evaluated in adults with C3G post-renal transplant recurrence, once again with variable outcomes [126, 127].

Summary

In summary, the therapeutic options in C3G depend on the level of proteinuria and kidney failure and on the results of the panel of diagnostic tests performed at diagnosis. In the vast majority of patients, the empiric use of ACEi or ARBs as drugs of first choice to treat hypertension and decrease proteinuria is a common practice, which may delay the progression of renal disease [90]. Plasma therapy or, in the future, purified FH may be useful in some cases in which there is clear evidence of a FH deficiency or of the presence of anti-FH autoantibodies. There is little evidence of the effectiveness of immunosuppression, which should therefore be employed only in cases where disease is very active and proliferative with intense inflammation in the renal biopsy and nephrotic-range proteinuria.

Preliminary results on the use of complement blockers such as eculizumab are encouraging in some but not in all patients.

Lastly, improvement in renal outcome for patients with C3G largely relies on the evaluation of more targeted agents in future studies [90].

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Part VI

The Kidney and Systemic Disease

Postinfectious Hemolytic Uremic Syndrome

24

Martin Bitzan and Anne-Laure Lapeyraque

Abbreviations

ADAMTS13	A disintegrin and metalloprotease with a thrombospondin type 1 motif, member 13
aHUS	Atypical hemolytic uremic syndrome
AKI	Acute kidney injury
AP(C)	Alternative pathway (of complement)
C3	Complement factor 3
C4	Complement factor 4
C5	Complement factor 5
CDC	Centers for Disease Control and Prevention
CFB	Complement factor B
CFH	Complement Factor H
CKD	Chronic kidney disease
CNS	Central nervous system
CRP	C-reactive protein
CRRT	Continuous renal replacement therapy
DIC	Disseminated intravascular coagulation
EAEC	Enteroaggregative *E. coli*
eGFR	Estimated glomerular filtration rate
EHEC	Enterohemorrhagic *Escherichia coli*
eHUS	Enteropathogen (or *Escherichia coli*) induced hemolytic uremic syndrome
ELISA	Enzyme-linked immunosorbent assay
EPEC	Enteropathogenic *Escherichia coli*
ER	Endoplasmic reticulum
ESKD	End stage kidney disease
Gb3	Globotriaosylceramide
Gb4	Globotetraosylceramide
Hb	Hemoglobin
HC	Hemorrhagic colitis
HD	Hemodialysis
HUS	Hemolytic uremic syndrome
IA	Immunoabsorption
iHUS	Influenza-induced hemolytic uremic syndrome
IPD	Invasive pneumococcal disease
LDH	Lactate dehydrogenase
LPS	Lipopolysaccharide
mAb	Monoclonal antibody
MAHA	Microangiopathic hemolytic anemia
NA	Neuraminidase
NanA	Neuraminidase A

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NM	Non-motile
NSAID(s)	Non-steroidal anti-inflammatory drug(s)
OR	Odds ratio
PCR	Polymerase chain reaction
PD	Peritoneal dialysis
PE	Plasma exchange
PI	Plasma infusion
pnHUS	Pneumococcal (*Streptococcus pneumoniae*) hemolytic uremic syndrome
PRBC	Packed red blood cell(s)
RBC	Red blood cell(s)
RRT	Renal replacement therapy
SC5b-9	Serum (soluble complement factor) C5b to 9 complex (see TCC)
SD1	*Shigella dysenteriae* type 1
SLT	Shiga-like toxin
SMX/TMP	Sulfamethoxazole/trimethoprim
STEC	Shiga toxin producing *Escherichia coli*
STPB	Shiga toxin producing bacteria
Stx	Shiga toxin
TCC	Terminal complement complex
TF	Thomsen-Friedenreich (antigen)
TMA	Thrombotic microangiopathy
TTP	Thrombotic thrombocytopenic purpura
UTI	Urinary tract infection
VT	Vero(cyto)toxin
VTEC	Vero(cyto)toxin producing *Escherichia coli*

Introduction

For the purpose of this chapter, we define postinfectious hemolytic uremic syndrome (HUS) as HUS [1] caused by specific infectious organisms in patients with no identifiable HUS-associated genetic variants or autoantibodies. HUS can follow infections by Shiga toxin (Stx) producing bacteria, mainly *Escherichia coli* (STEC) and *Shigella dysenteriae* type 1, [2, 3] neuraminidase producing organisms, mainly *Streptococcus pneumoniae* and *Clostridium species* [4–7], influenza virus [8], SARS-CoV-2 [9] or HIV [10]. Biologically diverse mechanisms affecting vascular endothelial cells, platelets and red blood cells (RBCs) have been uncovered involving Stx, neuraminidase and potentially complement that mediate thrombotic microangiopathy (TMA) [11, 12]. TMA is characterized by the simultaneous appearance of intravascular hemolysis, thrombocytopenia and acute organ injury, most commonly acute kidney injury (AKI). TMA is an umbrella term of diverse forms of HUS and thrombotic thrombocytopenic purpura (TTP). The majority of HUS episodes in children can be linked to STEC infections, depending on the geographical latitude and agricultural systems. This contrasts with HUS caused by the dysregulation of the alternative pathway of complement associated with pathogenic variants and/or autoantibodies (mainly to complement factor H), or to other genetic and metabolic causes (Box 24.1). However, HUS can arise following infection by a "specific" agent in a patient with a primary complement disorder; the "atypical" nature of such HUS is uncovered by its clinical presentation (relapsing course, recurrence after transplantation or family history) and/or genetic screening.

Box 24.1: Classification of Thrombotic Microangiopathies (TMA): HUS and TTP

1. Infection-induced HUS (caused by endothelial injury due to specific infectious agents)
   - (a) Shiga toxin-producing bacteria
     - (i) Shiga toxin-producing *Escherichia coli* (STEC)
     - (ii) *Shigella dysenteriae* type 1
   - (b) Neuraminidase-producing bacteria
     - (i) *Streptococcus pneumoniae*
     - (ii) *Clostridium spp.*
   - (c) Influenza virus (A/H3N2, A/H1N1, B)
   - (d) Severe acute respiratory syndrome corona virus-2 (SARS-CoV-2)
   - (e) Human immunodeficiency virus (HIV)

2. Hereditary/genetic forms of HUS
   (a) Mutations of regulatory proteins and components of complement and coagulation pathways (soluble and membrane-bound, usually heterozygous)
   (b) Genetic abnormalities without known complement dysregulation, usually autosomal recessive (examples: methylmalonic aciduria and homocystinuria, cblC type; diacylglycerol kinase-ε)
3. Autoimmune HUS
   (a) Autoantibodies against complement regulatory proteins (example: anti-CFH antibody)
4. Thrombotic thrombocytopenic purpura (TTP)
   (a) Hereditary TTP (Upshaw Shulman Syndrome)
   (b) Autoimmune TTP (anti-ADAMS13 antibody)
5. Other forms
   (a) Endotheliotoxic therapeutics (examples: cancer drugs, anti-VEGF antibody)
   (b) NYED (not yet etiologically defined)
6. Secondary forms
   (a) SLE, anti-phospholipid syndrome, bone marrow transplantation etc.

The historical terms diarrhea-positive (D^+) and diarrhea-negative (D^-) HUS, introduced to distinguish STEC-induced HUS from "atypical" forms, have become obsolete. At least 1/3 of patients with complement-mediated "atypical" HUS present with diarrhea or even colitis [13, 14]. The D^+/D^- dichotomy also failed to differentiate postinfectious forms, such as *S. pneumoniae* HUS (pnHUS), from "atypical" HUS. An etiology-based classification and diagnosis, where possible, is essential for patient management [15].

Finally, there is emerging evidence of transient complement activation in postinfectious forms of HUS in the absence of a demonstrable genetic defect or anti-CFH autoantibodies. The precise mechanism of complement activation and its pathological significance are presently under investigation. With the evolving understanding of the complement system and of the pathogenesis of different forms of HUS, some of the descriptions and assumptions in this chapter will have to be revised in the future [8, 11, 16–19].

Shiga Toxin-Producing *Escherichia coli* HUS

History of HUS and Definitions

The term "hemolytic uremic syndrome" was first used in 1955 by the Swiss hematologist Conrad von Gasser, who described five children presenting with the triad of acute hemolytic anemia, thrombocytopenia and kidney failure [1]. It was not until 1983 when Mohamed Karmali and his group identified vero(cyto)toxin producing *Escherichia coli* as the as the cause of typical childhood HUS [2, 20]. In the same year, Allison O'Brien recognized that a toxin, produced by a newly described *E. coli* O157:H7 serovar, bore close similarity with *Shigella dysenteriae* type 1 (Shiga). Soon it became clear that verotoxin and Shiga-like toxin belong to a family of closely related bacterial protein exotoxins, subsequently name Shiga toxins (Stx), with the major subdivision Stx1 and Stx2 (and several related variants) [21]. We will use the terms STEC-HUS to describe patients with "typical" HUS, the predominant form of pediatric HUS in many countries [22].

More than 200 *E coli* serotypes have been described carrying Stx phage(s) and producing Stx, but only a limited number has been associated with human disease [23]. Shiga toxin-producing bacteria encompass STEC and *S. dysenteriae* type 1, and the occasional *Citrobacter freundii* isolates capable of Stx production [24–26].

While *E. coli* O157:H7 is responsible for the majority of sporadic hemorrhagic colitis (HC) and HUS cases and outbreaks worldwide, non-O157:H7 STEC serotypes, including *E. coli*

O111:H11/NM and O26:H11/NM have also been implicated in severe human disease [27–29]. The large-scale *E. coli* O104:H4 outbreak in Germany in 2011 [30, 31] with >850 mostly adult victims of HUS and 50 deaths represents a unique scenario where an enteroaggregative *E. coli* incorporated an *stx2* phage; this novel strain contaminated sprouts, that are usually eaten raw [31, 32].

Epidemiology of STEC Infections and STEC-HUS

The annual incidence of sporadic STEC HUS is 1–1.5 per 100,000 pediatric population (median age 2.7 years) [33, 34]. The vast majority of patients is diagnosed during the warm season. The primary reservoir is cattle and cattle manure. STEC O157:H7 can survive for months or years and multiply at low rates even under adverse conditions.

Outbreaks of STEC infections and HUS in the early 1990s were linked to the consumption of STEC O157:H7-contaminated ground beef [35, 36]. They caused widespread media attention and expensive lawsuits, and eventually resulted in improved hygiene in slaughterhouses and warnings against the consumption of undercooked meat. Consequently, the role of processed meat as the predominant outbreak vehicle diminished, and more recent epidemics were due to contaminated well water [37], aquaculture spillover [38], or agricultural produce [31, 39–42].

Non-O157:H7 STEC serotypes have been increasingly recognized as a cause of sporadic and epidemic infections and HUS. Isolation frequencies of non-O157:H7 STEC strains belonging to more than 50 *E. coli* serogroups approach or exceed those of *E. coli* O157:H7 strains in North America, Europe and elsewhere [43–47]. Transmission occurs in about equal proportions through food and person-to-person spread [48–51].

The HUS risk following STEC infection varies substantially between STEC serotypes: in pediatric populations it is between 8 and 15% for O157:H7 [52, 53], and about ten-fold lower for most non-O157:H7 serotypes [45, 54], with some notable exceptions [28, 55–59]. The global burden of STEC infections is about 2.8 million annually leading to 3890 cases of HUS, 270 cases of end-stage kidney disease (ESKD), and 230 deaths [22]. Table 24.1 summarizes large or clinically significant outbreaks of STEC infections highlighting the spectrum of involved STEC serotypes and toxins, the vehicle of transmission and calculated HUS risks.

Pathogenesis of STEC Disease and HUS

STEC display a sophisticated machinery involving bacterial and host proteins, high contagiosity and resistance to environmental factors. The central pathogenic factor leading to HC and HUS is the ability to produce Stx and to deliver it into the blood stream. STEC are not tissue invasive, and bacteremia is not a feature of STEC diarrhea or HUS.

Ingested STEC bind to epithelial cells in the terminal ileum and Payer's patches. Bacterial interaction with host cells elicits signals that enhance bacterial colonization and release of bacterium-derived pathogenic factors including lipopolysaccharide (LPS) and Stx. Tight adherence of STEC to gut epithelium facilitates toxin translocation into local microvasculature and systemic circulation without killing the epithelial cell [60, 61] (Fig. 24.1). STEC and free fecal toxin excretion often diminish when HUS becomes manifest [53, 62–64]. The concentration of measurable, circulating toxin is extremely low. This can be attributed to its avid binding to endothelial cells and transport in shed microvesicles [65, 66]. Of note, evidence of coagulation system activation and intravascular fibrin deposition can be demonstrated well before, or even in the absence of the clinical manifestation of HUS [67, 68].

Table 24.1 STEC-HUS outbreaks and epidemics

Location [year]	Vehicle	Outbreak strain (Stx type)	Cases (hospitalized)	HUS	HUS risk	Mortality	Reference
Upper Bavaria, (Germany) [Sept–Nov 1988]	?	*E. coli* O157:NM (*stx2*)	6	6 (4–17 months; dialysis 6)	100%	0/6	[362]
Lombardia, Italy [Apr–May 1992]	?	*E. coli* O111:NM (*stx1* and *stx2*)	?	9	?	1/9 (11.1%)	[363]
West Coast (USA) [Jan–Feb 1993]	Beef patties (Hamburger; errors in meat processing and cooking)	*E. coli* O157:H7 (*stx1* and *stx2*)	501 (151; 31%) Children 278	45 (37 children)	9.0% (Children 13.3%)	3/45 (6.7%) 3/501 (0.60%)	[36] See also [126, 364–366]
South Australia [Jan–Feb 1995]	Dry fermented sausage (Mettwurst)	*E. coli* O111:NM (*stx1* and *stx2*)	?	21	?	1/21 (4.8%)	[367]
Sakai (Japan) [July 1996]	Bean sprout	*E. coli* O157:H7 (*stx1* and *stx2*)	12,680 (425; 3.4%)	12	0.09%	0/12	[39, 41, 172]
Scotland (UK) [Nov-Dec 1996]	Cold cooked meat from single butcher	*E. coli* O157:H7 (*stx2*, phage type 2)	512 (120; 23.4%)	36 (Children 6)	7.0%	17/36 (47.2%) 17/512 (3.32%; all deaths >65 years)	[368–370]
Walkerton, Ont. (Canada) [May 2000]	Contaminated municipal drinking water	*E. coli* O157:H7, *Campylobacter jejuni*	Symptomatic Self-reported 2300 (65; 2.8%)	HUS 30 (Children 22)	1.3% (total)	6/30 (20%) 6/2300 (0.26%)	[371–373]
Germany [2002]	Unknown	SF (sorbitol-fermenting) EHEC O157:NM	Unknown	38	Unknown	4/38 (10.5%)	[57]
Oklahoma (USA) [August 2008]	Food (diseased food workers in restaurant)	*E. coli* O111:NM (*stx1* and *stx2*)	344 (70; 20.3%)	25	7.3%	1/25 (4.0%) 1/344 (0.29%)	[374]
Northern Germany [May–June 2011]	Fenugreek	*E. coli* O104:H4 (*stx2*; STEC/EAEC hybrid strain)	3842	855 (children 90)	22.3%	54/855 (6.3%) 54/3842 (1.41%) Pediatric HUS 1/90 (1.11%)	[31, 239, 375, 376]

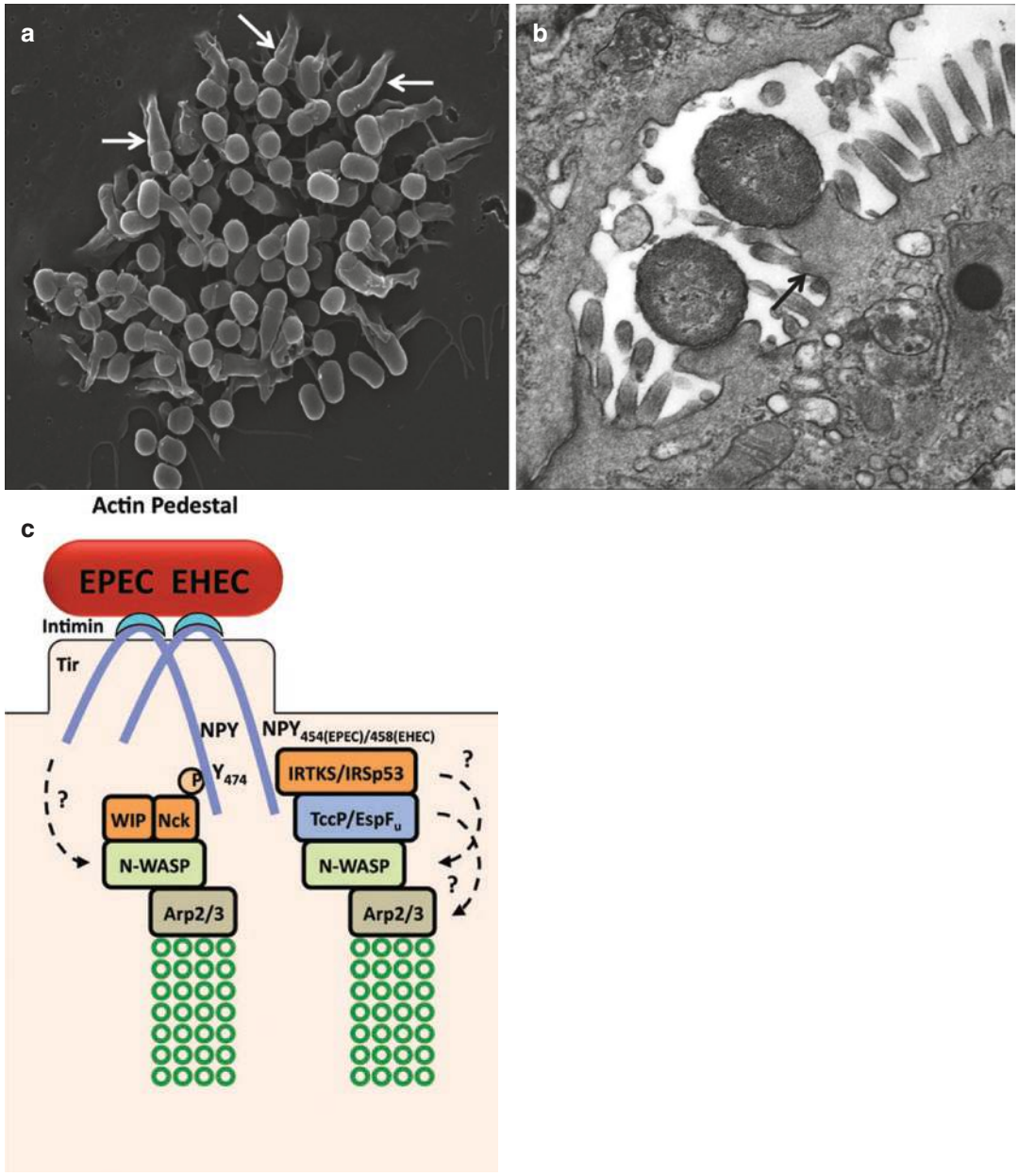

Fig. 24.1 STEC and related attaching and effacing (A/E) pathogens, such as enteropathogenic *E. coli* (EPEC), induce distinct histopathological lesions using the (bacterial) type III secretion system (T3SS) encoded by the "locus of enterocyte effacement" (LEE). (**a**) Scanning electron micrograph of pedestals induced by adherent bacteria (*arrows*). (**b**) Transmission electron micrograph showing intestinal A/E lesions (*arrow*). (**c**) Diagram depicting the actions of a subset of T3SS effectors of A/E pathogens on host cytoskeletal pathways and structures. *Green circles* represent actin filaments. (Used with permission of Jonn Wiley and Sons from Wong et al. [361])

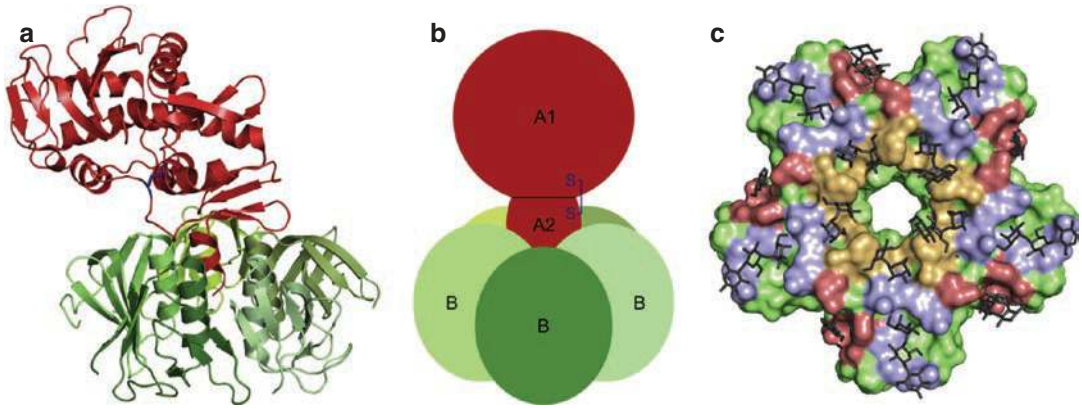

Fig. 24.2 (**a**) The structure of Shiga holotoxin as determined by X-ray crystallography. The A moiety is shown in *red*, the five B subunits in *green*, and the disulfide bridge linking the A1 and A2 fragments in *blue*. (**b**) Schematic representation of the Shiga toxin structure. (**c**) The surface of the B5 pentamer indicating the location of the 15 potential receptor binding sites, based on the structure of Shiga toxin 1. (Used with permission of Elsevier from Bergan et al. [69])

Shiga Toxin and Its Glycolipid Receptor

Shiga toxins consist of an enzymatically active 32.2 kDa A subunit and a receptor-binding B subunit, composed of 5 identical 7.7 kDa proteins (Fig. 24.2). The B subunit interacts with the terminal sugars of the glycolipid receptor, globotriaosylceramide (Gb3) [69]. Gb3 is identical with CD77 and the P^k blood group antigen [70–73] (Fig. 24.3). Although Stx1 and Stx2 display subtle differences in their affinity to Gb3, this does not readily explain why the vast majority of STEC-HUS cases are linked to Stx2 producing strains compared with Stx1 producers [74, 75].

Gb3 is the only functional receptor for Stx1 and most Stx2. Gb3 synthase knockout mice are resistant to the toxic actions of Stx [76]. Gb3 is found on microvascular endothelial cells (including glomerular and peritubular capillary endothelium), glomerular epithelial cells (podocytes), platelets and germinal center B-lymphocytes [77], and peripheral and central neurons [78, 79]. Cellular toxicity requires efficient transport of Stx to the endoplasmic reticulum and subsequent ribosomal RNA (rRNA) depurination [69, 80] (Fig. 24.4). Very few molecules are needed to paralyze the cell, making it one of the most potent known toxins. The action of Stx on the ribosome induces a ribotoxic stress response characterized by the activation of the MAP kinase pathway and apoptotic cell death [81, 82]. Evidence of apoptosis in kidney and other tissues has been demonstrated *ex vivo* in kidney biopsies from patients with STEC-HUS and in animal models of STEC infection [83–85].

Injured microvascular endothelial cells become prothrombotic. The mechanism(s) leading to intravascular hemolysis and acute thrombocytopenia are less well understood. Stx interacts with blood and plasma components and induces shedding of platelet and monocyte microparticles loaded with tissue factor and complement [86, 87] (Fig. 24.5). Biologically active microparticles and (direct) Stx-induced apoptosis of endothelial cells, including the externalization of plasma membrane phosphatidylserine [88], may provide a mechanism how microvascular thrombosis is initiated [89]. The molecular and human cellular biology of Shiga toxins has been summarized in excellent review articles [65, 90, 91].

Laboratory Diagnosis of STEC Infections

Tests must be sensitive, specific and easily accessible. Rapid microbiological diagnosis of STEC diarrhea is essential [92] and can be aided by PCR tests. The clinician should provide the labo-

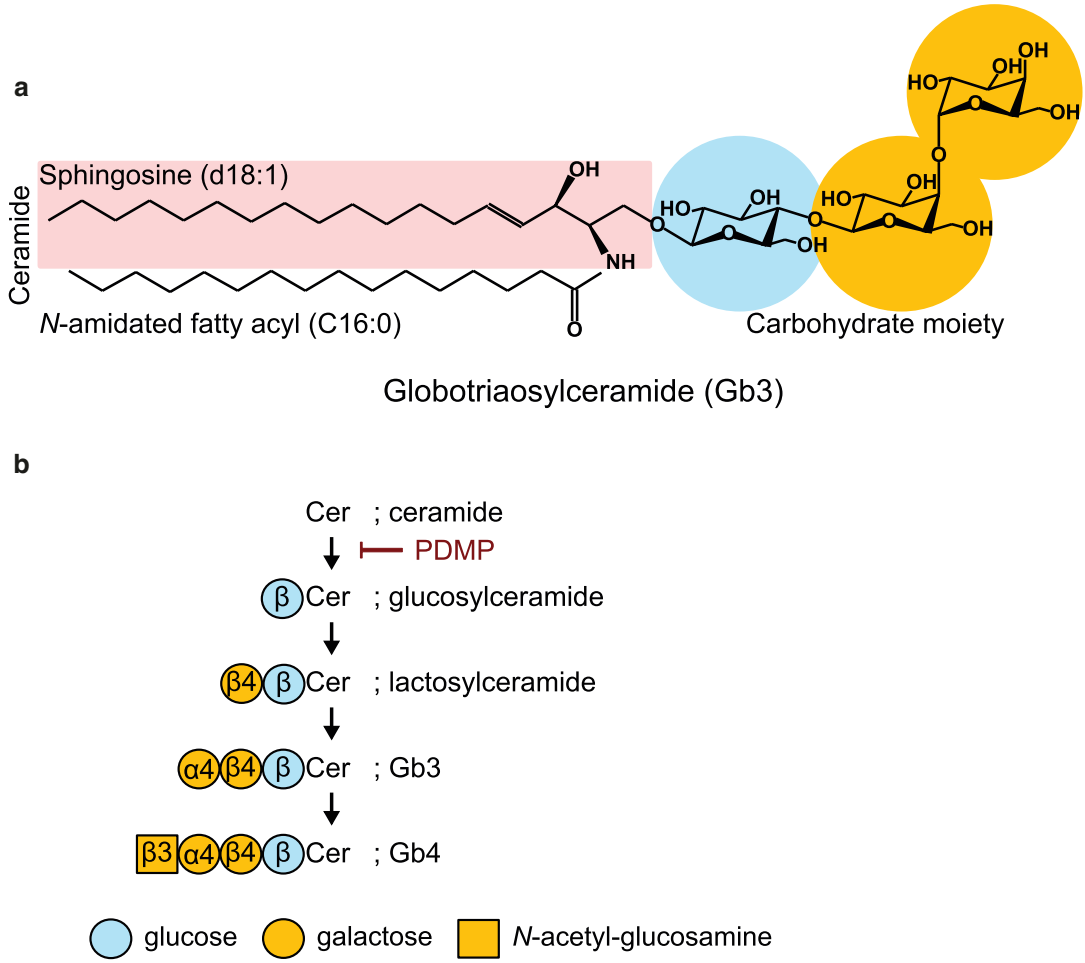

Fig. 24.3 (**a**) Structure of the Shiga toxin receptor Gb3. Glucose and galactose of the carbohydrate moiety are shown in *blue* and *yellow*, respectively. The ceramide moiety consists of a sphingosine backbone (in *pink*) and a variable fatty acid chain. (**b**) The steps in the synthesis of the globo-series of glycosphingolipids from ceramide, showing the sequential addition of carbohydrates represented by the glycan symbol system. (Used with permission of Elsevier from Bergan et al. [69])

ratory with meaningful clinical information, including the presence of painful or bloody diarrhea, or signs of HUS, and if there is a suspected outbreak. If the stool culture of an index patient is STEC negative, the pathogen may be identified in other family members [93].

An etiological diagnosis is important. It separates STEC-HUS from other HUS forms and impacts on acute treatment decisions and long-term follow-up [15, 94]. STEC detection has implications for close contacts, care facilities, restaurants/kitchens and the food industry. STEC infections are notifiable and require isolation measures to curb further transmission during an epidemic [95, 96]. In the bigger picture, bacterial isolation allows monitoring of epidemiological changes, such as the emergence of new strains and virulence traits.

Current recommendations stipulate that stools be plated simultaneously on an *E. coli* O157:H7 selective agar (or alternative bacteriological methods) and tested for the presence of Stx using a fresh stool suspension or overnight broth culture [97, 98]. Stx immunoassays or multiplex PCR

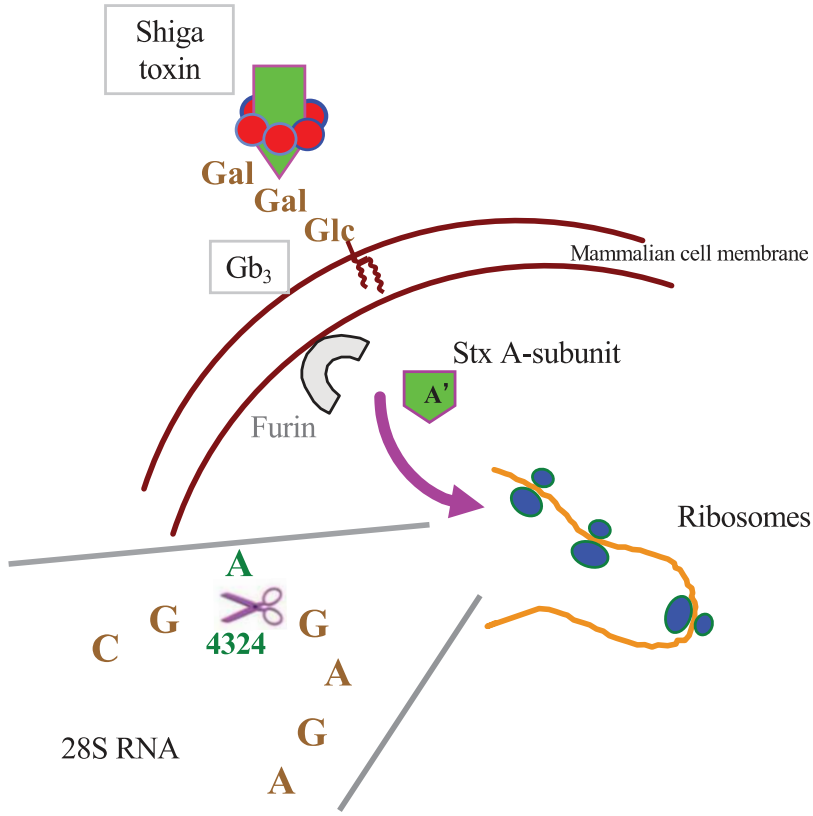

Fig. 24.4 Schematic diagram of the biological action of Stx in susceptible mammalian cells. The holotoxin binds to lipid raft-associated membrane globotriaosyl ceramide (Gb3) and enters the cell via clathrin-mediated endocytosis. A and B subunits become disengaged and the A subunit is cleaved and activated by intracellular furin. Upon "retrograde" passage through the Golgi apparatus, the A′ subunit selectively removes a specific adenine residue from the 28S RNA of the large ribosomal subunit (N-glycosidase activity). This results in (1) blockade of active (translating) ribosomes (translational inhibition), and (2) a ribotoxic cellular stress response with activation of c-jun and p38 (MAP) kinases and/or apoptotic cell death. (Used with permission of author and Elsevier from Loirat et al. [286])

alone are not sufficient for clinical samples [99]. Cell culture-based assays, although highly sensitive and specific, are not routinely performed.

Before the use of Shiga toxin assays and PCR, the isolation of non-O157:H7 STEC strains among commensal gut flora by traditional microbiological techniques was laborious, which contributed to the delayed appreciation of non-O157:H7 STEC clones as a cause of enterocolitis and HUS.

The yield of the stool diagnostic diminishes when the sample is delayed beyond 4 days after diarrhea onset [53, 63]. If stool culture or toxin assay(s) are negative, serological assays can be employed to search for elevated (or rising) IgM class antibodies to one of the more common STEC O-groups (LPS antigens) [62, 100–102]. Saliva IgA and IgM provide an alternative to serum antibodies [102, 103]. However, serodiagnostic assays are only offered in a few laboratories. Testing for Stx-specific antibodies has been used as an epidemiological tool [104, 105], but its clinical utility is limited [106].

From Colitis to HUS

The spectrum of STEC disease ranges from mild diarrhea and HC to severe HUS, and death [41, 53, 107]. The diarrhea is typically painful and

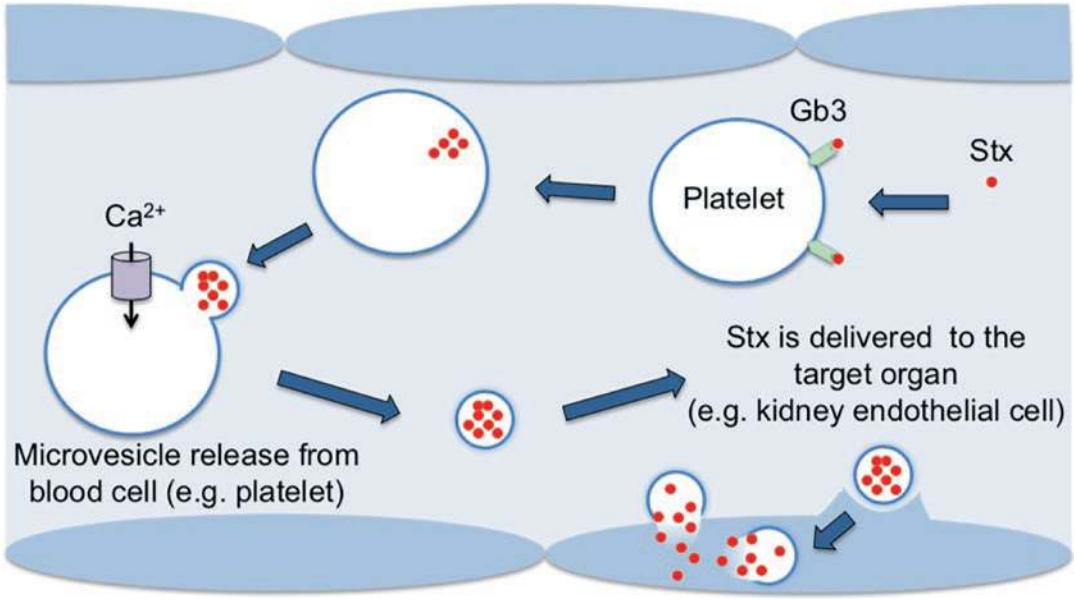

Fig. 24.5 Pathophysiological model incorporating the postulated role of microvesicles in the transfer of Shiga toxin (Stx) to target endothelium. Once in the bloodstream, Stx binds to platelets, neutrophils and red blood cells (RBC) via the toxin receptor, globotriaosylceramide (Gb3). The toxin is internalized and the activated blood cell releases microvesicles into the circulation containing the toxin. Upon reaching the target organ the microvesicles are taken up by microvascular endothelial cells, where Stx is released. In the kidney, this has been shown to occur within glomerular and peritubular capillary endothelial cells. (Used with permission from Karpman et al. [11])

frequent, with >15 small, soft or liquid stools that turn bloody by day 2 or 3 in >80% of children with STEC O157:H7 infection. The amount of visible blood varies from a few specks to frank hemorrhage. About 50% of patients develop nausea and vomiting; low-grade fever is present in one third [32, 53, 67, 92, 107]. Infections by non-O157:H7 STEC serotypes are generally milder [32, 108]. Exceptions are infections by STEC clones belonging to serogroups O26, O55, O91, O111, among others, that can be clinically indiscernible from those by classical *E. coli* O157:H7 [28, 55, 57, 109–115].

The HUS risk is greatest at the extremes of age, in children <5 years and the elderly; it decreases during childhood and adolescence, and is <0.1% in young and middle-aged adults [53, 116, 117]. Conversely, STEC colitis can lead to AKI and death without HUS, particularly in the elderly [116, 118]. Other variables impacting on the HUS risk are the STEC serotype or clone, the toxin type(s) produced, and preexisting immunity [105].

HUS starts abruptly, about 3–10 days (median 6 days) after the onset of diarrhea [53] (Fig. 24.6). Patients present from one day to the other with fatigue and pallor, become listless and may develop petechiae, often after transient clinical improvement of the colitis. Absent bowel movements during the acute phase of HUS should raise the suspicion of intussusception or ileus.

The clinical diagnosis of HUS is generally straightforward, based on the defining triad of intravascular hemolysis, thrombocytopenia and AKI. Hemolytic anemia of HUS—characterized by RBC fragmentation with schistocytes in the peripheral blood smear, with or without thrombocytopenia—is a microangiopathic hemolytic anemia (MAHA). With disease progression, serum creatinine levels rise and oligoanuria, hypertension and edema may appear, usually within 1–2 days of first HUS-related symptoms. Some patients may recover before the full picture of HUS develops. They may only demonstrate hemolytic anemia without apparent AKI, while

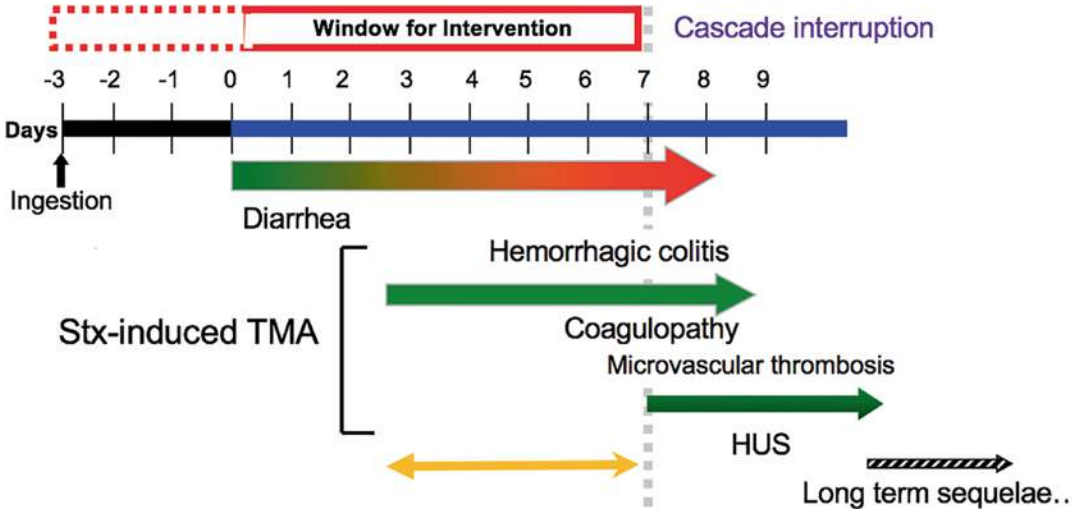

Fig. 24.6 Schematic diagram of the development of Shiga toxin-producing *E. coli* (STEC) colitis and HUS and possible window of therapeutic intervention

platelets may briefly dip within or slightly below the reference range ("partial HUS") [52, 119].

A predictive biological marker of impending HUS is the degree of systemic inflammation during the preceding colitis, particularly neutrophilia with a high-percentage shift to band forms, and a sharp rise of acute phase reactants, such as C-reactive protein or procalcitonin [41, 120–124]. Patients who progress to HUS more often have a peripheral neutrophil count above 20×10^9/L than patients without HUS [122, 125–127].

Hematologic Manifestations of STEC-HUS

Commonly, the hemoglobin level drops precipitously to <80 g/L in STEC-HUS. Hemolysis is accompanied by a rapid fall of platelet numbers, usually <50, at times $<30 \times 10^9$/L. Direct antiglobulin (Coombs) test is negative. Rising indirect bilirubin, free plasma hemoglobin and serum lactate dehydrogenase (LDH), the latter often more than five times the upper limit of normal, and haptoglobin depletion are consistent with rapid (intravascular) hemolysis. The peripheral WBC (neutrophil) count, already increased during the colitis phase, may continue to rise and, if associated with a leukemoid reaction, can herald a severe course with poor intestinal or kidney outcomes [33, 128, 129]. The severity of anemia and thrombocytopenia does not correlate with the degree of acute or chronic kidney injury.

Some authors noted an inverse relationship between Hb and disease severity [129–131]. A plausible explanation for the latter observation is the hemo concentration seen in patients with intravascular volume depletion during the first 4 days of STEC colitis [129, 132]. The recognition that hemo concentration increases the HUS risk in children with STEC diarrhea led to the development of an "HUS risk score." It is considered "high," where the sum of the concentrations of Hb plus 2x creatinine (in g/dL and mg/dL, respectively) exceeds a value of 13 [131]. The correlation between a high risk score and severe HUS/poor outcome was independently confirmed [133, 134], yet its clinical utility remains to be seen.

Thrombocytopenia and hemolysis usually resolve within 2 weeks. A rising platelet count heralds the cessation of HUS activity; platelets may transiently rebound to $>500 \times 10^9$/L. Anemia can persist for weeks after disease recovery without signs of active hemolysis.

AKI in STEC-HUS

Kidney injury in STEC-HUS ranges from microscopic hematuria and proteinuria to severe kidney failure and oligoanuria. Up to 50% of children with STEC-HUS will need acute dialysis [33, 34, 135, 136]. Arterial hypertension is common in the acute phase of HUS and may not be due to volume overload. Blood pressure instability and hypotension are not a typical feature of STEC-HUS and should raise the suspicion of sepsis. Time to recovery of kidney function ranges from a few days to weeks or even months. The risk of long-term kidney impairment (CKD, chronic hypertension, proteinuria) increases with the duration of oliguria (dialysis). A commonly cited threshold for the risk of diminished kidney recovery is anuria lasting >2–3 weeks [137, 138]. Primary ESKD is rare and should prompt investigations into a non postinfectious form of HUS [139].

Extrarenal Manifestations

There is hardly an organ system that may not be affected in STEC infection or STEC-HUS. Rare, but important extrarenal and extraintestinal manifestations are myocarditis and congestive heart failure, cardiac tamponade, pulmonary hemorrhage, pancreatitis, hepatic injury, and central nervous system (CNS) complications [140–143]. Patients with multiple organ involvement generally also have severe kidney failure and often poor outcome [141, 142, 144]. Postulated mechanisms underlying organ injury in STEC-HUS are Stx load and direct tissue toxicity, and ischemic injury [141], as evidenced by histological and autopsy findings.

Rectal prolapse and intussusception may result in transmural bowel necrosis with perforation and peritonitis [145–147]. Serum amylase and lipase activities, indicative of exocrine pancreas injury, are elevated in up to 20% of patients [145]. Transient glucose intolerance or, albeit rare, chronic insulin-dependent diabetes mellitus has been reported [146, 148–150]. Hepatomegaly and/or rising serum alanine amino transferase (ALT) are noted in up to 40% of cases. Acute myocardial insufficiency occurs in less than 1% of cases [141, 151–153]. Skeletal muscle involvement is exceedingly rare and may manifest as rhabdomyolysis [143].

Three to >41% of patients experience CNS manifestations [124, 143, 154–157]. Signs and symptoms can be vague and nonspecific and are probably underappreciated. Patients may present with irritability, lethargy or decreased level of consciousness. Brief seizures are relatively common and may reflect fluid and electrolyte imbalances and inadequate volume replacement [158]. Abnormal electroencephalograms have been reported in up to 50% of patients with HUS. Prolonged seizure activity, usually associated with acute respiratory deterioration, is an ominous sign and may indicate cerebral stroke or hemorrhage. Acute, transient or persistent isolated palsy, dysphasia, diplopia, retinal injury and cortical blindness have been noted [142, 143, 155]. Severe neurological events are associated with poor prognosis and even death [142].

Magnetic resonance imaging (MRI) of the brain is helpful in the differentiation of structural from ischemic or transient injury. In the acute phase, basal ganglia and white matter abnormalities with apparent diffusion coefficient restriction are common and reversible MRI findings [159–164] (Fig. 24.7). However, the described changes do not appear to be specific for STEC-HUS [164]. CNS lesions may be compounded by the occurrence of posterior reversible encephalopathy syndrome or hemodialysis-associated disequilibrium [164]. Peripheral nervous system involvement has been described, but pathogenesis and medical treatment of this rare complication remain unclear [165].

The *E. coli* O104:H4 HUS outbreak in Europe highlighted the occurrence of psychiatric symptoms [166, 167]. Described manifestations include cognitive impairment [168] and, in a few cases, hallucinations and affective disorders, such as severe panic attacks [166].

Histopathology of STEC-HUS

Few pathological descriptions are available from kidney biopsies of patients with acute STEC-HUS [85, 169–173]. Macroscopically, the kidneys appear swollen, with numerous petechial

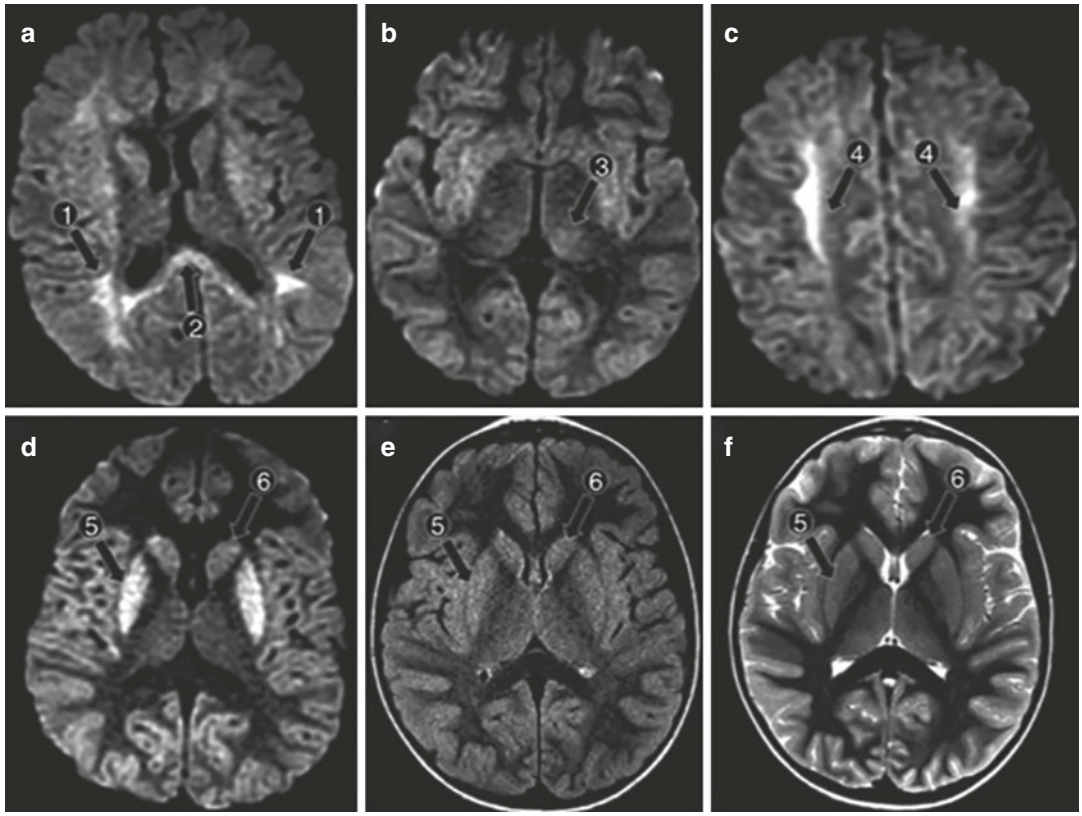

Fig. 24.7 Brain magnetic resonance imaging of patients with STEC-HUS acquired within the first 24 h after the onset of neurological symptoms. (**a–d**) Diffusion-weighted images (DWI) which demonstrate (*1*) hypersignal involving deep white matter; (*2*) corpus callosum; (*3*) thalamus, (*4*) centrum semiovale; (*5*) putamen; and (*6*) caudate nucleus. (**d–f**) Brain MRI of one of the two patients who died. (**d**) The images of this patient demonstrate deep hypersignal on DWI in putamen (*5*) and caudate nucleus (*6*), that can be detected in T2- (**e**) and fluid-attenuated inversion recovery (FLAIR) (**f**) weighted classical imaging. T2- and FLAIR-weighted images of all the surviving patients were normal (not shown). (Used with permission of John Wiley and Sons from Gitiaux et al. [164])

hemorrhages on the external surface; on section, the cortex will show areas of hemorrhage and infarction. Focal hemorrhage has also been noted in the collecting system and ureter (Chantal Bernard, unpublished communication). Prominent *light microscopic* features are the presence of fragmented RBC in *glomerular* capillary loops. Glomerular capillary and renal arteriolar microvascular thrombosis may demonstrate prominent fibrin staining. Endothelial and mesangial cell changes are also evident by *electron microscopy* (Fig. 24.8). *Immunofluorescence* is variably positive for fibrin. Immune deposits are not a feature of STEC-HUS. While glomerular histological changes dominate, the *tubulointerstitial compartment* can also be affected. In fact, all biopsies from a series of patients examined during the 2011 German HUS outbreak, including those without evidence of glomerular TMA, showed severe acute tubular injury [173].

Post-mortem studies of children with STEC-HUS [169, 170, 174] demonstrated that STEC-HUS is a systemic microangiopathy characterized by ubiquitous endothelial cell swelling and injury. There was diffuse hemorrhage with mucosal ulcerations and hemorrhage of the bowel wall as well as congestion of the serosa and extensive vascular thrombosis. The pancreas appeared enlarged and swollen, with areas of necrosis and hemorrhage (Chantal Bernard, unpublished

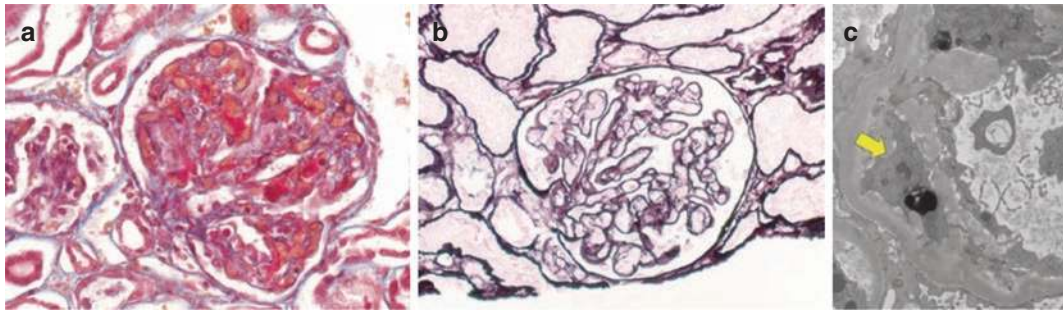

Fig. 24.8 Kidney biopsy, culture-proven STEC-HUS. (**a**) Trichrome stain of glomerulus showing fibrin and RBCs (*orange* and *red*, respectively). (**b**) Silver stain, emphasizing glomerular and tubular basement membrane structures. (**c**) Electron microscopy; a glomerular capillary is shown. *Arrow* indicates fibrin and proteinaceous material pushing toward the capillary lumen and creating the impression of double contours of the glomerular basement membrane. (Courtesy of Dr. Natacha Patey CHU Ste-Justine)

observations). CNS changes consisted of brain swelling and bilateral, symmetric necrotic lesions, mainly of the corpus striatum, and scattered necrotic lesions in the cortex and elsewhere [175].

Prevention and Treatment of STEC Disease

Preventive strategies focus on the implementation of hygienic measures. This applies to cattle farming, the management of drinking water and agricultural produce, safe practices of food preparation and consumption, and containment of the spread of the organism when cases are identified [95, 96, 176–178]. The risk of transmission is reduced by adherence to essential hygienic measures: frequent hand washing and avoiding touching the face [177, 179]. Children with proven STEC infection should only return to childcare or school 48 h after the cessation of diarrhea. Preventive measures and advice for patients and caregivers can be found in various publications [176, 177, 179].

Vaccines

Active immunization of humans, targeting the O157 LPS antigen [180, 181] and/or Stx and other bacterial antigens remains an elusive goal [182, 183]. Progress has been reported, however, in the vaccination of cattle [183, 184].

Therapeutic Interventions During STEC Colitis

Considerable work has been invested to better understand factors that facilitate the progression from colitis to HUS and to intervene at a stage where the process can be reversed, and HUS prevented or at least mitigated. Potential strategies are the elimination of STEC (or Stx) from the gut and toxin neutralization in the circulation [185–187]. None of these approaches have shown convincing results [188]. Randomized, controlled HUS prevention trials in a conventional format are extremely challenging due to the low incidence of STEC infections and the overall low risk of progression to HUS [187, 189–191].

Fluid Therapy

Volume expansion with isotonic saline administered intravenously during early STEC colitis may ameliorate the severity of HUS. In a retrospective cohort study of children with *E. coli* O157:H7 HUS [132], patients who became oligoanuric and needed dialysis had received significantly less intravenous fluid during the first 4 days of diarrhea than children who had preserved urine output and who were not dialyzed. The authors concluded that early parenteral volume expansion before the onset of HUS attenuates AKI and reduces the need for dialysis. Subsequent studies reproduced the initial findings [130, 190, 192, 193]. The “volume hypoth-

esis" is further supported by observations linking higher Hb concentrations at presentation—as a surrogate marker of volume depletion—to severe HUS, including neurological complications [75, 131, 134, 154, 193].

The administration of isotonic solutions is not expected to affect Stx production or tissue binding, but is intended to alleviate incipient acute tubular necrosis and AKI [132, 194]. Saline infusion may also mitigate abdominal cramps caused by STEC induced ischemic colitis [193, 195]. Considering HUS rates of 8–15% in an "under 5" target population, the number needed to treat to prevent one case of HUS is about 7–12.

For practical purposes, rapid confirmation of STEC infection, mainly by PCR (ideally with a multiplex platform that allows testing for *stx1* and *stx2*, and various pathogenic bacteria and viruses in the same stool sample) and easy-to-follow protocols targeting children with bloody and non-bloody diarrhea are needed. Including non-bloody diarrhea cases is relevant, since up to 57% of STEC-HUS patients lack a history of bloody diarrhea or colitis [75, 196].

Figure 24.9 shows an abbreviated, practical algorithm to estimate the individual child's risk of HUS and initiate treatment as soon as STEC colitis is diagnosed or suspected. The risk of fluid overload and cardiopulmonary complications due to saline infusion is negligible, provided the patient is hospitalized and supervised by an experienced team [190]. Hospital admission simplifies patient monitoring. It may also mitigate parental anxiety and reduce the spread of the potentially dangerous organisms [95, 96].

Analgesia

When volume expansion with isotonic saline fails to alleviate the ischemic colitis pain, pharmacological therapy may be warranted. Acetaminophen can be tried. Morphine may be administered sparingly, although it tends to worsen post-colitis constipation or ileus. Non-steroidal anti-inflammatory analgesic drugs

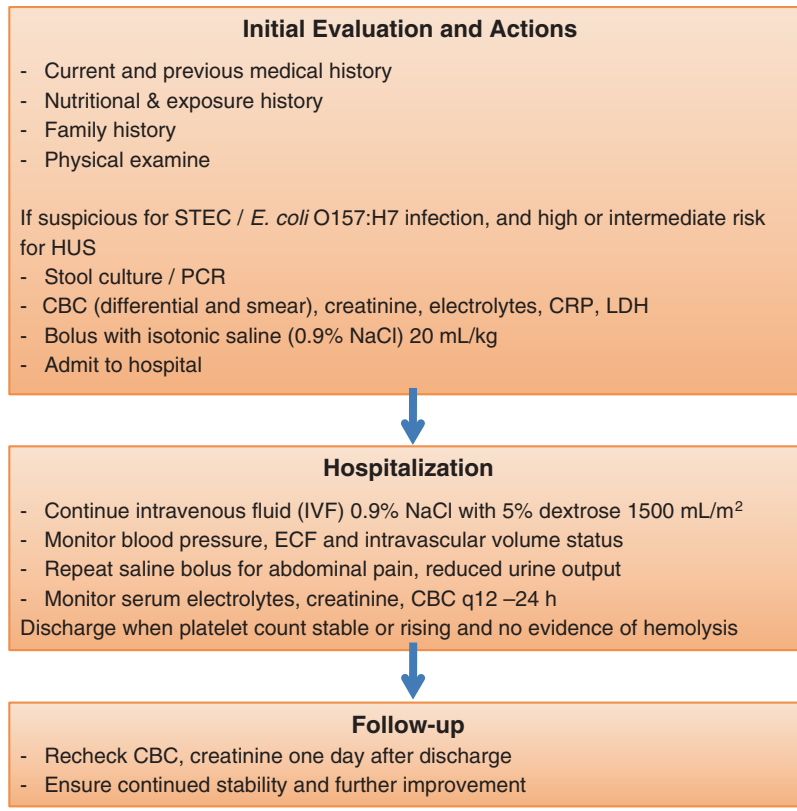

Fig. 24.9 Initial evaluation, monitoring and intervention for STEC infection. Risk of Stx HUS: Age group (>6 months) and diarrhea <4 to 7 days that is frequent, turned bloody after 2–3 days and/or is associated with abdominal cramps; recent or current hemorrhagic colitis or HUS in family or community. Rapid PCR (stool or rectal swab) or antigen assay, and culture for STEC (at least *E. coli* O157:H7), *Campylobacter*, *Salmonella*, *Shigella*, *Yersinia spp*. (Used with permission of AGA Institute from Holtz et al. [92], with modifications)

(NSAIDs) should not be used in patients with STEC colitis or HUS who may be intravascularly volume depleted and at risk of ischemic injury of the gut and kidneys.

Anti-Motility Drugs and Antibiotics

Antidiarrheal and antimotility agents have been associated with an increased risk of HUS and should therefore be avoided [32, 126, 154, 197–199]. The observed link between antimicrobial therapy and an increased HUS risk or fatal outcome is supported by experimental studies demonstrating that certain antibiotics stimulate Stx phage induction and toxin production [200–202]. However, the risk of HUS differs according to the class of the antibiotic and the timing of its administration [203, 204]. Evidence is emerging that rifaximin, azithromycin or fosfomycin may limit the spread of the organism and reduce the rate of complications during STEC epidemics [203–207].

Therapeutic Intervention During STEC-HUS

Symptomatic treatment of HUS follows general principles established for patients with AKI, but with specific recommendations related to the often profound hemolysis and thrombocytopenia. All patients need careful monitoring of vital signs, fluid balance and cardiac, respiratory, metabolic and neurological status. Treatment focuses on the normalization of intravascular volume status, acid-base balance, serum electrolytes, glucose and uric acid. As with other critically ill children, providing appropriate nutrition is part of the treatment. Early transfer to an experienced pediatric center is recommended, where extracorporeal purification techniques and a critical care environment can be provided 24/7. Most patients with HUS receive packed red blood cells (PRBCs) [135]. The threshold for PRBC infusions is clinically defined: symptoms (tachycardia and tachypnea) and the velocity of intravascular RBC destruction as gauged by LDH and free Hb levels in plasma. A practical cut-off is a Hb of 60 g/L. 12–15 mL of PRBC per kg body weight can be transfused over 2–4 h. A loop diuretic can be given in case of fluid overload or hyperkalemia and (some) preserved urine output. If necessary, PRBC transfusions can be timed to coincide with dialysis sessions, particularly if hyperkalemia or volume overload are a concern. RBCs should be leucocyte and platelet depleted, as practiced in most pediatric hospitals. Peritoneal and central venous catheters can be placed safely in most children with HUS without a need for platelet transfusion [208].

AKI Management in STEC-HUS

Assessment of intravascular volume status helps guide initial treatment toward fluid replacement or restriction, and administration of diuretics or dialysis. Patients are monitored for fluid intake and changes in urine output, along with frequent measurements of blood pressure (BP) and heart rate. Weight changes correlate poorly with effective circulatory volume. Intravascular volume may be decreased due to intestinal losses and reduced oral intake during the early phase of the disease and may result in hypoperfusion of the kidneys. Third spacing, especially in the gut, and generalized edema—due to endothelial injury and capillary leak—may mask intravascular depletion and, if not corrected, worsen ischemic injury. Patients warrant diligent intravascular volume expansion to improve organ perfusion, particularly of the gut, kidneys and brain.

Fluid restriction may be necessary in patients with fluid overload secondary to oliguric kidney failure. If intravascular volume is replete, a trial with furosemide at a dose of 1–2 mg/kg may be attempted to induce diuresis and delay dialysis, particularly in patients with hyperkalemia or cardiopulmonary compromise. Aggressive challenge with high-dose loop diuretics, advocated in the past to prevent progression to oligoanuric failure and avoid dialysis [209] should be avoided.

Antihypertensive Therapy

Arterial hypertension is common in STEC-HUS. It may be caused by renal microvascular thrombosis, direct vascular endothelial cell injury or activation, and intravascular fluid overload. Systemic hypertension can lead to CNS complications, such as PRES. It is reasonable to use dihydropyridine calcium channel blockers (nifedipine, amlodipine or PO/IV nicardipine).

Treatment with renin-angiotensin system (RAS) blockers has to be balanced against concerns over impairment of kidney perfusion and hyperkalemia. However, RAS inhibition is a rationale choice for the long-term treatment of patients with HUS-induced CKD and/or chronic hypertension or proteinuria [210, 211].

Kidney Replacement Therapy

There is no evidence that early dialysis changes the evolution of acute STEC-HUS or long-term outcome. However, delaying dialysis unduly increases the risk of complications. The North American Synsorb Pk® trial protocol mandated that dialysis not be started until 72 h post diagnosis of HUS, if clinically acceptable by the responsible physician. Under these restrictive conditions, 39% of the 49 placebo-treated patients were dialyzed for a mean of 3.6 days [135]. Indications for dialysis initiation in HUS are similar to those for other causes of AKI and may evolve rapidly: severe electrolyte imbalance (hyperkalemia, hyperphosphatemia), acidosis or fluid overload refractory to medical/diuretic therapy, or symptomatic uremia.

The choice of the dialysis modality depends on clinical and practical aspects, such as availability of PD and HD and adequately trained personnel, patient size for central access creation, local preference and experience, particularly when dialyzing young children and infants. Diarrhea or colitis are not considered contraindications for peritoneal dialysis (PD) [135, 189, 212].

Continuous renal replacement therapy (CRRT) or "slow" HD offer alternatives to conventional HD in patients with severe fluid overload, cardiovascular instability, with or without sepsis, and multiorgan failure. Both are typically performed in a critical care setting. HD and CRRT can be tried with minimal or no heparin, provided there is sufficient blood flow through the circuit. Regional, citrate-based anticoagulation offers an alternative to heparin, specifically in patients with cerebral stroke or hemorrhage, or after surgery.

Some pediatric centers consider severe, life-threatening STEC-HUS an indication for plasma exchange (PE) as "rescue" therapy, especially for patients with CNS complications [189, 213]. This approach may have been influenced by the success of PE in aHUS and TTP. However, the benefit of plasma therapy (PE or plasma infusion) in STEC-HUS remains unproven [214–217]. The European Paediatric Study Group for HUS excluded STEC-HUS from PE [94]. In young children, PE requires the insertion of a large-bore central venous access (HD line), which confers additional morbidity to children not already undergoing HD [19]. The guidelines for the use of apheresis by the American Society for Apheresis (ASFA) categorized STEC-HUS as III, i.e. a disorder where the role of apheresis treatment is not established, with weak recommendation and low-quality evidence (2C) [218].

Immunoadsorption (IA) is a specialized apheresis technique for the selective removal of humoral factors such as immunoglobulins and complement from plasma via a high-affinity adsorbent column [219]. However, reports of its use for STEC-HUS is anecdotal [220–222].

Anticomplement Therapy in STEC-HUS

The role of the complement system in the pathogenesis of STEC-HUS gained traction over the past decade [223–227]. Several events stimulated scientific inquiry and clinical interest: progress in the understanding of atypical HUS caused by genetic or acquired dysregulation of the alternative pathway, availability of a potent, well-tolerated anti-C5 antibody (eculizumab), and its compassionate use during the *E. coli* O104:H4 epidemic in Northern Germany.

Increased plasma levels of complement activation products derived from factor B (CFB) and C3, and of the terminal complement complex sC5b-9 [86, 227, 228] as well as the deposition of C5b-9 in post-mortem tissue (Fig. 24.10) suggests that complement is activated in STEC-HUS. Interestingly, no correlation was found between the levels of complement products in plasma or platelet derived microparticles and the presence or absence of kidney or extrarenal complications [86, 228].

Data on the use of eculizumab in (severe) STEC-HUS have been recently summarized [229, 230]. Despite signals indicating clinical improvement,

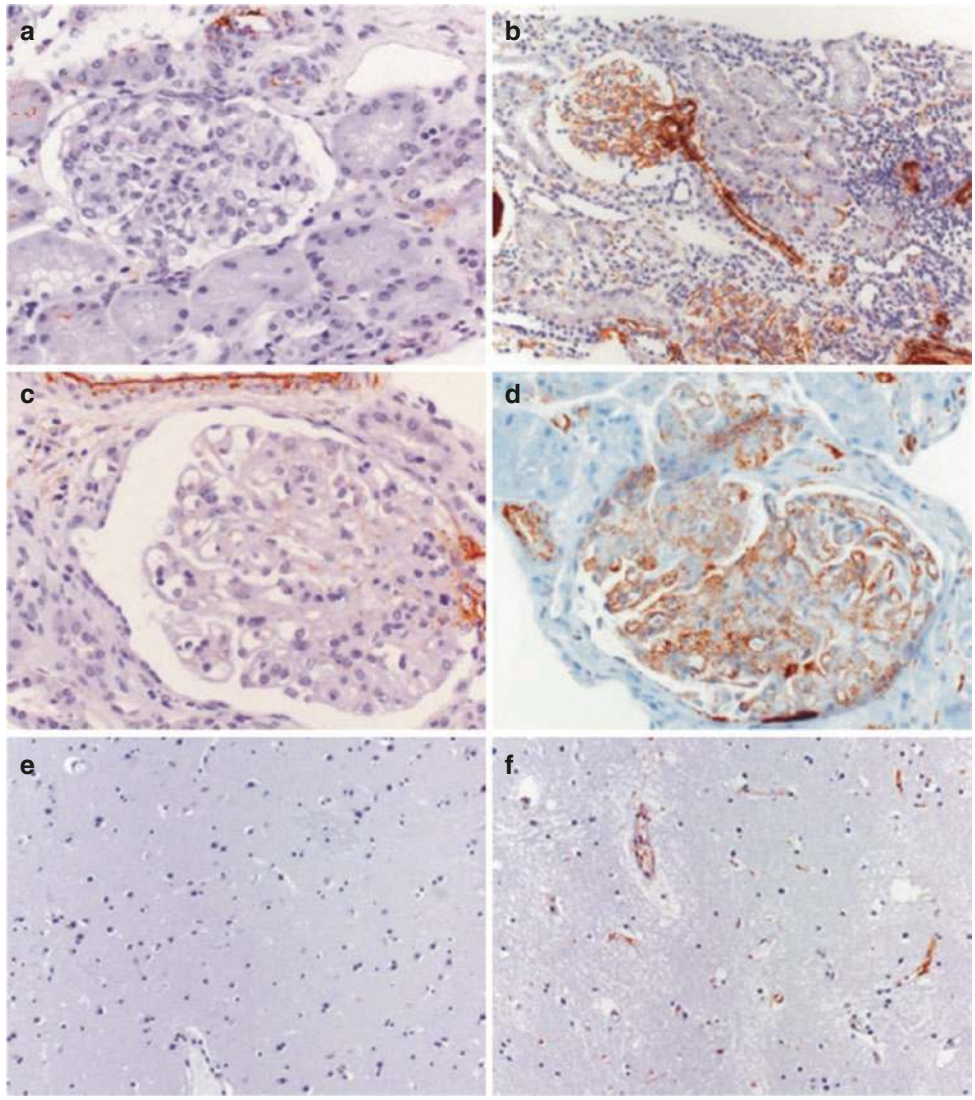

Fig. 24.10 C5b-9 (membrane attack complex, MAC) deposition in kidney and CNS sections of a child who died of STEC O157:H7 HUS. (**a**) Control (kidney). (**b**) Strong staining of kidney arteriole and glomerular capillaries with anti-MAC antibody (1:200). (**c**) Staining of afferent and efferent arterioles, minor staining within the glomerulus. (**d**) Ubiquitous glomerular capillary staining with anti- MAC antibody. (**e**) control (brain). (**f**) Staining of cerebral capillaries with anti-MAC antibody. (Courtesy of Dr. Natacha Patey CHU Ste-Justine)

mainly of neurological complications, the overall quality of evidence is low [230, 231]. It remains to be shown, if and under which conditions Stx-associated complement activation becomes deleterious and requires targeted treatment. While awaiting definitive trial results [232, 233], the off-label use of anticomplement agents in patients with STEC-HUS should be reserved for severe forms (especially in cases with CNS or cardiac involvement) and balanced against potential adverse effects and costs.

Complications and Long-Term Outcome

AKI due to STEC-HUS is frequently self-limited. Hematological improvement (decreas-

ing LDH, increasing platelet count) generally precedes kidney recovery. Overall, the outcome of STEC-HUS has improved substantially since its first description. Reported mortality rates typically vary between <1% and 5% [234], mostly due to CNS, cardiac, or gastrointestinal complications. About 70% of patients recover completely from the acute episode [125, 129, 142, 146, 152, 153, 234–236]. Complications appear to be more frequent in adults with STEC-HUS than in children, and the lethality in the elderly population may be as high as 50% [117, 237–240].

Long-Term Outcome and Monitoring

Long-term kidney complications have been reported in 5 to 25% of patients [113, 129, 241, 242]. 15–30% of patients have proteinuria, typically mild, and 5 to 15% have arterial hypertension. CKD has been noted in approximately 10% of surviving patients and ESKD in 3% [113, 125, 138, 234, 243]. Up to one-third of children with severe HUS (defined as anuria >8 days or oliguria >15 days) developed long-term sequelae [137, 138, 244]. Yearly evaluation of kidney function, BP and urinalysis has been recommended for at least 5 years, and indefinitely for patients with decreased GFR, proteinuria and/or hypertension [113, 138, 148, 234, 243].

Up to 30% of children with CNS manifestations during HUS will develop long-term neurological sequelae [235]. There is probably underdiagnosis of subtle neurological problems such as learning and behavioral difficulties, reduced fine motor coordination, and attention deficit and hyperactivity disorder [245].

STEC-HUS and Kidney Transplantation

For the small percentage of STEC-HUS patients who progress to ESKD, kidney transplantation is the optimal therapy [113, 246]. When a genetic cause of complement dysregulation is ruled out, graft and patient survival are comparable to other kidney transplant recipients [246–250].

Shigella dysenteriae HUS

HUS due to *Shigella dysenteriae* type 1 (SD1) is rare. Yet it is the leading cause of death in outbreaks of SD1 infections [251–254]. Unlike STEC, *S. dysenteriae* is an invasive organism. It penetrates the bowel wall and enters the blood stream. Patients with SD1 infection should be treated early with appropriate antibiotics [255]. The spread of highly resistant SD1 strains is a challenge [256]. Antimicrobial treatment after the first 4 days of diarrhea has been identified as a risk factor for HUS [257].

HUS occurs four to 17 days after the onset of bloody diarrhea, and occasionally after diarrhea has improved. Shiga toxin (Stx) is involved in the pathogenesis of *S. dysenteriae* colitis and HUS. The incidence of HUS among children with SD1 dysentery is less than 0.4% (median age 3 years) [258] (Table 24.2). A high peripheral neutrophil count has been associated with the development and the severity of HUS [259, 260]. Oliguric AKI has been described in 90% of SD1-HUS cases, dialysis in 52%, and disseminated intravascular coagulation in 21% [261]. Additional complications are listed in Table 24.3.

The reported mortality of SD1-HUS is substantially higher than the mortality due to STEC-HUS. However, most of the burden of S. *dysenteriae* infections and SD1-HUS is carried by under resourced countries [255, 257, 262].

Pneumococcal (*Streptococcus pneumoniae*) HUS

Epidemiology

Invasive pneumococcal disease (IPD) may lead to HUS, referred to as pneumococcal HUS (pnHUS). It occurs in <0.7% of IPD episodes [263, 264] and affects mostly infants before the age of 2–3 years [264–266], although older children and adults are also affected [267]. The 10-year incidence is about 1.2 per 100,000 children under 15 years [268]. Indigenous populations may have a higher

Table 24.2 Pediatric *Shigella dysenteriae* type 1 (SD1) HUS [a]

Country	# of reported cases	Age in years (mean or median)	Interval between onset of diarrhea and diagnosis of HUS (days)	Case fatality rate (%)	Reference
South Africa	151	4.6	7	17	[261, 377–379]
Zimbabwe	110	1.5	11	41	[379, 380]
India	74	2.3	8	59	[381, 382]
Nepal	55	2.1	17	23	[383, 384]
Saudi Arabia	33	3.0	8	26	[385, 386]
Bangladesh	30	3.3	6	37	[259]
Kenya	21	1.6	4	52	[387]

[a] Modified from Butler [257]

Table 24.3 Organ involvement in *Shigella dysenteriae* (SD1) HUS

Organ involvement	Details	Percentage (of SD1 HUS cases)[a]
Generalized	Septicemia	18.5
	Disseminated intravascular coagulation	21.0
	Hyponatremia	69.1
	Hypoalbuminemia	82.7
Gastrointestinal	Toxic megacolon	4.9
	Gastrointestinal perforation	9.9
	Protein-losing enteropathy	32.1
	Rectal prolapse	6.2
	Hepatitis	13.6
Kidney	Oliguric AKI	90.1
	Dialysis	51.6
Central nervous system	Encephalopathy Convulsions Hemiplegia	37.0 14.8 2.3
Heart	Myocarditis	6.2
	Congestive cardiac failure	3.7
	Cardiomyopathy	3.7
	Infective endocarditis	1.2
Hematological	Leukemoid reaction	91.3

[a] Extracted from Bhimma et al. [261]. Percentages are from 81 of 107 cases of HUS, admitted between July 1994 and February 1996 in KwaZulu/Natal

disease burden than other groups [265, 268, 269]. pnHUS accounts for approximately 5% of pediatric HUS cases, and 40% of non-STEC-HUS cases [264, 270, 271]. The majority of patients presents during the winter [265, 271].

Increased vaccine coverage may have led to a decline in the incidence of pnHUS, Yet *S. pneumoniae* serotype 19A was missing in the first generation pneumococcal 7-valent conjugate vaccine (PCV7) and has emerged as the predominant isolate during the last decade [265, 266, 272–275]. It is incorporated into the present 13-valent vaccine (PCV13) [276] and the 23-valent pneumococcal polysaccharide vaccine

(PPV23). pnHUS continues to occur, e.g., due to vaccine failure and emergence of non-vaccine replacement serotypes [7, 275].

Pathogenesis

pnHUS typically develops in a patient with pneumonia or meningitis [265, 266, 274, 277, 278]. HUS has been associated with abundant *in situ* production of bacterial neuraminidase [4, 279], in particular neuraminidase A (NanA) [280]. Neuraminidases cleave terminal sialyl residues from membrane glycoproteins and glycolipids [12, 280–282]. The exposed O-glycan core is known as Thomsen-Friedenreich disaccharide (TF antigen) [283]. The TF "neo" antigen is recognized by the lectin *Arachis hypogaea*, which has been used to detect and quantify the *in vivo* effect of neuraminidase on RBCs and tissues in patients with pnHUS [4, 5, 280] (Fig. 24.11).

It was postulated that the interaction of preformed anti-TF antibodies with the exposed neoantigen induces hemolysis, platelet agglutination, microvascular thrombosis, and tissue injury [5]. However, anti-TF antibodies are generally of the IgM class and of low affinity at body temperature [284–287]. Furthermore, desialylation of RBCs is not specific for HUS: it can be found in patients with IPD without progression to HUS [288–290], and pnHUS can develop in the absence of TF antibodies [282].

Presentation of pnHUS and Clinical Course

A practical approach for diagnosing pnHUS is shown in Table 24.4. Patients with pnHUS typically present with fever and respiratory distress due to lobar pneumonia (70–80%) that is complicated in two thirds of cases by pleural effusion or empyema [265, 291]. The remaining 20–30% have pneumococcal meningitis, acute otitis media or pneumococcal sepsis. The majority (80%) is bacteremic at the time of diagnosis [292]. The interval between onset of *S. pneumoniae* infection and HUS is 1–2 weeks [289].

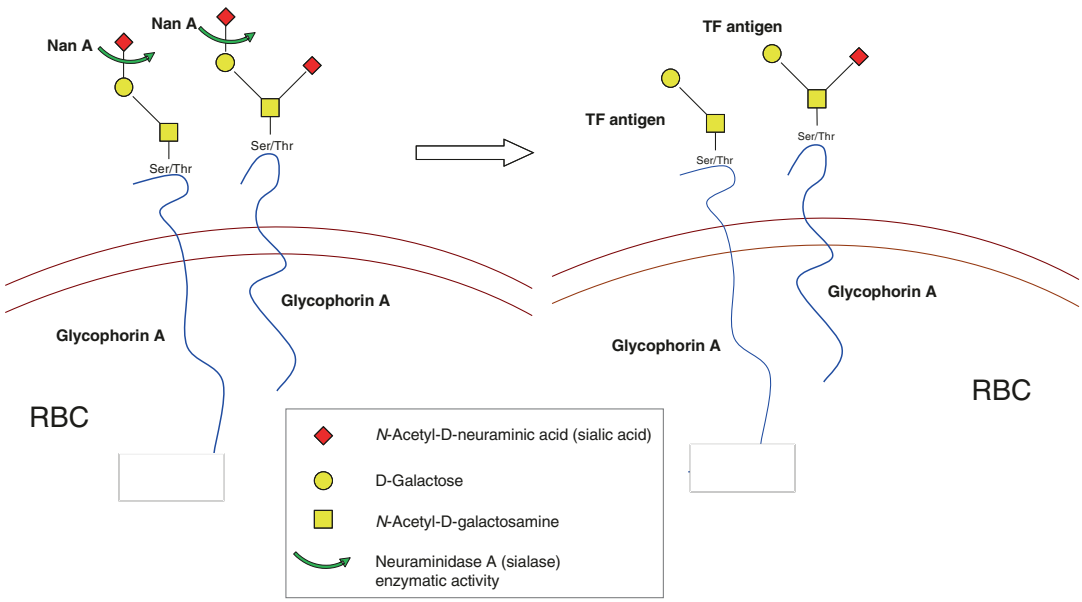

Fig. 24.11 Neuraminidase action on RBC membrane. Nan A (pneumococcal neuraminidase A) removes the terminal sialic acid. The *Arachis hypogea* lectin specifically recognizes the residual disaccharide β-D-galactose (1–3)-N-acetyl-D-galactosamine (Thomsen-Friedenreich antigen) that is O-glycosidically linked to the serine/threonine residue of glycophorin A (Used with permission of author and Elsevier from Loirat et al. [286])

The disease course can be severe or even fatal. A large proportion of patients will be admitted to the intensive care unit, of whom >50% will require mechanical ventilation and chest tube placement. About 70–85% of patients become oliguric or anuric, often with rapid clinical deterioration, and need acute dialysis [265, 291, 292]. Median time of dialysis in a large series was 10 days (range 2–240 days) [265].

In addition to microangiopathic hemolytic anemia with profound thrombocytopenia, the laboratory findings include a rapid rise of acute phase reactants (CRP, procalcitonin) [289]. The direct Coombs is positive in 58–90% of patients during the early phase of pnHUS [278, 293–295].

Patients may have DIC [278, 294, 296, 297]. *S. pneumoniae* sepsis with mild anemia, thrombocytopenia, DIC, hypotension and AKI can masquerade as HUS. Furthermore, Coombs positive hemolytic anemia may occur without thrombocytopenia and kidney injury [288, 298]. Hence, it is useful to choose a methodical approach diagnosing pnHUS [269, 274, 278] (Table 24.4).

Laboratory Studies and Biomarkers

The defining criterium is the detection of *S. pneumoniae* in a sterile fluid (blood, pleural effusion, cerebral spinal fluid, middle ear aspirate, etc.). In case of preceding antibiotic therapy, pneumococcal PCR or antigen detection should be tried.

Laboratory workup includes demonstration of TF exposure on PRBCs, a direct Coombs test and screening for DIC. It is recommended to measure C3, C4, CH50, and serum immunoglobulin levels and exclude congenital or acquired immune deficiencies that are known to predispose to invasive pneumococcal disease [299]. Serial CRP measurements are useful to confirm effective antimicrobial therapy.

No other HUS presents with a positive Coombs test or TF antigen exposure. However, the frequency of Coombs test positivity in IPD without HUS is unclear [278]. Sensitivity and specificity of TF antigen detection was reported as 86% and 57%, respectively, for pnHUS or isolated hemolytic anemia. The positive predictive value for HUS was 76%. Conversely, in

Table 24.4 Diagnostic Criteria for pnHUS[a]

pnHUS	Criteria		Details
Definite	1	Evidence of HUS	Intravascular hemolytic anemia, thrombocytopenia and acute kidney injury
	2	Evidence of invasive *S. pneumoniae* infection	Pneumococcal growth/antigen detection or positive PCR from physiologically sterile biological fluid
	3	No evidence of disseminated intravascular coagulation (DIC)	Fibrinogen consumption, prolonged prothrombin or partial thromboplastin time, and/or d-dimers *at the time of diagnosis*
Probable	1	Evidence of HUS	See above
	2	Evidence of invasive *S. pneumoniae* infection	See above
	3	a) Evidence of DIC ***and*** b) Positive Coombs test and/or evidence of TF antigen exposure	Usually cold agglutinins; TF antigen detection by *Arachis hypogaea* or specific lectin/monoclonal antibody binding [298]
Possible	1	Evidence of HUS	See above
	2	Suspected (undocumented) invasive *S. pneumoniae* infection	Negative culture/antigen detection or PCR from sterile fluid
	3	(a) No evidence of DIC, ***or***	
		(b) Positive Coombs test and/or TF antigen exposure	With or without evidence of DIC (see above)

Abbreviations: *TF* Thomsen-Friedenreich

[a]Modified from Copelovitch et al. [274]

children with IPD, positive and negative predictive values of TF antigen detection for pnHUS were 52 and 100% [290]. Among hospitalized patients with severe pneumococcal disease, TF antigen exposure levels peak 5–10 days after disease onset [300].

Complement and pnHUS

Informative studies on underlying complement abnormalities in pnHUS patients are scarce [287, 301, 302]. Complement consumption, primarily due to activation of the alternative pathway, is common in patients with pnHUS. Removal of sialic acids by NanA was noted to increase complement activity in whole blood, while absence of NanA blocked complement activation implying that the enzymatic removal of sialic acids can trigger pnHUS [12]. Transient CFH desialylation may play a role in disease pathogenesis [303]. While *S. pneumoniae* infection can trigger HUS in children with damaging mutations of complement regulator genes, similar to patients with "atypical" HUS, disease relapses due to *S. pneumoniae* have not been described. The emergence of specific (protective) immunity is surmised.

Treatment of pnHUS

Treatment of patients with pnHUS includes appropriate antibiotics and supportive care [278, 286]. Dialysis is required in up to 80% of patients. Previous recommendations to restrict blood transfusion to "washed" RBCs and to avoid administration of plasma which contains anti-TF antibodies [304, 305] are not based on evidence [17, 268, 278, 286, 287, 306, 307]. Indeed, plasma infusion and plasma exchange therapy have been described in pnHUS patients without apparent worsening of hemolysis or kidney function [265, 306, 308, 309].

Therapeutic complement blockade in the absence of pathogenic complement gene variants has not been systematically studied [307, 310]. Pneumococcal vaccination is an important measure for disease prevention [7].

Outcome of pnHUS

Unfavorable outcome has been noted in 20% of patients [265]. In another series, 3% of patients had died [292], 13% had neurologic sequelae, and 10% underwent kidney transplantation [292]. Important CNS complications are intracranial hemorrhage and infarction that can result in obstructive hydrocephalus [265, 268]. Waters et al. found that only 2 of 13 patients with pnHUS and meningitis had a normal neurodevelopmental outcome [265]. Pulmonary complications in addition to empyema include pneumatoceles and necrotic pneumonia [268]. Most deaths are not caused by HUS or kidney injury, but are due to meningitis and septic shock. Residual kidney dysfunction and proteinuria are expected in 20–25% [265, 311]. Kidney transplantation following pnHUS is rare, but HUS recurrence has not been reported [275, 312, 313].

Clostridium HUS

Early descriptions of HUS were traced to infections by *Clostridium perfringens*, the cause of gas gangrene, necrotizing fasciitis, and necrotizing enterocolitis [306, 314]. *C. perfringens* and other clostridial species produce neuraminidases, along with a range of other proteases and toxic enzymes [315]. A pathogenetic role of neuraminidase in clostridium HUS has been postulated [306, 314]. Newer reports have also implicated *C. difficile* in cases of HUS [316–322]. Treatment is supportive, in addition to appropriate antibiotics.

Influenza HUS

There is an established link between influenza virus infection, mostly influenza A, and HUS, but the mechanism remains speculative [8, 16].

Influenza virus shares with *S. pneumoniae* the production of neuraminidase (NA). Hemagglutinin (HA) and NA are defining and important viral pathogenicity factors in infections. NA shedding is minimal compared to *S. pneumoniae*, and it remains to be shown whether and how much it contributes to the pathogenesis HUS.

Infection of endothelial cells by influenza virus can induce apoptosis, [323] which triggers platelet adhesion directly and via extracellular matrix exposure [88, 324]. The virus also activates platelets and causes thrombin generation [325, 326]. Furthermore, influenza virus is a potent activator of complement [327–329]. Excess complement activation may lead to temporary consumption of C3 or may trigger HUS in individuals with risk variants of complement genes.

Patients with influenza HUS should be tested for plasma C3 and sC5b-9 concentrations, and ADAMTS13 activity. The presence of concomitant or complicating pneumococcal pneumonia or sepsis must be ruled out [16].

Supportive care is the main therapeutic intervention. It is unknown if the NA inhibitor oseltamivir prevents or ameliorates influenza HUS. The role of plasma therapy (PI, PLEX) or eculizumab in influenza HUS is unproven. However, recommendations for the treatment of aHUS should be followed if defective complement regulation is proven or suspected, particularly in instances of preceding HUS episode(s), a positive family history of aHUS, or recurrence of HUS after kidney transplantation [13, 94, 286].

COVID-19 and HUS

SARS-CoV-2, the virus causing COVID-19, injures the vascular endothelium, which can result in thrombotic complications [330]. SARS-CoV-2 can induce a fulminant cytokine response, including activation of the complement system and release of the chemokines C3a and C5a [331]. Multisystem inflammatory syndrome in children (MIS-C) is the most severe form of this pathologic reaction [332, 333], which may also involve the kidneys, but generally without overt TMA [334–336]. However, TMA has been observed in SARS-Cov-2 infected patients [337, 338] and after COVID-19 vaccination [339–342].

COVID-19 may cause TMA in patients without genetically determined complement abnormalities [9, 343, 344]. SARS-CoV-2 activates the alternative and lectin pathways of complement, likely via its spike surface protein [337, 345]. The pathogenic role of complement activation in COVID-19 is supported by the experimental observation that C3 deficient mice are protected against SARS-CoV-2 disease [346]. Finally, complement activation may also contribute to the hypercoagulable state in COVID-19 patients [347]. Some clinical observations, however, indicate that eculizumab may not prevent severe COVID-19 and associated endothelial cell injury [348], suggesting that blocking the terminal complement pathway is not sufficient.

HIV HUS

TMA in the context of AIDS has been variably described as HUS or TTP, and TTP has been listed as an AIDS defining condition [349, 350]. The incidence of HIV TMA has decreased since the advent of effective HIV therapies [351].

Clinical observation and animal experiments suggest that HIV can cause HUS directly [352], although the mechanism(s) remain unclear. HIV infects glomerular endothelial and mesangial cells, but not epithelial cells *in vitro* [353]. Intriguingly, a viral surface glycoprotein, pg120, binds to Gb3, the Stx receptor; this interaction has been linked to the occurrence of HIV HUS [352, 354–356]. Conversely, Gb3 has been described as natural resistance factor for the prevention of HIV infection, e.g. when given as a soluble agent [356, 357]. Kidney biopsies may show features of TMA [351, 358, 359].

Treatment of HIV TMA consists of effective antiviral therapy and supportive care, with the anecdotal use of fresh-frozen plasma infusions and plasmapheresis [351, 358–360].

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Renal Vasculitis in Children

25

Mojca Zajc Avramovič, Tadej Avčin, and Marina Vivarelli

Abbreviations

AAGN	ANCA-associated glomerulonephritis
AAV	ANCA-associated vasculitis
ACR	American College of Rheumatology
ANA	Anti-nuclear antibody
ANCA	Antineutrophil cytoplasmic antibody
BAFF	B-cell-activating factor
BVAS	Birmingham vasculitis activity score
CHC2012	Chapel Hill Consensus Conference on the Nomenclature of Systemic Vasculitides, 2012
EGPA	Eosinophilic granuloma with polyangiitis (Churg-Strauss syndrome)
ENT	Ear, nose, and throat
ESR	Erythrocyte sedimentation rate
GBM	Glomerular basement membrane
GFR	Glomerular filtration rate
GN	Glomerulonephritis
GPA	Granulomatous polyangiitis (WG)
GWAS	Genome wide association study
HLA	Human leucocyte antigen
HUVS	Hypocomplementemic urticarial vasculitis syndrome
IgAV	IgA vasculitis
KD	Kawasaki disease
LAMP-2	Lysosome-associated membrane protein-2
MCP-1	Monocyte chemoattractant protein-1
MHC	Major histocompatibility complex
MMF	Mycophenolate mofetil
MMI	Methimazole
MPA	Microscopic polyangiitis
MPO	Myeloperoxidase
NCGN	Necrotizing crescentic glomerulonephritis
NETs	Neutrophil extracellular traps
PAN	Polyarteritis nodosa
PLEX	Plasma exchange
PR 3	Proteinase-3
PTU	Propylthiouracil
PVAS	Pediatric vasculitis activity score
pVDI	Pediatric vasculitis damage index
RCT	Randomized control trial
RLV	Renal-limited vasculitis
RPGN	Rapidly progressive glomerulonephritis
SLE	Systemic lupus erythematosus
SNP	Single nucleotide polymorphism

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SOV	Single-organ vasculitis
VDI	Vasculitis damage index
VVV	Variable vessel vasculitis
WG	Wegener's granulomatosis (GPA)

Introduction

Vasculitides are a wide range of disorders where the primary pathological feature is inflammation in a blood vessel wall. The clinical presentation depends on the site of involvement, vessel size, and the type and severity of pathological changes. Patients usually present with fever and constitutional symptoms, such as fatigue, weakness, weight loss, and skin lesions. Laboratory markers show inflammation, and signs of multiorgan involvement may be present at presentation. Nevertheless, the most common vasculitis in childhood, IgA vasculitis (IgAV), can present mildly, with cutaneous purpura of the lower limbs, or as a severe renal vasculitis. Vasculitis is often aggressive and can be organ- or life-threatening. Renal involvement, when present, is often rapidly progressive glomerulonephritis (RPGN) since it is extremely aggressive with severe hypertension and rapid deterioration of renal function, requiring intervention within hours of initial presentation to preserve residual kidney tissue. Classic histological features are the presence of severe inflammation with intense endocapillary and extracapillary proliferation, leading to crescents and necrosis, and in many cases (excluding IgAV and lupus nephritis) a negative (pauci-immune) immunofluorescence. Vasculitis may affect any part of the body, and patients should be treated by a multidisciplinary team including rheumatologists, nephrologists, pulmonologists, dermatologists, and neurologists. Although more common in adults, renal vasculitis occurs in childhood and may present as an isolated kidney disorder or associated with a systemic disease.

Classification of Vasculitides

Traditionally, vasculitis has been classified according to the size of the affected vasculature (Figs. 25.1 and 25.2). Large-vessel vasculitis or

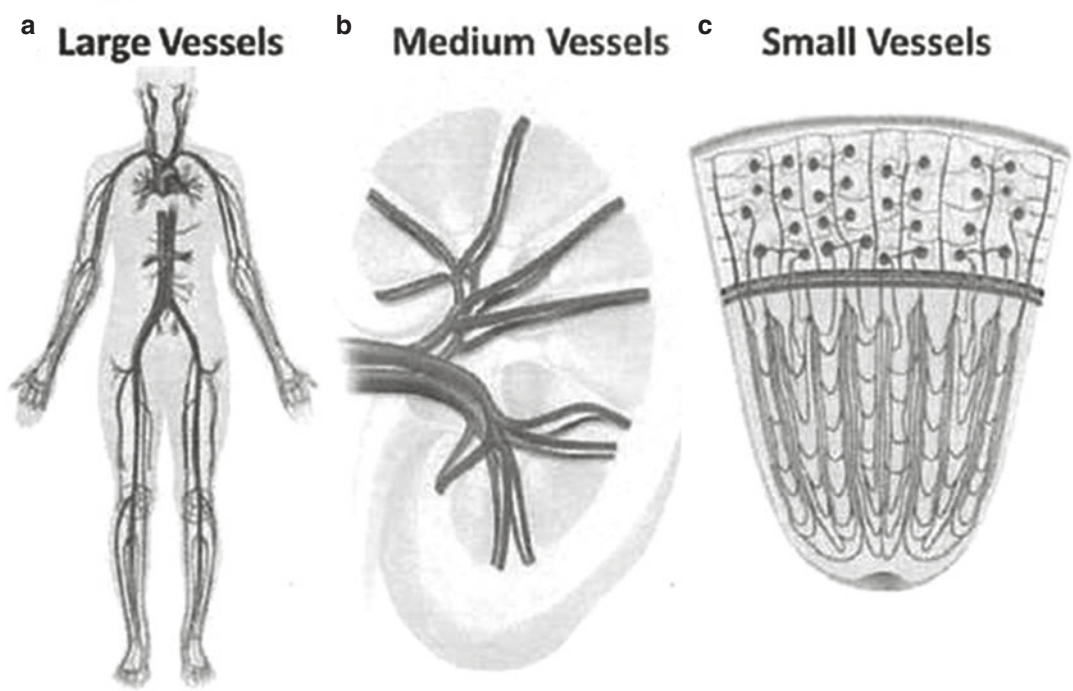

Fig. 25.1 The definition of vessel size [1]. (Reference: Jennette JC et al.:2012 Revised International Chapel Hill Consensus Conference Nomenclature of Vasculitis Arthritis & Rheumatism, 65:1–11, 2013)

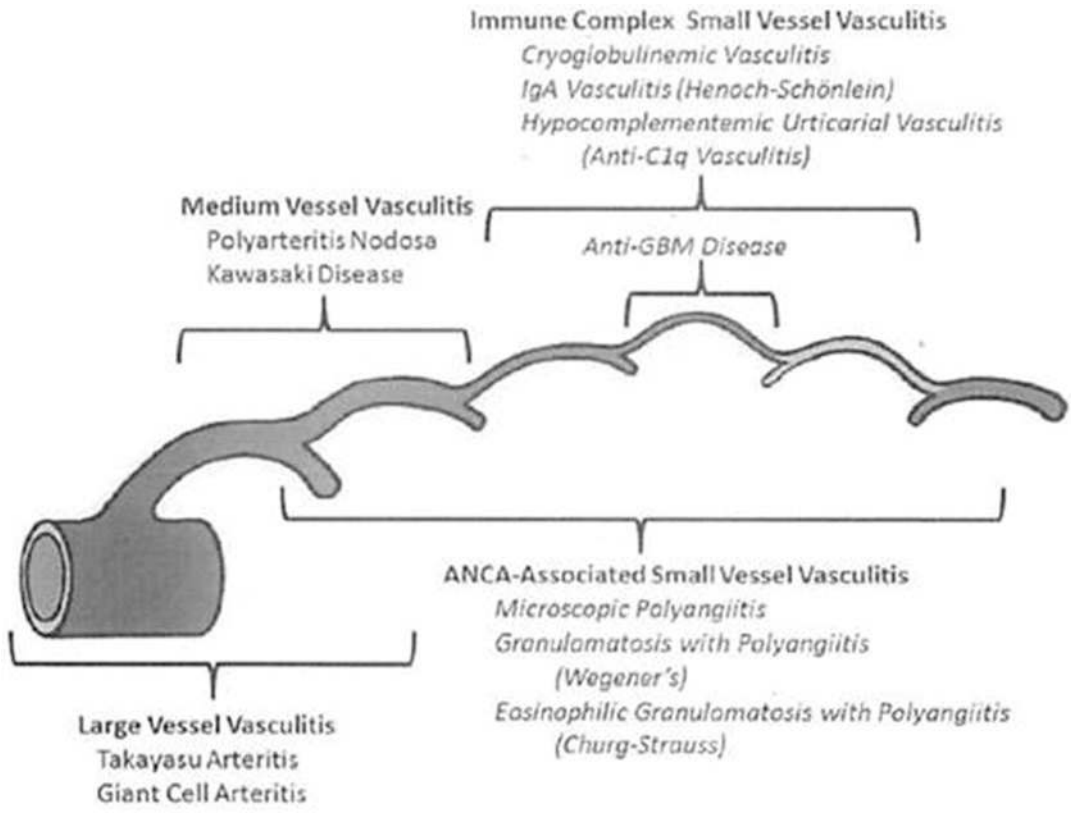

Fig. 25.2 Vasculitis and vascular size (CHCC 2012) [1]. (Reference: Jennette JC et al.:2012 Revised International Chapel Hill Consensus Conference Nomenclature of Vasculitis Arthritis & Rheumatism, 65:1–11, 2013)

arteritis affects the aorta and its major branches in diseases such as giant-cell arteritis and Takayasu arteritis, which can reduce the caliber of the abdominal aorta or of renal arteries, leading to renal ischemia and renovascular hypertension. Medium-vessel vasculitis involves medium or small arteries, leading to infarction and hemorrhage of the affected organ, and includes polyarteritis nodosa (PAN) and Kawasaki disease (KD), the only large-vessel or medium-vessel vasculitides that involves mainly infants and small children. Small-vessel vasculitides, affecting capillaries and venules, are the only forms which can by definition cause glomerulonephritis (GN). They comprise a large group of diseases; the two most common are IgAV and antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV). The current nomenclature system was established at the International Chapel Hill Consensus Conference on the Nomenclature of Systemic Vasculitides in 1994 and further modified in 2012 (CHC2012), presented in Table 25.1 [1]. The most prominent modifications comprise the adoption of new names for several diseases, consistent with the trend of replacing eponyms with names that reflect the pathophysiology, the formalisation of the term AAV and categories for secondary forms of vasculitis. Monogenic forms of vasculitis, recognized more recently, were not included in the 2012 classification. The chapter includes information about rare monogenic forms of vasculitis that can affect the kidney and occur in children. The American College of Rheumatology (ACR) published a classification in 1990 of 7 vasculitides [2], which has become inadequate, and currently a joint ACR/The European Alliance of Associations for Rheumatology (EULAR) classification that will include new diagnostic criteria is being developed [3].

Table 25.1 Names for the vasculitides adopted by the CHC2012 [1]

Large-vessel vasculitis	Takayasu arteritis Giant cell (temporal) vasculitis Both of these occur usually in patients over 50 years old
Medium-sized vasculitis	Polyarteritis nodosa Kawasaki disease (KD)
Small-vessel vasculitis	Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) Microscopic polyangiitis (MPA) Granulomatosis with polyangiitis (Wegener's) (GPA) Eosinophilic granulomatosis with polyangiitis (Churg-Strauss syndrome) (EGPA) Immune complex small-vessel vasculitis Anti-glomerular basement membrane disease Cryoglobulinemic vasculitis nephritis IgA vasculitis (Henoch-Schönlein purpura) (IgAV) Hypocomplementemic urticarial vasculitis (anti-C1q vasculitis)
Variable-vessel vasculitis	Behcet syndrome Cogan's syndrome
Single-organ vasculitis	Cutaneus leukocytoclastic angiitis Cutaneus arteritis Primary central nervous system vasculitis Isolated aortitis Others
Vasculitis associated with systemic disease	Lupus vasculitis Rheumatoid vasculitis Sarcoid vasculitis Others
Vasculitis associated with probable etiology	Hepatitis C virus-associated cryoglobulinemic vasculitis Hepatitis B virus-associated vasculitis Syphilis-associated aortitis Drug-associated immune complex vasculitis Drug-associated ANCA-associated vasculitis Cancer-associated vasculitis Others

Classification of Childhood Vasculitis

The most common vasculitis in childhood is IgAV, followed by KD. In certain parts of the world, such as Japan, KD is more common. Although KD may cause infarction of any organ, kidney involvement is rare, and management is the same as for any other severe form of KD. Other forms of vasculitis are rare in childhood, and there are large-vessel vasculitides, such as temporal arteritis and giant cell arteritis, that usually occur in patients over 50 years of age, and are not seen in pediatric patients. The 2005 EULAR/Paediatric Rheumatology European Society (PReS) criteria [4], which were revised and validated in children in 2008 [5], are generally used for classification of vasculitis in pediatric patients. These criteria provide detailed classification for the most common childhood vasculitides, while rare forms are under the umbrella of "other vasculitides" (Table 25.2).

Table 25.2 EULAR/PReS classification of childhood vasculitis [5]

Predominantly large vessel	Takayasu arteritis
Predominantly medium-sized vessel	Childhood polyarteritis nodosa
	Cutaneus polyarteritis nodosa
	Kawasaki disease
Predominantly small vessel	Granulomatous Eosinophilic granulomatosis with polyangiitis (EGPA or Churg Strauss) Granulomatosis with polyangiitis (GPA) Nongranulomatous Microscopic polyangiitis (MPA) IgA vasculitis Isolated cutaneus leukocytoclastic vasculitis Hypocomplementemic urticarial vasculitis
Other vasculitides	Behcet syndrome Secondary vasculitides due to infection, malignancy, or drugs, including hypersensitivity vasculitis Isolated vasculitis of the central nervous system Cogan's syndrome Unclassified

Large Vessel Vasculitis

Takayasu Arteritis

The EULAR recommendations on adult-onset Takayasu arteritis may be used in paediatric Takayasu arteritis patients [6].

Medium-Vessel Vasculitis

Polyarteritis Nodosa

PAN is a necrotizing vasculitis of medium-sized arteries that rarely occurs in children. Peak age of childhood onset is 7–11 years, with a slight male predominance [7]. The histological hallmark is a necrotizing vasculitis associated with aneurysmal nodules along the walls of medium-sized arteries, which can be visualized by conventional angiography. Importantly, a renal biopsy is not recommended due to a high risk of bleeding and arteriovenous fistulae, and it may not be informative. In the recent pediatric classification of PAN [5], characteristic histology or angiography findings are mandatory for diagnosis of PAN in addition to at least one of five criteria that include skin involvement, myalgia/muscle tenderness, hypertension, peripheral neuropathy and renal involvement. PAN limited to the skin is defined as cutaneous PAN and is more common in children. It can vary from classic purpura to necrotic lesions associated with peripheral gangrene. Renal involvement can be visualized by angiography of renal arteries with the characteristic "pruned tree" images (aneurysms, perfusion defects, arterial cutoff and lack of crossing of peripheral renal arteries) and may be indirectly proven by demonstrating patchy areas within the renal parenchyma of decreased isotope uptake on Tc-99 m dimercaptosuccinic acid (DMSA) scan [7].

PAN can be caused by a viral illness or cancer, and there are genetic etiologies [8]. Renal involvement was present in 19% of cases in the largest described cohort of 69 children with PAN followed in a single center [9]. Of the eight renal biopsies performed in this study, two showed focal segmental GN, two showed >10% crescents and the remaining 4 showed membranoproliferative GN with necrotizing vasculitis. Fibrin deposition with vessel occlusion was noted in 3 of the 8 renal biopsies. Management approach is the same as for AAV. Notably, a multicenter, open-label, randomized clinical trial (RCT) comparing mycophenolate mofetil (MMF) versus cyclophosphamide for the induction of remission of childhood PAN (the MYPAN trial) was completed, although not yet published as of 2021 [10].

Kawasaki Disease

KD is an acute, self-limited vasculitis, occurring most frequently in infants and small children. Its main manifestations are fever (persisting for 5 days or more), peripheral extremity changes, a polymorphous exanthema, bilateral conjunctivitis, oral mucosal changes (e.g. red, dry, cracked lips and strawberry tongue) and non-purulent cervical lymphadenopathy [7]. It frequently affects the coronary vessels, causing aneurysms and other cardiac abnormalities; KD is one of the main causes of acquired cardiac disease in children. Renal manifestations are infrequent and their pathogenesis is unclear: pyuria, proteinuria and renal failure have been described, as well as tubulointerstitial nephritis which may be secondary to intravenous (IV) immunoglobulin, which together with aspirin is the mainstay of therapy [11].

Small-Vessel Vasculitis

The most common vasculitides affecting kidneys in children are IgAV (covered in Chap. 54) and AAV, which includes granulomatosis polyangiitis (GPA), *microscopic polyangiitis (MPA) and eosinophilic granulomatosis with polyangiitis (EGPA)*. AAV can be classified based on the autoantigen specificity. The two most recognized autoantigens are myeloperoxidase (MPO) and proteinase 3 (PR3). The categorization of a patient AAV should include both the clinicopathological phenotype and the autoantigen. The other forms of vasculitis are briefly addressed below, and the remainder of the chapter focuses on AAV.

Anti-Glomerular Basement Membrane Disease

Anti-glomerular basement membrane (GBM) disease is a rare form of small-vessel vasculitis caused by autoantibodies directed against the so-called Goodpasture antigen or non-collagenous domain 1 (NC-1), a neo-epitope of the α3 subunit of type IV collagen expressed in the GBM, where autoantibodies cause a rapidly progressive crescentic and necrotizing GN, and in pulmonary alveoli. The disease can occur in adolescents, and may be renal-limited or may, in approximately 50% of cases, involve small-vessels in the lungs as well, leading to pulmonary hemorrhage (Goodpasture syndrome). Exposure of the neo-epitope NC-1 is due to a perturbation of the structure of type IV collagen, which may be secondary to environmental exposure to reactive oxygen species (e.g. industrial hydrocarbon, cigarette smoke), systemic inflammation (e.g. AAV, which can co-exist with anti-GBM disease in approximately 25% of cases) or membranous nephropathy. Without aggressive and timely treatment, the disease is generally fulminant, and both patient and kidney survival are poor. Recommended treatment relies on the use of plasma exchange (PLEX) or immune-adsorption and immunosuppression with glucocorticoids and cyclophosphamide [12]. Treatment should be commenced without waiting for a renal biopsy when this diagnosis is suspected, and should be continued until the anti-GBM antibodies are undetectable [13].

Cryoglobulinemic Vasculitis

Cryoglobulinemic vasculitis is a rare systemic vasculitis resulting from circulating immune complex deposition in the small vessels which occurs when the temperature falls below 37° C. It has variable clinical features, including signs of hyperviscosity syndrome such as Raynaud's phenomenon, cold-induced acral ulcerations, livedo reticularis, headache and confusion, or signs of immune complex-mediated vasculitis of small vessels such as purpura, arthralgia, GN, and peripheral neuropathy. Cryoglobulinemia can result from infections (mainly hepatitis C virus and hepatitis B virus), cancer or autoimmune disease, or it can be essential. In children, essential forms predominate (72%), and renal involvement occurs, as in adults, in approximately 10% of cases [14]. Treatment is aimed at eliminating the cause, and rituximab (RTX) has been used with

success. In severe forms, such as those with RPGN or symptomatic hyperviscosity, PLEX is necessary. IV immunoglobulin is contraindicated in cryoglobulinemic vasculitis as it can exacerbate immune complex precipitation, leading to multiorgan failure.

ANCA-Associated Vasculitis

Renal Pathology

The pathology of renal vasculitis is characterized by its grade of severity and anatomical location. Although there may be several stimuli that trigger vasculitis, endothelial cells are its basic target. The early pathology is an endothelitis, which is frequently seen with any endothelial injury. The more severe phenotypes are fibrinoid necrosis and occasional rupture of the corresponding vasculature, resulting in interstitial hemorrhage (Fig. 25.3a). In some cases, vasculitis is recognized by severe inflammatory infiltrates in the vascular wall; other cases show fewer inflammatory infiltrates with necrosis, such as PAN (very rare) or KD.

The key histological feature of AAV is necrotizing vasculitis, with few or no immune deposits, predominantly affecting small vessels. This can be associated with granulomatous lesions in GPA and with eosinophil-rich (and often granulomatous) inflammation in EGPA. Tissue biopsies are essential to make the diagnosis of AAV.

When renal involvement is present in patients with suspected or confirmed AAV, renal biopsy is the gold standard for diagnosis, and is an important predictor of renal outcome. ANCA-associated GN is characterized by pauci-immune immunofluorescence; necrotizing and crescentic lesions in the affected glomeruli by light microscopy; and subendothelial oedema, microthrombosis, and degranulation of neutrophils by electron microscopy [15].

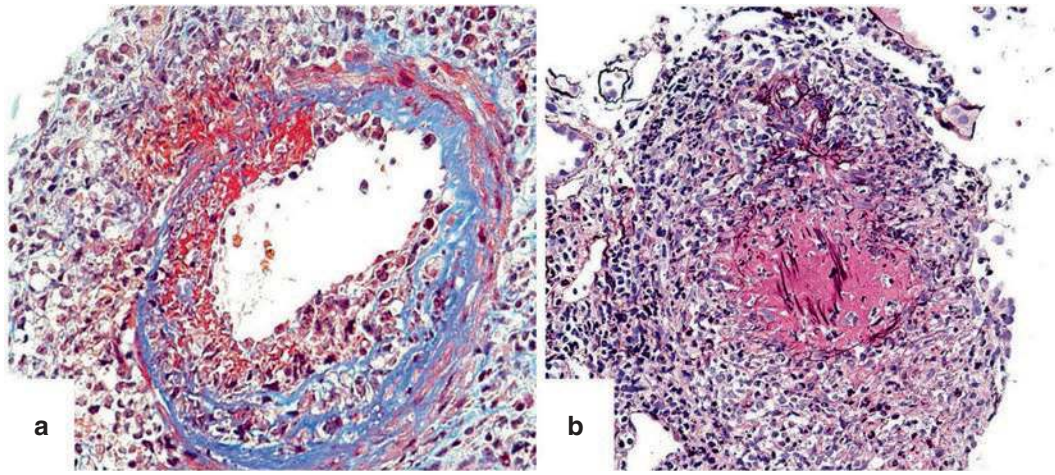

Fig. 25.3 Necrotizing angiitis and glomerulonephritis in patients with ANCA-associated vasculitis/glomerulonephritis. (**a**) Necrotizing vasculitis. Interlobular artery is affected by intimal inflammatory infiltrates particularly neutrophils. Note rupture of vascular wall with substantial fibrin deposition (Masson Trichrome Stain, ×400). (**b**) Severe vasculitis involves entire glomerulus with granulomatous changes (Periodic Acid Methenamine-Silver Stain, ×400). (**c**) Necrotizing capillaritis in the glomerulus. Note numerous neutrophils accumulation at the site of fibrinoid necrosis (Masson Trichrome Stain, ×400). (**d**) Segmental fibrinoid necrosis with nuclear fragmentation in the neutrophils (Periodic Acid Schiff Stain, ×550). (**e**) Peritubular capillaritis and interstitial inflammatory infiltrates (Masson Trichrome Stain, ×400). (**f**) Interstitial inflammatory infiltrates by electron microscopy. Note neutrophils predominant infiltration (×2000)

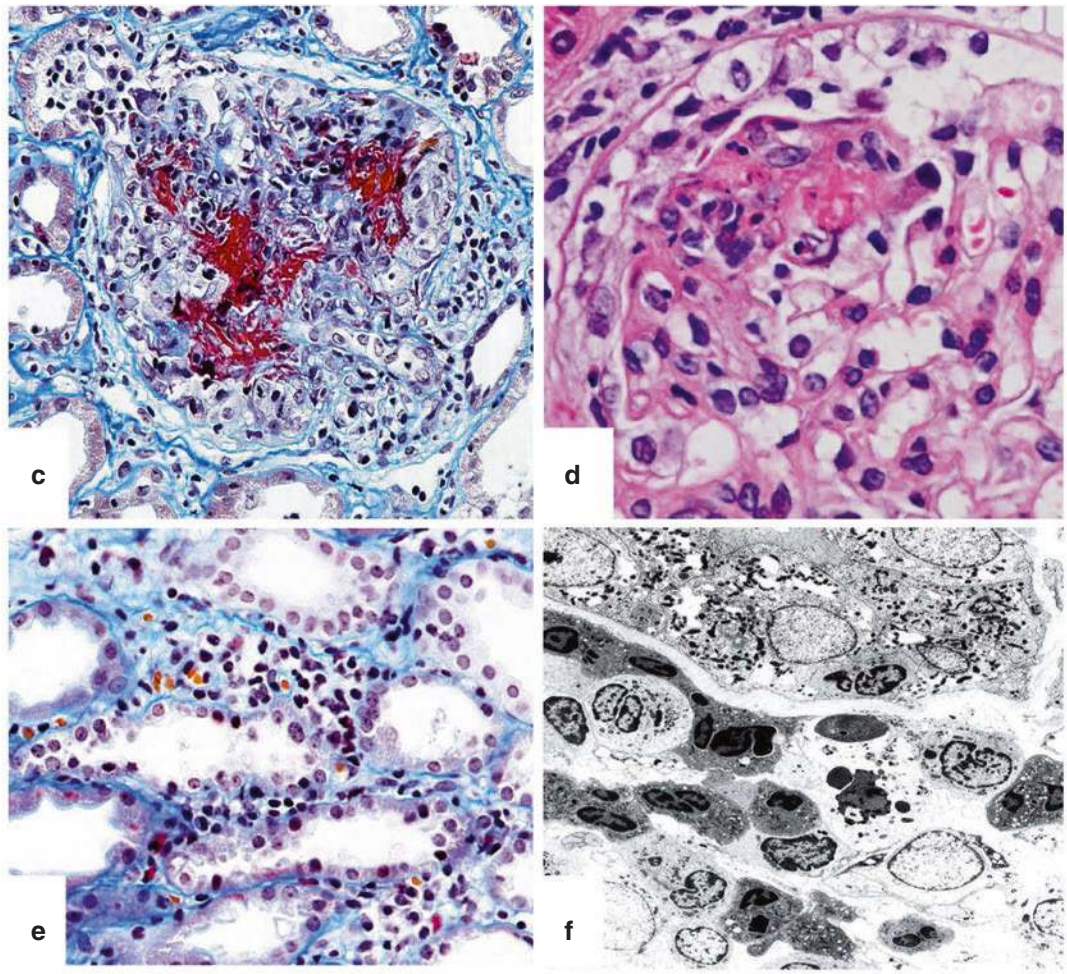

Fig. 25.3 (continued)

Light Microscopy

Renal vasculitis affects endothelial cells, causing inflammation, which manifests as endothelial cell swelling and proliferation and by inflammatory cell infiltration, in AAV mainly by neutrophils, into the subendothelial space. The typical sites of vasculitis in AAV are arterioles, glomeruli, and peritubular capillaries. In arterioles, following endothelial cell swelling, accumulation of inflammatory cells leads to intimal thickening, deposition of active coagulation product (fibrinoid necrosis), and thrombosis (Fig. 25.3a). If the lesion progresses, it leads to a defect or rupture of the vascular wall. In GPA-associated forms of AAV, angiitis can occur, targeting the glomerular hilum and resulting in the destruction of the entire glomerular structure, which is replaced by granulomatous inflammation (Fig. 25.3b). In the glomeruli, capillaritis causes necrotizing crescentic GN, which frequently progresses to glomerulosclerosis (Fig. 25.3c, d). In addition to glomerular involvement, there is also a tubulointerstitial nephritis in AAV; tubular lesions are important predictors of outcome, especially in patients treated with B-cell depleting therapy [16]. Peritubular capillaritis (Fig. 25.3e) is characterized by dilatation and adherence of inflammatory cells on the endothelium of cortical

peritubular capillaries. Occasional rupture leads to interstitial hemorrhage.

GN in MPA differs from GN in GPA by the absence of granulomatous lesions and the more frequent occurrence of chronic lesions (e.g. glomerulosclerosis, fibrous crescents, interstitial fibrosis).

In 2010, an international working group of renal pathologists proposed a histopathologic classification of ANCA-associated GN that was predictive of long-term renal outcome in adult patients. Renal pathological features were divided into four categories: focal, crescentic, sclerotic, and mixed (Table 25.3). These categories predicted renal outcomes at 1 and 5 years (Fig. 25.4). Patients with focal lesions had an excellent renal outcome, whereas renal survival was poor in the sclerotic class, and severe patients also presented an increased mortality risk [17]. This classification system was confirmed in other adult cohorts [18] and in a pediatric retrospective study with biopsy-proven ANCA-associated GN (MPA and GPA) [19]. The biopsy specimens were focal in 13 patients (32.5%), crescentic in 20 (50%), mixed in 2 (5%), and sclerotic in 5 (12.5%). Mixed and crescentic were combined for analyses. This study showed rapid progression to end-stage kidney disease (ESKD) for patients in the sclerotic class, mild disease in the focal, and a slower decline over 2 years in the crescentic/mixed classes. The probability of having an estimated glomerular filtration rate (eGFR) >60 ml/min per 1.73 m^2 at 2 years was 100% for the focal, 56.5% for the crescentic/mixed, and 0% for the sclerotic biopsy categories. Renal biopsies were repeated to determine progression or because of relapses in a subset of patients. Among children with crescentic patterns who had a repeat biopsy, 80% progressed to sclerosis on a second biopsy. Among the focal group, only one child had a repeat biopsy and was reclassified as mixed. These data demonstrate a clear prognostic value of the histopathological classification and suggest for children with sclerotic class (at least 50% globally sclerotic glomeruli) that aggressive immunosuppression is unlikely to result in recovery of renal function. Therefore, it is likely that risks outweigh benefits in this setting and that when extensive sclerosis is present intense treatment should be avoided. Conversely, patients with more florid lesions require early and intensive treatment. Prospective studies are needed to achieve the objective of tailored management based on histological parameters.

Table 25.3 Classification scheme for ANCA-associated glomerulonephritis [17]

Class	Inclusion criteria[a]
Focal	≥50% normal glomeruli
Crescentic	≥50% glomeruli with crescents
Mixed	<50% normal, <50% crescentic, <50% globally sclerotic glomeruli
Sclerotic	≥50% globally sclerotic glomeruli

[a]Pauci-immune staining pattern on immunofluorescence micrography and ≥1 glomerulus with necrotizing or crescentic glomerulonephritis on light microscopy are required for inclusion in all four classes

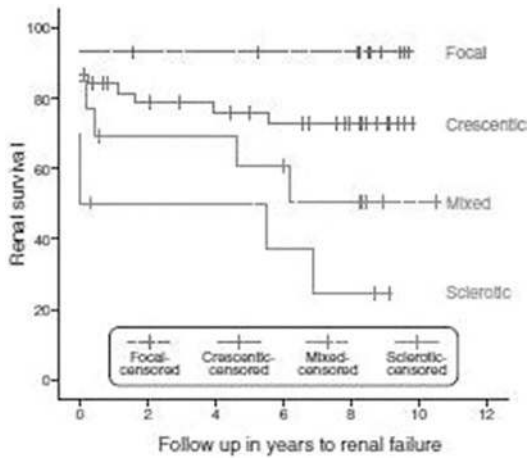

Fig. 25.4 Renal survival is depicted according to the four histological categories [17]. (Reference: Berden et al. Histological classification of ANCA-associated glomerulonephritis. JASN 21:1628–1639, 2010)

Immunofluorescence

Falk and Jennette defined "pauci-immune" as not more than 2+ staining of any immunoglobulin (on a scale of 0 to 4+) and the absence of immune-complex type electron-dense deposits by electron microscopy [20]. However, in a study of electron micrographs of 124 cases of ANCA-associated crescentic GN, 54% of the biopsies had glomeru-

lar immune-complex deposition [21], although the immunofluorescence staining was relatively weak (≤2+). Moreover, Manenti et al. evaluated 27 renal biopsies of adult patients with AAV for glomerular complement deposition by immunohistochemistry and found strong C3c staining in 11 of 27 patients (41%) and positive discrete glomerular deposition of C4d in 8 patients and of C5b-9 in 5 cases (20%), while Bb was usually negative. In this study, there was no association between tissue deposition of complement fractions and outcome [22]. In a subsequent study, patients with glomerular C3d deposition had a worse prognosis [23].

Electron Microscopy

By electron microscopy, neutrophil infiltration into the interstitium and degranulation is visible (Fig. 25.3f), together with neutrophil degranulation, subendothelial edema, and microthrombosis. Moreover, as reported above [21], in about 50% of cases immune-complex deposition was visible. These immune complexes were often few in number. Nonetheless, their presence was associated with more frequent glomerular tuft hypercellularity, greater proteinuria, and trends toward higher serum creatinine level and more widespread crescent formation compared with cases lacking deposits on electron microscopy.

Pathogenesis

The initiating events leading to AAV are multifactorial, but several risk factors and possible triggers have been recognized, including genetic factors, environmental exposures, and previous infections. The pathogenicity of ANCA plays an important role in initiating and amplifying the inflammation, and the ANCA-induced neutrophil activation is an important cause of vascular injury. The pathogenesis is presented in Fig. 25.5 [24].

Genetic Factors

Differences in the prevalence of AAV between ethnic groups, familial association studies, and genetic associations studies, including large genome-wide association studies (GWAS) [25–28], all support the role of genetics. The strongest associations are those in the genes for human leukocyte antigen (HLA) [25–28], *SERPINA1* [25, 26, 29] and multiple genes encoding inflammatory mediators. Links with the HLA region support the central role of autoreactivity and autoimmunity in AAV. Candidate gene and GWAS studies not only indicated a highly significant association between AAV and HLA regions, but also showed genetic distinctions between different clinical phenotypes and ANCA specificity. In general, genetic associations are more closely linked with the auto-antigen specificity than with the clinical syndrome. GPA and PR3-ANCA AAV are associated with HLA-DPB1 and HLA-DPA1, while MPA and MPO-ANCA AAV are associated with HLA-DQB1 and HLA-DQA2 [25, 26, 30]. HLA-DPB1 is associated with EGPA [28]. Moreover, HLA alleles are associated with the mortality and relapse of AAV [30]. *SERPINA* codes for α1-antitrypsin, which is a major inhibitor of PR3. Involvement of the *SERPINA1* gene confirms the role of ANCA in the pathogenesis of AAV as the genetic variants may lead to decreased function α1-antitrypsin, resulting in PR3 accumulating in tissues and potentially triggering ANCA formation. Nevertheless, in a meta-analysis, not only PR3-ANCA positive patients but MPO-ANCA-positive patients and both c-ANCA and p-ANCA-positive patients are associated with *SERPINA1* [29]. One proposed hypothesis is that patients with AAV and these genetic variants have a reduced ability to inhibit PR3 released by activated neutrophils; consequently, these variants increase PR3-mediated proteolytic vessel damage. Nevertheless, the changes in *PRTN3*, which encodes PR3, were present only in PR3-ANCA-positive patients, independent of the clinical diagnosis [25, 26]. A wide range of associations in immunoregulatory genes were found, the strongest with *CTLA-4, PTPN22,* and *TLR9* [29, 30].

Environmental Factors

Infections are a known potential trigger of autoimmune disease. In AAV, more than 50% of

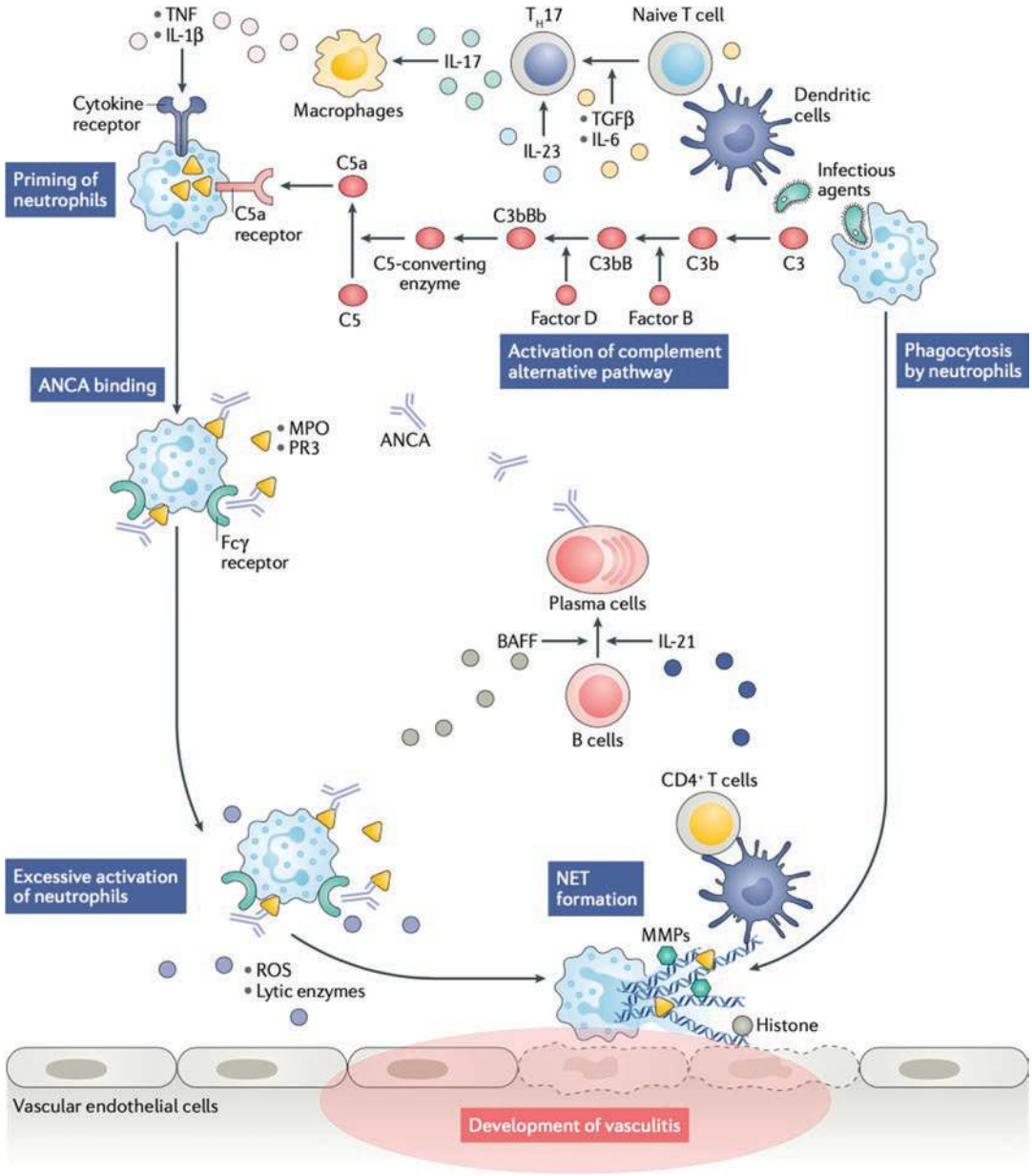

Fig. 25.5 Pathogenesis of AAV. Modified from Nature reviews Rheumatology [24]. (Reference: Nakazawa D, Masuda S, Tomaru U, Ishizu A. Pathogenesis and therapeutic interventions for ANCA-associated vasculitis. Nat Rev. Rheumatol. 2019;15(2):91–101)

patients are t nasal carriers of *Staphylococcus aureus* at the time of diagnosis, which is significantly higher than the general population. Nasal carriage of *Staphylococcus aureus* is associated with an increase risk of disease relapse [31, 32]. The underlying mechanisms is not known [32]. The human lysosome-associated membrane protein 2 (LAMP-2) epitope has complete homology with the bacterial adhesin FimH, and human LAMP-2-ANCA may cross react with this microbial protein. This supports the hypothesis of infection driven immune dysregulation in

AAV. The human antibodies directed against LAMP-2 are somewhat controversial because they have been shown in a rat model to elicit ANCA-associated GN [33], but the results were not replicated [34]. Drugs, including hydralazine, minocycline, propylthiouracil, and levamisole, may cause the development of ANCA [35, 36]. In a case series of AAV in seven children who received propylthiouracil, the most common of the recognized drugs used in childhood, the prognosis was better than non-drug induced AAV [37]. Geographic clustering, temporal clustering, seasonal variation in disease onset, and differences in urban/rural disease prevalence all suggest an environmental trigger [38]. Environmental exposure to air pollutants was investigated, with the strongest evidence for silica dust [39], which is further supported by outbreaks of AAV, mainly MPO-ANCA positive with intense pulmonary involvement, after three large earthquakes in Asia [38]. Pesticides, ultraviolet radiation, and smoking are all believed to contribute to the pathogenesis, but none is by itself sufficient to trigger the disease [38].

The Pathogenicity of ANCA

Substantial clinical and experimental data show that ANCAs are pathogenic. First, there is a strong association of the disease with ANCA, as >90% of MPA and GPA patients and >75% of EGPA patients and GN have positive ANCA. Second, there is also a partial correlation of titer with disease activity. Third, transplacental transfer of ANCA was able to induce disease in humans [40], as was the infusion of anti-MPO IgG in rat and mice models [41, 42]. Fourth, drug-induced ANCAs have a similar clinical presentation [35]. However, healthy individuals can have circulating autoantibodies against PR3 and particularly MPO, also called natural or non-pathogenic ANCAs. In comparison with ANCA from patients, the natural ANCAs have lower titers, lower avidity, less epitope specificity, and less capability to activate neutrophils [43, 44]. The transformation of natural ANCA to pathogenic is most probably an interplay between previously mentioned genetic, environmental and immunologic triggering events. The current hypothesis is that the first reaction is not against autoantigen, but against a peptide that is complementary to the autoantigen. The anti-idiotypic antibody is a response to this first immune response and it cross-reacts with the autoantigen epitopes that are complementary to the initial immunogenic peptide [45, 46]. Multiple microbial peptides are homologues of complementary PR3, including *S. aureus*.

Pathogenesis of Vascular Inflammation

Primed neutrophils, activated by ANCA, are the primary drivers of vascular inflammation in AAV. Different inflammatory signals, for example C5a, bacterial lipopolysaccharide, and TNF, can prime neutrophils to release the MPO/PR3 target autoantigens to the cell surface, where they interact with ANCA. This immune complex further activates neutrophils and causes a respiratory burst; neutrophil enzymes are degranulated; free radicals are released; and neutrophil extracellular traps (NETs) are extruded. NETs in AAV contain MPO and PR3, and further interact with ANCA. All of the above leads to injury and death of endothelial cells. Disrupted endothelium allows plasma to enter the vascular and perivascular tissue, and the following coagulation cascade results in fibroid necrosis [34, 47]. In addition, monocytes can express autoantigens (MPO and PR3) and can be activated by binding of ANCA to Fc-γ receptors (FcγRs). When activated, monocytes release proinflammatory cytokines, such as interleukin-8 (IL-8) and monocyte chemoattractant protein-1 (MCP-1). While IL-8 further activates neutrophils, MCP-1 attracts monocytes and macrophages, presumably shifting the inflammation into the next stage, from predominantly neutrophil-rich to predominantly monocyte/macrophage-rich, which includes granulomatous inflammation.

Pathogenesis of Granulomatosis

Granulomas are foci of extravascular inflammation; the exact mechanism of their formation is not known. It is believed that there is no antigen-specific response, but rather the secondary innate

monocyte/macrophage response, triggered by acute vascular inflammation caused by ANCA-primed neutrophiles [47].

The Role of the Alternative Complement Pathway

The significance of the alternative complement pathway in AAV was first shown in animal studies. Ablation of C5, depletion of C3 and factor B deficiency completely inhibited disease development in a transferred anti-MPO IgG mouse model, while deficiency of classical pathway components did not prevent disease [48]. Later studies documented that the inflammatory process initiated by anti-MPO IgG is further amplified by C5aR activation on inflammatory cells, and a C5 inhibiting monoclonal antibody prevented or strongly attenuated GN development [49, 50]. The human biopsy specimens of MPO-ANCA associated GN had deposition of factor B, C3d, and the membrane attack complex (MAC) in glomeruli and small blood vessels [51]. In addition, increased levels of C3a, C5a, soluble C5b-9, and Bb were observed in the plasma of patients with active AAV, and lower levels of properdin were seen in the plasma of patients in remission. The levels of classical pathway proteins did not differ [52, 53]. Neutrophils, C5a and ANCAs create an inflammatory amplification loop, with C5a attracting and priming more neutrophils for activation by ANCAs, which causes further activation of the alternative complement pathway and consequent release of more C5a [54].

The Role of B and T Cells

The pathogenicity of ANCA, neutrophils, and complement are enabled by a defect in the regulation of the immune system. The regulatory FOXP3+ T cells are decreased and dysfunctional in AAV patients [55], even more so during active disease [56]. The subset of B cells with mostly regulatory suppressive function highly expresses CD5, and in the sera of patients with active AAV, the CD5+ B cells are reduced in number, which normalizes in remission after treatment with RTX [57, 58]. Furthermore, the sera of patients with active AAV has elevated levels of B-cell-activating factor (BAFF), which stimulates B cells and inhibits apoptosis. The ligand for BAFF is released by activated neutrophils [59, 60]. In this way, ANCA-activated neutrophils, along with dysfunctional T- and B-cell regulation, augment the production of more ANCA.

Clinical Features

Patients typically present with non-specific systemic symptoms, such as fatigue, malaise, fever, anorexia, and weight loss. Rhinosinusitis and epistaxis can be early signs that sometimes go unnoticed, while a purpuric rash may be the first obvious reason to seek medical care. Arthralgia or overt arthritis, hematuria, hypertension, cough, diarrhea, and dyspnea can also be among the presenting features. The clinical symptoms may start suddenly or develop slowly over days or weeks.

Granulomatosis with Polyangiitis (GPA)

Upper and lower respiratory tract inflammation together with GN and positive ANCA, especially PR-3 ANCA, is typical for GPA in childhood. Granulomas are found in the tissue. The largest published cohort is the multicenter ARChiVe (A Registry for Children with Vasculitis) study, which included 183 children with GPA. A comparison of presenting features is shown in Table 25.4 [61]. Over half of the patients were female and white, while the median age at disease onset was 14 years. The median time to diagnosis was 2.1 months, but varied from 0–71 months. The most common presenting features were constitutional (88%), renal (83%), pulmonary (74%), ear, nose and throat (70%), musculoskeletal (65%), and cutaneous (47%). Renal manifestations included proteinuria (72%), hematuria (72%), decreased GFR creatinine clearance (54%), and nephrotic syndrome with edema (11%). Almost all of the patients with a biopsy had GN (94%). Thirteen percent had renal failure requiring dialysis and 7% progressed to ESKD. In the available renal biopsy specimens, pauci-immune and/or necrotizing GN was pres-

Table 25.4 Presenting features of MPA and GPA in ARChiVe study (n = 231). Adapted from Cabral et al. [61]

Clinical feature, n (%)	MPA (n = 48)	GPA (n = 183)
Constitutional/general	41 (85)	160 (88)
Malaise/fatigue	37 (77)	152 (83)
Fever	25 (52)	97 (53)
Weight Loss	15 (31)	80 (44)
Renal	36 (76)	151 (83)
Hypertension (age-adjusted)	16 (33)	39 (21)
Clinically nephrotic with edema	11 (23)	20 (11)
Renal failure requiring dialysis	12 (25)	24 (13)
End-stage renal disease	5 (10)	12 (7)
Impaired creatinine clearance or abnormal protein/creatinine ratio	28 (58)	99 (54)
Proteinuria	33 (69)	132 (72)
Hematuria >1+ or <10 hpf od red cell casts	29 (60)	132 (72)
Biopsy-proven glomerulonephritis	30 of 32 (94)	101 of 108 (94)
Pulmonary	21 (44)	136 (74)
Chronic cough	11 (23)	99 (54)
Wheeze or expiratory dyspnea	2 (4)	15 (8)
Alveolar hemorrhage/massive hemoptysis	7 (15)	76 (42)
Pleurisy	4 (8)	25 (14)
Supplemental oxygen requirement	6 (13)	40 (22)
Respiratory failure	2 (4)	22 (12)
Ear, nose, throat	0 (0)	128 (70)
Septal perforation or nasal collapse	0 (0)	15 (8)
Recurrent nasal bloody discharge/crusting/obstruction/ulcer	0 (0)	98 (53)
Chronic recurrent sinusitis	0 (0)	71 (39)
Conductive or sensorineural hearing loss	0 (0)	19 (10)
Otitis/mastoiditis	0 (0)	31 (17)
Subglottic involvement	0 (0)	19 (10)
Oral ulcers/granulomata	2 (4)	27 (15)
Eyes	15 (31)	78 (43)
Conjunctivitis	3 (6)	21 (11)
Non-specific red eye	1 (2)	19 (10)
Episcleritis	2 (4)	15 (8)
Proptosis or retro-orbital mass	0 (0)	3 (2)
Retinal exudates of hemorrhages or aneurysms or vessel thrombosis	1 (2)	0 (0)
Cutaneus	25 (52)	86 (47)
Palpable purpura/petechial rash	15 (31)	49 (27)
Gastrointestinal	28 (58)	66 (36)
Non-specific abdominal pain	18 (38)	41 (22)
Chronic nausea	16 (33)	22 (12)
Musculoskeletal	25 (52)	118 (65)
Arthralgia or confirmed arthritis	20 (42)	112 (61)
Myalgia, muscle weakness, or confirmed myositis	9 (19)	24 (14)
Nervous system	10 (21)	36 (20)
Headache	6 (13)	20 (11)
Dizziness	2 (4)	12 (7)
Cardiovascular	3 (6)	10 (5)
Venous thrombosis	0 (0)	3 (2)

ent in 80% of GPA patients [61]. In a single-center Canadian study of 25 patients with GPA, 88% had GN and 20% required dialysis at disease onset. Twenty-eight percent had elevated creatinine at disease onset and an additional 16% had an elevated creatinine during follow-up [62]. A large cohort from the PRINTO vasculitis database included 56 children with GPA. The demo-

graphic and most of the clinical data confirms results of the ARChiVe data, but patients from the PRINTO registry were younger (median 11.7 years), and had more frequent inflammation of the ears, nose, and throat (91%) and the eyes (35%) [63]. Sixty-seven percent were ANCA-PR3 positive [61, 63].

Microscopic Polyangiitis (MPA)

MPA characteristically involves small vessels of the kidneys and lungs and patients are positive for MPO-ANCA, and there are no granulomas in the pathologic specimens. The ARChiVe database included 48 pediatric MPO patients, who were significantly younger than GPA patients, with a median age at the time of diagnosis of 12 years. At presentation, the most common clinical manifestations were constitutional symptoms (85%), renal (75%), gastrointestinal (58%), musculoskeletal (52%), and cutaneous (52%). Results are shown in Table 25.4 [61]. The kidneys were more severely affected in MPA compared to GPA. The serum creatinine was moderately to severely elevated in almost half of the MPA (48%) patients. The rates of proteinuria and hematuria were 69% and 60%, similar to GPA, but almost one-quarter of patients presented with nephrotic syndrome with edema, and one-quarter of patients required dialysis. Ten percent progressed to ESKD [61]. Where renal biopsies were obtained, pauci-immune and/or necrotizing GN was seen in 78% [61]. Renal disease was a hallmark of MPA, present in 94% of MPA patients in the meta-analysis that included 130 pediatric patients [64]. The study bias was that it included studies from 1950 and MPA was previously not clearly defined. In ARChiVe, more than half of the patients had MPO-ANCA and/or p-ANCA (55%) and only 17% had PR3-ANCA and/or c-ANCA. ANCA was not present in 26% of the patients [61].

Eosinophilic Granulomatosis with Polyangiitis

EGPA is characterized by asthma or allergic rhinitis, skin disease, and vasculitis, which mostly involves small vessels and eosinophilic infiltration accompanies extravascular granulomas [65]. It is very rare in childhood, with only two out of 114 children with AAV having EGPA in the Canadian registry [66]. The largest pediatric cohorts include from 9–14 children with EGPA [67–69].

Laboratory Results

Marked elevation of inflammatory markers is typical, both erythrocyte sedimentation rate (ESR) and c-reactive protein. In half of GPA patients and one-third of MPA, total white blood cells and neutrophils are elevated. Eosinophil levels are normal for most patients with GPA and MPA. Over 80% of all patients have anemia and approximately one-third have elevated platelet counts. Von Willebrand antigen may also be elevated [61]. More than half of MPA patients and around 26% of GPA patients have MPO-ANCA and/or p-ANCA; whereas 67% of patients with GPA and 17% of patients with MPA have PR3-ANCA [61, 63]. ANCA, tested by either immunofluorescence or ELISA, was not present in 26% of MPA patients and 5% of GPA patients in ARChiVe [61, 66].

Disease Activity Assessment

Assessing disease activity and disease damage are important to help guide treatment decisions. Standardized tools are lacking in the field of childhood vasculitides. The most commonly used score in adult systemic vasculitides is the Birmingham Vasculitis Activity Score (BVAS), that was designed in 1994 [70], while the BVAS version 3 from 2009 is the most recent [71]. It is a composite score of 56 clinical features from 9 organ systems. Each feature is enumerated, according to the severity. Symptoms are categorized as new, persistent, or worsening [71]. The BVAS/WG is a disease-specific, modified version, validated for use in GPA [72]. To acknowledge the differences in childhood vasculitis, the Pediatric Vasculitis Activity Score (PVAS) was developed and validated [73]. PVAS was created by modifying BVAS. The pediatric vasculitis reg-

istry was reviewed to identify clinical features missing in the BVAS version; consequently, eight additional pediatric items were added, and others redefined, making 64 active items in nine categories. Age-specific reference ranges for blood pressure and renal function, and a pediatric definition of weight loss are incorporated. The score correlated well with physicians' global assessment, treatment decisions, and ESR [73].

Disease Damage Assessment

In 1997 the Birmingham Vasculitis Group developed and validated the Vasculitis Damage Index (VDI), a standardized tool for clinical assessment of damage in the systemic vasculitides [74]. Damage was defined as irreversible change resulting from scars. Currently, the PReS.

Childhood Arthritis and Rheumatology Research Alliance joint effort to make a pediatric version of VDI (pVDI) is underway. Vasculitis damage is defined as the presence of irreversible features present for at least 3 months since the onset of vasculitis and the pVDI contains 72 items in 10 systems [75]. Morishita et al. used pVDI to assess early outcomes in children with AAV [76].

Table 25.5 represents a roadmap of investigations suggested in all (first-line) and in certain (second-line) children with renal vasculitides [77].

Renal Transplantation

Due to the severity and rapid progression of the renal involvement in AAV, a substantial proportion of children progress to ESKD and require renal transplantation. In a recent single-center study assessing outcome of renal transplantation in these patients, seven children (3 with GPA, 4 with MPA) were described [78]. Time from diagnosis to transplant was 30 ± 12 (range, 17–48) months. Median duration of follow-up post transplantation was 27 months (range, 13–88 months). Median eGFR at last follow-up was 77 ml/min/1.73 m^2 (range, 7.9–83.5). One patient lost her transplant to acute cellular rejection following non-adherence to immunosuppression after

Table 25.5 Laboratory investigations in pediatric AAV. Modified from Plumb et al. [77]

First-line investigations	Second-line investigations
Hematology: Full blood count, Erythrocyte sedimentation rate, coagulation profile	Histology: renal/lung biopsy
Immunology: Lymphocyte subsets with CD19 count (pre-RTX), Immunoglobulins (Ig): IgG, IgM, IgA, IgE	Imaging: fibroendoscopy/X-ray sinuses, Chest high resolution computed tomography, MRI/MR angiography head
Biochemistry: Urea, creatinine and electrolytes, Liver function tests, Lactate dehydrogenase, Creatinine kinase, Thyroid function, Pancreatic function (amylase/lipase), Urine protein: creatinine ratio	Immunology: Anti-glomerular basement membrane antibodies, Coeliac screening
Infectious: Serology HBV, HCV, parvovirus B19, HIV, EBV, CMV, Mycoplasma, VZV and Anti-Streptolysin titre; Mantoux and/or QuantiFERON-TB testing, Blood culture, Urine microscopy and cell culture	Neurological: Nerve conduction studies
Autoantibody panel: Anti-neutrophil cytoplasmic antibodies, Antinuclear antibodies, Anti-double stranded DNA antibodies, Anti-cardiolipin and anti-phospholipid antibodies, Lupus anticoagulant	Ambulatory blood pressure monitoring; fundus oculi
Complement (C) function: C3, C4	Birmingham vasculitis activity score
Chest X-ray, Electrocardiography, Echocardiography	Renal angiography, Renal dimercaptosuccinic acid (DMSA) scan

21 months of stable transplant function. No patient had recurrence of vasculitis, either renal or extrarenal.

Monogenic Forms of Renal Vasculitis

Recently, monogenic forms of autoinflammatory diseases have been identified that present predominantly with features of systemic vasculitis in childhood. Their discovery has shed light on new pathophysiological pathways which may allow innovative management, both in terms of monitoring biomarkers of disease activity and in terms of targeted therapeutic approaches [79].

Type I Interferonopathies

Type I interferons (IFNs) are regulatory proteins involved in immune response against viral infections. Their activation is induced by recognition of foreign nucleic acids and is tightly regulated by a complex network of pathways that involve both the innate and adaptive immune system. Type I interferonopathies are a group of rare monogenic disorders associated with constitutive overproduction of type I IFNs and provide unique insights into the mechanisms of differentiating self nucleic acids from non-self nucleic acids [80]. There is a marked overlap of clinical features across different type I interferonopathies, particularly the involvement of the central nervous system and skin as well as lung inflammation in certain genotypes. The clinical expression of type I interferonopathies may be influenced by differential exposure to environmental) triggers such as infection [81, 82].

Stimulator of interferon genes -associated vasculopathy with onset in infancy.

Stimulator of interferon genes (STING)-associated vasculopathy with onset in infancy (SAVI) is a type I interferonopathy caused by a gain-of-function mutation in the transmembrane protein 173 gene (*TMEM173*) [83]. *TMEM173* encodes STING, which is a transmembrane protein localized in the endoplasmic reticulum and functions in the transduction of a type I IFN response to different) types of cytosolic DNA. In patients with SAVI, constitutively activated STING leads to increased transcription of the type I IFN gene *IFNB1* and production of IFN-β, which further up-regulates the transcription of IFN-response genes.

SAVI is clinically characterized by neonatal-onset systemic inflammation, a severe cutaneous vasculopathy and major interstitial lung disease [83]. Over-expression of STING induces endothelial cell activation resulting in a severe inflammatory vaso-occlusive process leading to tissue loss on hands and feet at an early age. Renal involvement in SAVI is rare and usually mild, with microscopic hematuria and mild proteinuria associated) with hypertension [84]. One patient with SAVI developed kidney involvement with apolipoprotein L1 (APOL1)-associated collapsing glomerulopathy suggesting the role of IFN pathways in the pathogenesis of APOL1-associated nephropathy [85]. Systemic inflammation in patients with SAVI is refractory to conventional immunosuppressive medications, but treatment with JAK inhibitors (tofacitinib, ruxolitinib and baricitinib) could suppress the expression of STING-induced IFN-response genes and lead to clinical improvement.

Coatomer Associated Protein Subunit Alpha Gene Syndrome

Coatomer associated protein subunit alpha gene (COPA) syndrome is an autosomal dominant syndrome caused by mutations in the coatomer associated protein subunit alpha (COPα) gene (*COPA*) [86]. It is characterized by interstitial lung disease, inflammatory arthritis and kidney disease in childhood. COPα is part of the coatomer protein complex I involved in retrograde movement of vesicles from the Golgi apparatus to the endoplasmic reticulum (ER). Mutations in COPA lead to defective intracellular transport, resulting in immune dysregulation that can promote both autoinflammation and autoimmunity. Immunologically, COPA syndrome is associated with autoantibodies, significant skewing of CD4 T cells toward a T helper type 17 (Th17) phenotype implicated in autoimmunity, and proinflammatory cytokine expression such as IL-1β, IL-6

and IL-23. Patients with COPA syndrome have a strong upregulation of type I IFN-stimulated genes similar to patients with SAVI [87, 88].

Renal disease was reported in 45% of patients with COPA syndrome and typically presents as GN with proteinuria and decreased renal function [88, 89]. Kidney biopsy findings were heterogeneous, including crescentic GN and focal mesangial hypercellularity with immune complex deposition ranging from isolated IgA deposits to a "full house" immunofluorescence (IgM, IgG and C1q) resembling lupus nephritis. Increased type I IFN signaling suggests that JAK inhibitors as a possible treatment for COPA syndrome [87, 88].

Deficiency of Adenosine Deaminase 2

Deficiency of adenosine deaminase type 2 (DADA2) is a monogenic vasculitis syndrome caused by loss-of-function mutations in the adenosine deaminase type 2 (*ADA2*) gene, and clinically presents as an inflammatory vasculopathy [90, 91]. *ADA2* is a highly polymorphic gene, and more than 60 disease-causing mutations have been described, mostly missense variants.

Adenosine deaminase proteins regulate purine metabolism, and in the absence of ADA1 toxic deoxyadenosine nucleotides accumulate in lymphocytes leading to the severe combined immunodeficiency. ADA2, in addition to its deaminase activity, functions as a growth factor for endothelial cells and is involved in leukocyte development and differentiation. ADA2 appears to be critical for the maintenance of vascular integrity and in cross-talk between macrophages and pericytes. Increased production of proinflammatory cytokines was found in skin biopsies and blood samples from patients with DADA2 [92].

The disease has a highly variable clinical presentation, with 77% of patients presenting before the age of 10 years. A characteristic clinical feature is an inflammatory vasculopathy of small- and medium-sized arteries, with manifestations ranging from livedo reticularis to PAN and life-threatening ischemic or hemorrhagic stroke. Patients with DADA2 usually present with fever, increased acute phase reactants and a vasculitic skin rash such as livedo reticularis, nodules, purpura, skin ulcers and digital gangrene. Vasculitis in DADA2 can affect other organs, including intestine, liver and kidney. The most common renal manifestations in patients with DADA2 are arterial hypertension, renovascular aneurysms, renal artery stenosis and kidney inflammation with dense lymphocytic infiltration and glomerular scarring [91, 93].

Genetic testing for DADA2 and measurement of ADA2 enzymatic activity should be considered for patients with early-onset PAN-like systemic vasculitis, refractory PAN and familial vasculitis. The mainstay of treatment for DADA2 is anti-TNF-α biologic drugs, which are effective in reducing fever episodes, vasculitic disease activity and preventing ischemic strokes, but are not effective for the immunodeficiency [94, 95].

Hypocomplementemic Urticarial Vasculitis Syndrome

Hypocomplementemic urticarial vasculitis syndrome (HUVS), or McDuffie syndrome, is a small vessel vasculitis associated with urticaria, hypocomplementemia (both C3 and C4) and anti-C1q circulating antibodies [96]. It affects the skin, joints, eyes, lungs and kidneys. Diagnostic criteria have been formulated [97] and diagnosis requires the presence of chronic urticarial exanthema and hypocomplementemia plus at least two minor diagnostic criteria (leukocytoclastic vasculitis, arthralgia/arthritis, GN, uveitis/episcleritis/conjunctivitis, abdominal pain, positive anti-C1q antibodies) and exclusion of autoimmune disease (systemic lupus erythematosus [SLE], Sjögren syndrome, cryoglobulinemia). It affects predominantly females with an 8:1 ratio, typically in the fifth decade of life. In a recent review of 60 cases described in the literature [98], renal involvement was identified in 14–50% of cases of HUVS. The most frequent presenting symptoms were hematuria and proteinuria (70% of patients). One third of patients with renal involvement had reduced kidney function at pre-

sentation (eGFR below 60 ml/min/1.73 m^2). The most frequent glomerular pattern of injury was membranoproliferative GN (35%), followed by mesangioproliferative (21%) and membranous (19%) GN. Crescents were found in 23% of cases and were associated with a more severe presentation, as expected. By immunofluorescence, positivity for IgG, IgM, C1q and C3 was found in the mesangium and along the glomerular capillary walls, indicating immune-complex deposition. This finding, together with the observation that 50–55% of patients with HUVS have a positive ANA, indicates that this disease has similarities to SLE. Indeed, in about 50% of patients disease progression, if not prevented, leads to the development of SLE [99].

In the cohort of HUVS patients with renal involvement [98], 18% were children, and in children the male to female ratio was 1:1. Renal involvement in HUVS led to ESKD in 15% and 17% of cases in adults and children, respectively. Treatment should therefore be aggressive and timely, following the indications given below for other forms of renal vasculitis in children.

Studies in familial forms have revealed that HUVS can be caused by a homozygous mutation in *DNASE1L3* [100]. The protein encoded by *DNASE1L3* is an endonuclease capable of cleaving both single- and double-stranded DNA. Individuals harboring mutations in this gene develop HUVS in infancy/childhood, have a substantial risk of developing severe organ (kidney, lung) involvement and full-blown SLE, and may require life-long immunosuppressive treatment.

Treatment and Management of Renal Vasculitis

Renal vasculitides in children are extremely rare, though they determine significant morbidity and mortality, particularly if there is diagnostic delay [101]. Therefore, conducting traditionally designed randomized clinical trials is not feasible in children, and current therapeutic strategies are based on small case series or data derived from RCTs performed in adults. Despite these limitations, there have been substantial improvements in management of these conditions. This progress is related to the use of different immunosuppressive medications and newer biologic agents, and to the broader recognition of monogenic forms, which may benefit from targeted therapy. Moving forward, innovative trial design tailored to small numbers is necessary, as is a standardized approach to the management of these rare pediatric diseases. Consequently, there are now recommendations based on consensus and, where possible, on evidence, for management of these conditions. The European consensus-based recommendations on diagnosis of rare pediatric vasculitides are a successful example of this approach [6]. As described above, the AAV include GPA (formerly known as Wegener's granulomatosis), MPA, EGPA (previously referred to as Churg-Strauss syndrome) and renal-limited microscopic vasculitis, also known as pauci-immune or crescentic GN (GN). Pauci-immune GN is a fulminant, relapsing disease in children [102, 103] and is frequently associated with RPGN, characterized by clinical features of GN and rapid decline of renal function, with pathology exhibiting crescent formation affecting the majority of glomeruli. RPGN and its treatment are covered in Chap. 50 of this textbook.

A possible therapeutic approach is outlined in Table 25.6. Treatment consists of an aggressive induction treatment, aimed at switching off the fulminant inflammatory disease process, followed by a maintenance phase aimed at consolidating remission. There is no clear consensus as to when and how treatment should be discontinued. However, following induction, assessment of organ damage and of the side effects of therapy should be considered when designing a maintenance strategy that balances risks and benefits. Even in terms of induction, recent positive results from studies assessing RTX and avacopan should be considered, with the objective of minimizing long-term toxicity, such as sterility, malignancy, stunted growth and osteoporosis/aseptic necrosis, which is especially important in children. While GPA and MPA are considered separate entities, they are managed identically.

Table 25.6 AAV treatment. Modified from Pediatric Kidney Disease 2nd edition [144]

Agent	Route	Administration dosage and duration
Induction treatment		
Pulse Methylprednisolone[a]	IV	400–600 mg/m^2/day (max 1000 mg/day) for 3–5 consecutive days
Prednisone[a] Prednisolone[a]	Oral	1.5–2.0 mg/kg/day (max 60 mg/day) for 4 weeks Gradually taper down over 6–12 months
Cyclophosphamide[b]	IV	Start at 500 mg/m^2/day Increase monthly by 125 mg/m^2/day to 750–1000 mg/m^2/day (max 1000 mg/day) for 6–10 times
Cyclophosphamide	Oral	2 mg/kg/day for 2–3 months
Rituximab[c]	IV	375 mg/mg/m^2/day weekly for 4 times
Plasmapheresis		Double volume on alternate day for 2 weeks
Maintenance treatment		
Azathioprine	Oral	2.0 mg/kg/day for 9 months Switch from cyclophosphamide at 3 months
Rituximab[d]	IV	Optimal dose and timing in children not available

IV intravenous
[a] Methylprednisolone pulses followed by oral prednisone or prednisolone 1.5–2.0 mg/kg/day for 4 weeks, with gradually tapering until discontinuation over 6–12 months
[b] Cyclophosphamide followed by azathioprine switching from cyclophosphamide at 3–6 months
[c] Given with pulse and oral corticosteroids
[d] No data available on use of RTX for maintenance if it has been used at induction

Induction Treatment

Cyclophosphamide and Glucocorticoids

Cyclophosphamide and glucocorticoids are well-established induction treatment of AAV. Therapy with cyclophosphamide and oral prednisone/prednisolone for 3–6 months was found to lead to clinical remission in 93% of adult patients with AAV [104], and improved remission rate from 56% to 84.7% and decreased relapse rate by approximately 50% [105]. At disease onset, given the rapidity and severity of renal manifestations, pulse IV methylprednisolone is recommended [6] at 400–600 mg/m^2/day (maximum dose 1000 mg/day) for 3–5 consecutive days. This treatment should be followed by oral prednisone/prednisolone 1.5–2.0 mg/kg of ideal body weight daily (maximum dose 60 mg/day) for 4 weeks, followed by gradual tapering over 6–12 months. Cyclophosphamide can be administered in oral or IV pulse regimens. Daily oral cyclophosphamide should start at a dose of 2 mg/kg/day and continue for 2–3 months while adjusting the dose to keep the nadir leukocyte count above 3000/mm^3. When a regimen of IV pulsed cyclophosphamide is used, the initial dose should be approximately 500 mg/m^2 and increased monthly by 125 mg/m^2 to 750–1000 mg/m^2 (maximum dose 1000 mg/day) every 4 weeks for 6–10 doses. Cyclophosphamide should be given with adequate oral or IV hydration and with mesna, a drug which binds the cyclophosphamide metabolite which is toxic for the bladder mucosa, to minimize the risk of hemorrhagic cystitis. Subsequent doses should be adjusted depending on the 2-week post-treatment nadir leukocyte count. A RCT of IV pulse cyclophosphamide versus daily oral cyclophosphamide for induction of remission in AAV with renal involvement has been conducted [106]. This trial demonstrated that IV pulse and daily oral cyclophosphamide had similar remission rates and times to remission. Patients receiving the pulse regimen were administered approximately one-half of the cumulative dose of cyclophosphamide of the oral regimen and experienced a significantly lower rate of leukopenia for the same duration of therapy [106]. In a meta-analysis of RCTs, the pulse regimen was associated with fewer infections, increased risk of relapse, less leukopenia, and a trend toward a higher rate of requiring renal replacement therapy [107]. Because of a lower cumulative dose and a lower risk of side effects, the pulse regimen is recommended as the first line of induction therapy for pediatric pauci-immune GN. Combination therapy with oral cyclophosphamide (2 mg/kg/day) and pulse methylprednisolone followed by oral prednisolone resulted in a high remission rate (70–100%) and low mor-

tality in pediatric ANCA-associated GN [108–110]. Thus, the duration of continuous oral cyclophosphamide should usually be limited to 3 months, with a maximum of 6 months, but whether the same duration can be applied to IV pulsed cyclophosphamide is unclear [111].

Other agents employed in remission induction include anti-TNF-α antibodies and MMF. It is now recognized that RTX is as effective as cyclophosphamide in severe or relapsing disease, particularly for those patients at risk for glucocorticoid or cyclophosphamide toxicity. PLEX and IV immunoglobulin therapy may also be used as adjuvant therapy to induce remission in more severe cases.

The only randomized study of pulse methylprednisolone is the MEPEX trial [112]. This study investigated whether the addition of PLEX to oral corticosteroids and cyclophosphamide was more effective than pulse methylprednisolone (1 g × 3) for renal recovery in patients who presented with renal failure. In this study, there was no difference in mortality and safety, but PLEX appeared more effective than pulse methylprednisolone in preserving kidney function.

Rituximab

Despite its effectiveness, the induction regimen with high-dose glucocorticoids and cyclophosphamide has significant morbidity in the long-term, which is particularly relevant in children. In a cohort of eight adults who presented with childhood-onset AAV, at a median of 19 years of follow-up, seven suffered from infections, four were infertile, two had skeletal complications, and one developed malignancy [113].

RTX is a chimeric monoclonal antibody that targets the CD20 antigen on the surface of B cells. Several case series and small studies have reported the efficacy of RTX in refractory AAV. Two randomized trials examined RTX as induction therapy for AAV. In the RITUXVAS trial, 44 patients with newly diagnosed AAV were randomized to either RTX or cyclophosphamide groups. The RTX group received four 375 mg/m^2 doses of RTX given weekly and IV cyclophosphamide at a dose of 15 mg/kg, 2 weeks apart for a total of two doses. The cyclophosphamide group received 15 mg/kg of IV cyclophosphamide every 2 weeks × 3 doses, and then every 3 weeks for a maximum of 10 doses. Both groups received IV methylprednisolone, followed by oral corticosteroids. There were no significant differences in the rates of remission and serious adverse events [114]. In the RAVE trial, 197 ANCA-positive patients with either GPA or MPA were randomized to treatment with either RTX or conventional cyclophosphamide followed by azathioprine. The RTX group received four weekly doses of 375 mg/m^2. The cyclophosphamide group received 2 mg/kg/day orally for 3 months, followed by oral azathioprine at a dose of 2 mg/kg/day for 3 months. Both groups received 1–3 pulses of methylprednisolone (1000 mg each), followed by prednisone at a dose of 1 mg/kg/day, tapered by 5 months. At 6 months, 64% of the patients in the RTX group, compared with 53% of the patients in the cyclophosphamide–azathioprine group, experienced complete remission. There were no significant differences between the two treatment groups in the rates of complete remission, adverse events, or relapse [115]. At 12 and 18 months, 48% and 39%, respectively, of the patients in the RTX group had maintained complete remission, compared with 39% and 33%, respectively, in the cyclophosphamide–azathioprine group. There were no significant differences between the two groups in the duration of complete remission, the frequency or severity of relapses, and adverse events. This study shows that RTX is equivalent to cyclophosphamide in efficacy for the induction and maintenance of remission over 18 months [116] Following these important results obtained in adult clinical trials, the use of RTX in children with AAV is being evaluated in the Pediatric Polyangiitis Rituximab Study (PEPRS) [117]. This is an open-label study which has enrolled 25 children with newly diagnosed or relapsing GPA or MPA. They have received weekly IV RTX for 4 weeks at a dose of 375 mg/m^2 as well as glucocorticoids (1 mg/kg/day [max 60 mg/day]) tapered to 0.2 mg/kg/day (max 10 mg/kg/day) by 6 months. All patients received three doses of IV methylprednisolone at 30 mg/kg/day with a maximum of 1 g/day prior to the first RTX infusion.

The safety profile and pharmacokinetics were comparable to adults with GPA or MPA. No new safety signals have emerged thus far [118]. Basu et al. reported a retrospective analysis of 11 pediatric MPA patients treated with a cyclophosphamide free, RTX- and MMF-based protocol with a median follow-up period of 20.9 months [119]. Patient and renal survival at 1 year were 100%. Despite varying degrees of renal involvement at presentation, kidney function recovered in all patients, with a median eGFR of 79.5 ml/min/1.73 m^2. At last follow-up, 91% of patients were in complete remission and one (9%) child was in partial remission.

Hence, it seems reasonable to use RTX in children with severe AAV when cyclophosphamide is not available or not advisable. In terms of RTX dose, it is possible that an alternate regimen of 2 doses at 750 mg/m^2 (max 1000 mg) administered 14 days apart as in rheumatoid arthritis may be comparable to the classic regimen [77] RTX in AAV and other autoimmune disorders has been shown to induce hypogammaglobulinemia [120]: 56% of adults had IgG hypogammaglobulinemia during follow-up; IgG replacement was initiated because of recurrent infection in 4.2% of patients. No association was found between IgG levels and cumulative RTX dose.

Plasma Exchange

In severe AAV, especially with pulmonary haemorrhage or RPGN, or when the patient has significant deterioration despite an appropriate induction regimen, therapy is often augmented by PLEX, a strategy targeted towards removing the pathogenic antibodies [121–123]. Following the first positive report on PLEX in nine patients with crescentic GN, of whom five rapidly recovered renal function, [25, 124] the use of PLEX was recommended only in patients with the most severe renal disease (creatinine >500 μmol/L equivalent to >5.66 mg/dl) [125] due to the results of studies demonstrating a beneficial effect of PLEX only in dialysis-dependent patients [126, 127]. The MEPEX trial [112] compared PLEX to pulse methylprednisolone in addition to oral prednisolone and oral cyclophosphamide in patients with a new diagnosis of AAV and serum creatinine >500 μmol/L. PLEX was associated with a significantly higher rate of kidney recovery at 3 months (69% with PLEX vs. 49% with pulse methylprednisolone), and also with a reduction in risk for progression to ESKD at 12 months. On the other hand, patient survival and the rate of severe adverse events were similar in both groups [112].

More recently, the PEXIVAS trial included all patients with a GFR <50 ml/min/1.73 m^2 and thus aimed to answer the question of whether PLEX is a good option for patients with moderate kidney function impairment. PEXIVAS showed that after a follow-up of almost 3 years routine PLEX did not provide reduce the rate of the composite outcome of ESKD or death. Taken altogether, these results indicate that PLEX should be reserved for severe cases of AAV with marked reduction in kidney function or requiring dialysis [128].

The 2021 KDIGO guidelines suggest to consider plasmapheresis for patients with refractory disease due to drug intolerance, non-adherence, concomitant morbidities, a secondary drive for vasculitis such as malignancy, drugs or infection, and true treatment failure and for patients with diffuse alveolar bleeding with hypoxemia. They recommend plasmapheresis for patients with combined AAV and anti-GBM GN, according to the proposed criteria and regimen for anti-GBM GN [129].

Mycophenolate Mofetil

Another agent which has been investigated in AAV induction as a safer, less toxic alternative to cyclophosphamide is MMF, an orally administered lymphocyte suppressive agent with short duration of action, which in small studies appeared beneficial in AAV, both in adults and in children [130, 131]. TA RCT assessed whether MMF was non-inferior to cyclophosphamide for remission induction in newly diagnosed AAV patients [132]. All patients received the same oral glucocorticoid regimen and were switched to azathioprine following remission. The primary endpoint was remission by 6 months and compliance with the tapering glucocorticoid regimen. MMF was non-inferior to cyclophosphamide for

remission induction in AAV, but resulted in a higher relapse rate. Therefore, if other options are unavailable or unadvisable, MMF can be considered for induction in patients with non-severe or life-threatening AAV.

Anti-TNF Therapy

In AAV, TNF-α may have a pathogenic role, both in the formation of granulomas and in neutrophil priming, which enhances the expression of endothelial adhesion molecules on the cell surface and the capability of ANCA to stimulate neutrophil degranulation, a driver of vascular damage [77]. Etanercept, infliximab and adalimumab have been investigated in this setting with mixed results, and evidence is insufficient to recommend their use at this time [118].

Avacopan

Evidence of a role of complement, especially of C5a, a powerful anaphylatoxin which recruits neutrophils to the inflammatory site, and of the C5aR, in the pathogenesis of AAV [18] led to the use of complement inhibition with the oral C5aR antagonist avacopan. Efficacy, safety and steroid-sparing effects of avacopan in patients with GPA/MPA were shown in two phase II trials [133, 134] and a phase III trial [135]. In the phase III RCT, adult patients with AAV were randomized in a 1:1 ratio to receive oral avacopan at a dose of 30 mg twice daily or oral prednisone on a tapering schedule. All patients received either cyclophosphamide (followed by azathioprine) or RTX. The first primary end-point was remission, defined as a BVAS score of 0 at week 26 and no glucocorticoid use in the previous 4 weeks. The second primary end-point was sustained remission, defined as remission at both weeks 26 and 52. Both end-points were tested for non-inferiority and for superiority. Avacopan was non-inferior, but not superior to prednisone taper with respect to remission at week 26 and was superior to prednisone taper with respect to sustained remission at week 52. This remarkable result needs to be confirmed in a pediatric population, but suggests the possibility of a prednisone-free induction treatment for AAV.

Adjunctive Measures

The SHARE initiative European recommendations on management of pediatric vasculitides suggest the use of antiplatelet agents to prevent thrombotic complications associated with systemic vasculitis in the young [136]; antibiotic prophylaxis to prevent Pneumocystis jiroveci pneumonia at induction; osteoporosis prophylaxis with vitamin D in children treated with prednisone/methylprednisolone; and gastric protection (e.g. with protein pump inhibitors) in case of gastric pain.

Maintenance Treatment

Following induction of remission, maintenance therapy is necessary to prevent relapse. Long-term toxicity (infertility, risk of bladder cancer and lymphoproliferative disorder) makes cyclophosphamide an unattractive option for maintenance after successful induction. The CYCAZAREM trial (cyclophosphamide vs. azathioprine for early remission phase of vasculitis) found that azathioprine was as effective as continuous cyclophosphamide at maintaining remission and was associated with fewer side effects [104]. In this study, both study groups received the same induction therapy, consisting of oral cyclophosphamide and prednisolone. Once remission had been achieved, between 3 and 6 months, patients were randomly assigned to treatment with azathioprine (2 mg/kg/day) or to continued cyclophosphamide therapy (1.5 mg/kg/day), with the same dose of prednisolone (10 mg/day) up to 12 months. Subsequently, from 12 to 18 months, all patients received azathioprine (1.5 mg/kg/day) and prednisolone (7.5 mg/kg/day). The primary outcome was relapse at 18 months, and there was no difference between the groups. Once azathioprine was established as a suitable alternative therapeutic agent for maintenance, its optimal duration was not evaluated. The REMAIN study addressed this question and found that azathioprine given in association with low-dose glucocorticoids for 48 months compared to 24 months reduced the risk of relapse threefold. Moreover,

the 48-month group had improved renal survival and reduced incidence of ESKD compared to the 24-month group [137]. The use of LEF in maintaining remission is less well studied, but results of 1 prematurely ended randomized study indicated that, at a dose of 30 mg/day, leflunomide was more effective than methotrexate at preventing relapse despite being associated with a higher rate of adverse events [138].

The use of MMF was evaluated in the IMPROVE study, which at a median follow-up of 39 months showed that MMF was inferior to azathioprine at preventing relapses during maintenance [139]. In this study, patients with AAV who attained remission with cyclophosphamide and prednisolone, were randomized to either MMF at a dose of 2000 mg/day or azathioprine at a dose of 2 mg/kg/day. Relapse was more common in the MMF group than in the azathioprine group. Both groups had similar adverse event rates. Therefore, azathioprine is preferred to MMF or cyclophosphamide for maintenance therapy in AAV.

Recently, the use of RTX for the maintenance of AAV has been investigated. The MAINRITSAN trial compared the use of repeated doses of RTX (500 mg at weeks 0 and 2, then every 6 months for 5 courses) with daily azathioprine after cyclophosphamide induction for new and relapsing patients [140]. The adult patients received azathioprine or RTX. RTX was significantly better than AZA since at 28 months major relapse had occurred in 29% of patients in the azathioprine group and in 5% of patients in the RTX group, without significant differences in severe adverse events. In considering the results of this trial, it is important to note that only a cyclophosphamide induction was used. This trial does not provide information about RTX maintenance after RTX induction. The RTX dose utilized was also different from previous trials (500 mg twice at a 14-day interval after remission is achieved, with subsequent 500 mg doses every 6 months for 5 courses), while the azathioprine dose was tapered between months 12–22, possibly to a sub-therapeutic dose [118]. The RITAZAREM trial investigated the use of RTX for the treatment of relapses in adults with AAV. RTX in conjunction with glucocorticoids demonstrated a high level of efficacy for the reinduction of remission in patients with AAV who have relapsed [141]. Seventy-nine percent and 36% had previously received cyclophosphamide and RTX, respectively. The vast majority (90%) achieved remission by 4 months. The use of RTX therefore appears promising, although not validated in a pediatric cohort, and the optimal dose and timing are not yet established.

The use of a B cell modulator, belimumab (a monoclonal antibody directed against BAFF, a B cell survival factor), has been investigated in the BREVAS study [142]. In this double-blind, placebo-controlled study, adult patients with AAV were randomized 1:1 to receive azathioprine (2 mg/kg/day), low-dose oral glucocorticoids (≤10 mg/day), and either IV belimumab (10 mg/kg) or placebo, following remission induction with RTX or cyclophosphamide along with glucocorticoids. Belimumab, although safe, did not reduce the risk of relapse of vasculitis; therefore, its use is not warranted for maintenance of AAV.

Treatment of Relapses

Relapses are not infrequent in AAV. A relapse is defined as the reactivation of vascular inflammation. It is important to assess the severity of the relapse by identifying which organs are affected. Most guidelines recommend basing treatment on what was used previously at induction and then switching (for example from cyclophosphamide to RTX or vice versa). However, if the patient responded fully to RTX at induction, it can be reasonable to repeat this therapeutic approach [143].

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Lupus Nephritis

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Introduction

Juvenile onset systemic lupus erythematosus (JSLE) is a lifelong, life-limiting, multi-system, autoimmune disorder, which is episodic in nature with a broad spectrum of clinical and immunological manifestations. JSLE is characterised by widespread inflammation of blood vessels and connective tissues affecting the skin, joints, kidneys, heart, lungs, nervous and other systems. There is a higher rate and more severe organ involvement in children than in adults (especially with respect to haematological and renal disease) [1–4]. Renal involvement with biopsy-proven lupus nephritis (LN) occurs in up to 80% of all cases of JSLE and is a major determinant of the prognosis. We currently have an increasing armamentarium of immunosuppressive agents that can be used to treat active disease with newer agents on the horizon. However, there is still a significant morbidity and mortality for severe disease with considerable physical and psychosocial morbidity due to the variable, and often progressive, clinical course of JSLE. This results from both the sequelae of disease activity and the side-effects of medications, including the infectious risks from over-immunosuppression, and longer-term risks with accelerated atherosclerosis [5].

There are differences between clinical diagnosis of JSLE and the utility of classification criteria of patients, which can be used in clinical trials. The American College of Rheumatology classification criteria for SLE gives 95% sensitivity and 96% specificity in clinical practice when 4 of 11 criteria are met (Table 26.1) [6, 7]. The Systemic Lupus International Collaborating Clinics (SLICC group) revised and validated the ACR classification criteria with improved methodology in order to improve clinical relevance and incorporate better understanding of the aetiopathogenesis and immunology of SLE [8]. These classification criteria have been validated in multicenter studies of children and young people with SLE [9, 10]. There has been a recent adaptation with the development of a Childhood Lupus Improvement Index (CHILI) as a tool to measure response to therapy in JSLE [11].

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Table 26.1 American College of Rheumatology Criteria for classification of SLE

1.	**Malar rash**
2.	**Discoid rash**
3.	**Photosensitivity**
4.	**Oral ulcers**
5.	**Arthritis**
6.	**Serositis**
	Pleuritis
	Pericarditis
7.	**Renal disorder**
	Proteinuria (>0.5 g/day) or persistently 3+
	Red blood cell casts
8.	**Neurological disorder**
	Seizures
	Psychosis (after excluding other causes)
9.	**Hematological disorder**
	Hemolytic anemia
	Leucopenia ($<4 \times 10^9$/L on two occasions)
	Lymphopenia ($<1.5 \times 10^9$/L on two occasions)
	Thrombocytopenia ($<100 \times 10^9$/L)
10.	**Immunological disorder**
	Elevated anti-double stranded DNA
	Elevated anti-Smith antibodies
	Positive antiphospholipid antibodies (previously lupus erythematosus cell tests or false positive *Treponema pallidum* immobilisation/Venereal Disease Reference Laboratory)
11.	**Elevated anti-nuclear antibodies (after exclusion of drug-induced lupus)**

Epidemiology

JSLE accounts for up to 20% of all SLE cases, with epidemiological studies demonstrating a minimum incidence in a paediatric population of 0.28 per 100,000 children at risk per year [12] with a prevalence in children and adults from various epidemiological studies of between 12.0 and 50.8 per 100,000 [13–20]. However, SLE has been reported to be more common in children from China, Hong Kong and Taiwan and three times more frequent in Afro-Caribbean than Caucasian children [21, 22]. The prevalence is increased in minority ethnic backgrounds in the United Kingdom where patients were diagnosed sooner as age at diagnosis was lowest (but not age at symptom onset) in Black African/Caribbean patients compared to White Caucasians [23]. In addition, the severity of renal and neuropsychiatric lupus is increased in Afro-Caribbean children [24]. Asian and Afro-Caribbean children are over six times more likely to be affected when compared to Caucasian children in the United Kingdom [25]. SLE is more prevalent in females of childbearing age, possibly due to the hormonal influences, and is commoner over the age of 10 years [26, 27].

Etiopathogenesis

SLE is a multifactorial disorder with multigenic inheritance and various environmental factors implicated in its etiopathogenesis with abnormal regulation of cell-mediated and humoral immunity that lead to tissue damage. The developing immune system is immature compared to adults and the heterogeneity of the clinical manifestations probably reflects the complexity of the disease pathogenesis.

The immune system in SLE is characterised by a complex interplay between overactive B cells, abnormally activated T cells and antigen-presenting cells, which lead to the production of an array of inflammatory cytokines, apoptotic cells, diverse autoantibodies and immune complexes. They in turn activate effector cells and the complement system leading to tissue injury and damage; these are the hallmarks of the clinical manifestations [28]. Several autoantibodies against cell wall components or circulating proteins can produce specific disease manifestations. However, it is interesting that some healthy children have positive ANA titres and that 88% of adult SLE patients have autoantibodies (including ANA, anti-dsDNA and anti-Smith) present up to 9.4 years before SLE is ever diagnosed [29]. It is generally assumed that anti-dsDNA antibodies play an important role in the pathogenesis of LN. This is because an increase in anti-dsDNA titre often precedes onset of renal disease, immune deposits are present in glomeruli and eluates of glomeruli are enriched for anti-dsDNA. However, the classical concept of deposition of DNA-anti-DNA complexes inciting glomerular inflammation is questionable as free, naked, DNA is not present in the circulation and

injection of these complexes hardly leads to glomerular localisation. The pathogenicity of anti-DNA has been proven with circulating immune complexes, in situ immune complexes, direct binding to renal and non-renal antigens, penetration into cells, and stimulation of cytokines in the form of immune complexes.

Neutrophil extracellular traps (NETs) are fibrous networks found in different clinical situations from infection to malignancy and from atherosclerosis to autoimmune diseases, such as SLE, where there is an imbalance between the process by which NETs are formed, called NETosis and their degradation. The key players in NETosis are neutrophils, interleukin-8 and anti-neutrophil cytoplasmic antibodies where prolonged exposure to NETs increases the change of organ damage. Neutrophils accumulate in the kidneys of patients with LN where the pathogenesis may be due to neutrophil products and low-density granulocytes [30].

Genomic and gene expression studies in patients with SLE have revealed novel gene mutations and cytokine alterations that may explain many of the features of the disease as well as the genetic susceptibility. There is a familial incidence of SLE in 12–15% of cases with a 10–20-fold increased risk of developing the disease if a sibling is affected compared to the general population (prevalence increases from 0.4% of populations up to 3.5% if there is a first degree relative with SLE) [14]. The concordance rate of SLE in monozygous twins is 24% compared with 2% in heterozygous pairs highlighting the importance of genetic (including HLA haplotypes, complement components and Fcγ receptor polymorphisms) and environmental factors in the etiology of SLE [31–34].

The genetics of SLE is now better understood with inroads made in the last decade in identification of susceptible loci, as there is a complex, multifactorial inheritance with associated environmental factors. Genetic linkage studies using microsatellite markers and single nucleotide polymorphisms have identified at least seven loci displaying significant linkage to SLE, including 1q23 (FcγRIIA, FcγRIIB, FcγRIIIA), 1q25-31, 1q41-42, 2q35-37, 4p16-15.2, 6p11-21 (MHC haplotypes), and 16q12. Genome-wide association studies have revealed further loci which are associated with susceptibility to lupus and specifically to LN [35]. New loci continue to be discovered and relate to various biological pathways associated with lupus risk, for example B-cell receptor signaling and CTLA4 co-stimulation for T-cell activation [36]. Findings from such genetics studies not only help further understanding of etiopathogenesis to develop new therapeutic options, but also may enable better diagnosis and prognostication in the future, with the publication of genetic risk scores to aid prognostication which may become applicable in future clinical practice as genetic technology advances [37].

Complement activation is involved in tissue damage with initial murine lupus models and later human studies revealing homozygous deficiencies of the components of the classical complement pathway (C1q, C1r, C1s, C2 and C4) predispose to the development of SLE. The complement system is an important part of the immune system which when dysregulated can result in the development of SLE, which occurs in 75% and 90% of patients with complete deficiencies of C4 and C1q, respectively [38]. Although initially anti-C1q was neither specific nor sensitive for SLE, in vitro testing has shown that anti-C1q is pathogenic in conjunction with complement-fixing antibodies and immune complexes with an increased prevalence in LN. Anti-C1q auto-antibodies are strongly associated with renal involvement in SLE and deposit in glomeruli together with C1q [39]. Anti-C1q antibodies are especially pathogenic in patients with SLE as they induce overt renal disease in the context of glomerular immune complex disease [40].

There are profound alterations in the B cell compartments of both children and adults with SLE [41, 42] with characteristic hypergammaglobulinemia and increased serum autoantibody titres, explaining why B cell depletion may be an effective therapy [43, 44]. In addition to autoantibodies and immune complexes, autoreactive T cells cause tissue damage in SLE with evidence of alterations in human SLE T cell signalling molecules and loss of self-tolerance [45]. Compared to healthy T cells, there are increased

and accelerated signaling responses in T cells from patients with SLE with hyper-reactivity to antigenic triggers, which may be due to genetic influences [46, 47]. Many cytokines, including interferon and interleukins (IL-6, IL10, IL12 (p40) and IL-18), which are elevated in the serum of SLE patients, correlate with disease activity [48].

DNA microarray technology has helped in understanding some of the complex pathogenesis of SLE through genome-wide profiling and earlier studies using microarray analysis of peripheral blood mononuclear cells (PBMCs). There is evidence of dysregulation of inflammatory cytokines, chemokines, and immune response-related genes, as well as genes involved in apoptosis, signal transduction, and the cell cycle. Interferon (IFN)-regulated genes are highly overexpressed in the peripheral blood and kidney glomeruli, supporting a crucial role for interferon in SLE. Future studies focusing on target tissues or organs in SLE may further contribute to our understanding of the etiopathogenesis while providing new targets for therapy [49].

Type I interferons are associated with SLE and genes that are regulated by IFN-alpha are upregulated in JSLE patients, with gene deletion of the IFN-alpha/beta receptor in experimental lupus-like NZB mice resulting in reduced disease activity (although conversely, IFN-beta is a well-established treatment in multiple sclerosis). There are several underlying mechanisms of IFN-beta therapy involving cellular (decreased T cell proliferation and infiltration of leucocytes into the kidney) and humoral (decrease in IgG3 isotypes) immune responses and a reduction in nephrogenic cytokines have been identified. IFN-beta treatment of LN in MRL-Fas(lpr) mice is beneficial and suggests that IFN-beta may be a therapeutic candidate for subtypes of human SLE [50]. IFN-alpha-inducible proteins represent a novel class of autoantigens in murine lupus, and experiments suggest additional roles for IFN-alpha in SLE [51].

The increase in autoantigens in SLE may be due to impaired immune complex clearance and apoptosis. There is evidence of defective clearance of apoptotic cells in some SLE patients, due to the genetic deficiency of molecules, including complement component deficiencies, with autoantigens undergoing structural modifications during the process of apoptosis that may induce immunogenicity [52]. The development of SLE may be attributable to genetic susceptibility with changes in the hormonal milieu, environmental, pharmaceutical and toxic agents (including crystalline silica, solvents and pesticides) [53]. However, there is also an association with infectious conditions influencing the developing immune system of children who develop SLE, including Epstein-Barr virus [54, 55].

Clinical Presentation

Children and young people with JSLE have different clinical presentations, although typically there are non-specific symptoms of being generally unwell with fatigue, malaise, lethargy, aches, pains, episodic fever, anorexia, nausea, vomiting and weight loss with a typical butterfly rash over a period of a few weeks or months. Most organ systems can be involved (Table 26.2) [56] although unusual presentations are sometimes encountered, which is why SLE has been called one of the great mimickers [57]. In view of the relatively low incidence of JSLE compared to many other paediatric

Table 26.2 Presenting symptoms of SLE

Malaise, weight loss, growth retardation	96%
Cutaneous abnormalities	96%
Hematological abnormalities	91%
Fever	84%
Lupus nephritis	84%
Musculoskeletal complaints	82%
Pleural/pulmonary disease	67%
Hepatosplenomegaly and/or lymphadenopathy	58%
Neurological disease	49%
Other disease manifestations (including cardiac, ocular, gastro-intestinal, Raynaud's phenomenon)	13–38%

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health problems, there can be a significant delay between onset of symptoms and eventual diagnosis. Pediatricians require a high index of suspicion to ensure this disease is not missed.

The majority of children with SLE present during their adolescence. From one of the largest cohort of 201 children with SLE from Toronto, Canada, 6 children (3%) presented before the age of 6 years, 41 (20%) between 6 and 10 years, 62 (31%) between 11 and 13 years and 92 between 14 and 18 years of age [58]. There was a female predominance of 80%, with a slightly higher proportion of male patients than in adulthood.

Lupus Nephritis

There is evidence of renal involvement in up to 60–80% of children and young people with SLE close to the onset of the disease [56]. In a review of the presentation of LN from different studies involving 208 children, 55% presented with nephrotic syndrome and 43% with proteinuria of lesser degrees (Table 26.3). Most children have microscopic haematuria while few (1.4%) presented with macroscopic haematuria. Fifty percent of children and young people have impaired renal function at onset while only 1.4% have acute kidney injury requiring renal replacement therapy. A small proportion will present with a rapidly progressive glomerulonephritis with biopsy-proven crescentic glomerulonephritis. Hypertension was found in 40% of children.

Table 26.3 Presenting features of lupus nephritis

Nephrotic syndrome (>3 g/day)	55%
Proteinuria (<3 g/day)	43%
Macroscopic haematuria	1.4%
Microscopic haematuria	79%
Hypertension	40%
Reduced GFR (<80 mL/min/1.73 m^2)	50%
Acute renal failure	1.4%

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Other Organ Systems

Dermatological

The butterfly or malar rash is the classical rash over the cheeks and nose with photosensitivity to sunlight. However, other rashes can be present, including maculopapular eruptions or purpuric rashes, discoid lesions, livedo reticularis, urticaria and more severe cutaneous vasculitis with ulceration, including mucosal ulceration. Hair loss and brown discolouration of the nails are common findings.

Cerebral

Neurological symptoms, including headache, migraines, seizures and mood disorders are among the most severe manifestations in SLE. There are more severe forms of cerebral lupus with ataxia, chorea, cerebrovascular accidents and deteriorating level of consciousness. The psychiatric symptoms can range from fatigue and depression to confusion, delirium, frank psychosis, hallucinations and catatonic states. Poor academic achievement is a common problem of multifactorial origin that is important to address in these children and young people [59].

Haematological

Coombs-positive haemolytic anaemia, leucopenia, thrombocytopenia and pancytopenia are common findings in children with SLE. Erythrocyte sedimentation rate (ESR) is markedly raised in most children with SLE, while high C-reactive protein (CRP) is found in only a small minority. Therefore, CRP can be helpful in differentiating between flares of lupus disease activity or an infectious complication, such as septicaemia due to the disease or treatment.

Rheumatological

Generalised pain involving the musculo-skeletal system is a very common finding in SLE patients due to myalgia and arthralgia, with severe arthritis less common.

Other Organs

All serous membranes including pleura and pericardium are frequently affected so presentation may be with dyspnoea or pleuropericardial and intermittent chest pain. Hepatosplenomegaly and lymphadenopathy are commonly found in children and young people with SLE. Growth delay is often seen in children, partly related to pubertal delay. Primary and secondary amenorrhoea are manifestations of SLE and LN, but also complications of high doses of cyclophosphamide treatment.

Antiphospholipid Syndrome

Antiphospholipid syndrome (APS) was first described in 1987 as an autoimmune disorder characterised by hypercoagulability of any blood vessel size or type of any organ with the presence of antiphospholipid antibodies [60–62]. Thromboembolism results from the involvement of larger vessels (arteries and veins), whereas thrombotic microangiopathy results from involvement of smaller vessels (capillaries, arterioles and venules). APS with anticardiolipin antibodies and/or lupus anticoagulant are found in 65% of children and young people with SLE [63]. Livedo reticularis has been documented as a marker of APS with increasing tendency to develop both venous and arterial thrombosis. Primary APS is rare in children and is unlikely to progress to SLE. APS is an independent risk factor for more severe renal disease due to microangiopathy in the kidneys and may require treatment as outlined below.

Classification Criteria

The American College of Rheumatology (ACR) classification criteria are utilised in classifying and not diagnosing patients with SLE (Table 26.1). The diagnosis of SLE is made in typical cases with classical organ involvement, elevated autoantibodies and hypocomplementaemia. However, in some cases, the initial diagnosis is more difficult due to the evolution of disease and these cases may not initially fulfil the criteria developed by ACR which have been refined for children [6, 7]. They consist of 11 different criteria of which four should be fulfilled for the diagnosis of SLE; however, meeting these criteria is not sufficient for a diagnosis of SLE because many children with other diseases can also formally match a number of these criteria.

The Systemic Lupus International Collaborating Clinics (SLICC) classification criteria for SLE was published in 2012 [8] where patients are classified if they have biopsy-proven LN with either positive ANA or anti-dsDNA antibodies or at least four criteria (at least one clinical [acute and chronic cutaneous lupus, oral or nasal ulceration, non-scarring alopecia, arthritis, serositis, renal, neurologic, haemolytic anaemia, leucopenia and thrombocytopenia] and one laboratory [ANA, anti-dsDNA, anti-Smith, anti-phospholipid antibodies, hypocomplementaemia (C3, C4, CH50) and direct Coombs test (which is not counted if haemolytic anaemia is present)] criteria and this has now been validated in children [9].

Disease Activity Scoring Systems

There are various disease activity and damage scoring systems, which are very helpful in monitoring disease activity and damage in children and adolescents with SLE with respect to both clinical long-term follow-up and scientific studies. Scales of indices of disease activity continue to evolve and include SLEDAI (Systemic Lupus

Erythematosus Disease Activity Index), SLAM (Systemic Lupus Activity Measure) and ECLAM (European Consensus Lupus Activity Measure). The British Isles Lupus Activity Assessment Group (BILAG) index is another scoring system that can be used and has been evaluated in children [64]. The BILAG index is based on the principle of the physician's intention to treat and is a clinical measure of disease activity in SLE patients, which has been validated to be reliable, comprehensive and sensitive to change. It was developed to report disease activity in eight different systems (general, mucocutaneous, neurological, musculoskeletal, cardio-respiratory, vasculitis, renal and haematological), which differentiates it from other lupus activity indices.

Investigations

The initial investigations of a child with suspected SLE include haematological, biochemical and immunological investigations. Further investigations are warranted depending on organ involvement so a percutaneous renal biopsy and imaging of relevant organ systems are often required.

Blood Investigations

The initial blood test should include a full blood count with a blood film, ESR and reticulocyte count. Anaemia, leucopenia and thrombocytopenia are common findings during active disease that normally improve with effective treatment. Iron studies should also be considered when there is anemia, but caution must be used in the interpretation of these in the setting of systemic inflammation. The leucocyte count, in particular the neutrophil count, should be monitored during active immunosuppressive treatment as the presence of neutropenia influences the doses of immunosuppressive therapies. However, lymphopenia is often seen with treatment and is mostly regarded as a "desired" side effect, which can sometimes be a marker of the effectiveness of treatment. ESR is a marker of disease activity, which can be clinically useful, although it is not uncommon for it to be markedly elevated even during clinical and serological remission. A direct Coombs test should be performed to look for evidence of haemolysis. Macrophage activation syndrome should be screened for in those children and young people with unexplained fever with check of ferritin, lactate dehydrogenase and consideration of bone marrow aspirate and trephine biopsy to exclude malignancy.

The biochemistry profile should include estimation of renal function with plasma creatinine and urea, serum electrolytes, bone, thyroid and liver function tests (including serum albumin), pancreatic enzymes, 25-hydroxyvitamin D levels and CRP (where sepsis is clinically suspected). Creatinine kinase may be useful if evidence of myalgia and parathyroid hormone level when there is evidence of chronic kidney disease. It is useful to calculate the estimated glomerular filtration rate using the Schwartz formula [65].

Immunology Testing

There is evidence of immune dysregulation in almost all children with SLE with positive immunological tests and anti-nuclear antibodies (ANA). ANA can sometimes be a non-specific finding but the use of anti-double-stranded DNA (dsDNA) and the extractable nuclear antibodies (ENA) and C1q levels and anti-C1q antibody testing increases the specificity (Table 26.4). The

Table 26.4 Auto-antibodies in patients with lupus nephritis

	Frequency	Specificity	Association with disease activity
Anti-dsDNA	40–90%	High	Yes
Anti-SSA/Ro	35%	Low	No
Anti-SSB/La	15%	Low	No
Anti-Sm	5–30%	High	No
Anti-C1q	80–100%	High	Yes

dsDNA double stranded DNA, *Anti-SSA/Ro* anti-Sjögren's syndrome A, *Anti-SSB/La* anti-Sjögren's syndrome B, *Anti-Sm* Anti-Smith, *Anti-C1q* Anti-complement factor C1q

pathogenic significance of these antibodies is debated and they can be found in serum sometimes several years before the development of symptoms [29]. However, it is clear that dsDNA and anti-C1q can be used to monitor disease activity, as a marker of improvement or a pending flare of disease. Anti-C1q antibodies have also been shown to predict more severe renal involvement [66]. Knowledge of patients' VZV IgG levels is important for future exposure to varicella. In some settings where the prevalence of tuberculosis is high, the Quantiferon-TB (interferon gamma release assay) may also be indicated. Antibodies against aquaporin-4 (AQP4) and myelin oligodendrocyte glycoprotein (MOG) may be helpful in patients with cerebral lupus.

Complement C3 and C4 are mostly reduced during the active phases of disease and can also be useful markers of disease activity, although some patients with inherited complement deficiencies may never normalise their serum values. Anticardiolipin antibodies and lupus anticoagulant should be regularly monitored. Hypergammaglobulinaemia is a feature of SLE and it is useful to monitor serum immunoglobulins, especially in patients treated with B-cell depletion with intravenous (IV) rituximab. It remains controversial whether hypogammaglobulinaemia should be supplemented with IV immunoglobulin if patients are free of infections. B-lymphocyte counts should be monitored in children treated with IV rituximab by measuring the number of CD19 positive cells.

Urine Investigations

Early morning urine should be regularly monitored in all children and young people with SLE with urinalysis by dipstick performed for haematuria and proteinuria. Urine microscopy is also helpful in looking for red blood cells and casts during the acute phase of LN. Some standardised measurement of proteinuria or albuminuria should be regularly followed, which in most centres is carried out by analysing an early morning spot urine sample relating the urine excretion of protein or albumin to the urine levels of creatinine. Evidence of tubular dysfunction may help to identify LN prior to the onset of albuminuria by measuring NAG (N-acetyl-beta-D-glucosaminidase):creatinine ratio, RBP (retinol binding protein):creatinine ratio or other tubular markers [67].

Other Investigations

It is important to base treatment decisions on the histopathology of percutaneous renal biopsies as it has been shown that the severity of the renal involvement sometimes is difficult to predict from clinical symptoms and signs. Estimated or formal measurements of glomerular filtration rate should be performed when there is a clinical suspicion of impaired renal function. Even in patients with unimpaired GFR the histological findings can be significant and therefore a "normal" eGFR or serum creatinine should not be overly reassuring. Pulmonary function tests, electrocardiography, echocardiography and chest X-rays are important investigations in selected children. Electroencephalography and cerebral imaging with cranial MRI and MRA is advocated in children with neuropsychiatric evidence of cerebral lupus, when lumbar puncture examining cerebrospinal fluid may be performed in appropriate cases.

Follow-Up

Each child should at every clinic visit have a full clinical evaluation including weight, height and a disease activity score (as above). They should have their blood pressure monitored and their urine tested for proteinuria and haematuria. Regular blood tests should include full blood count, ESR, CRP, renal and liver function tests, electrolytes, C3 and C4, and autoantibodies, including dsDNA. Anticardiolipin antibodies and in particular in children treated with rituximab

immunoglobulins and lymphocyte subsets should be regularly monitored. Fasting blood lipids including cholesterol, triglycerides, HDL, LDL and VLDL should be monitored at least once annually. Bone density should be measured in children with long-term daily corticosteroid therapy on a regular basis.

Histological Classification of Lupus Nephritis

The histological classification of LN was initially formatted in 1975 by the World Health Organisation (WHO) and modified in 1982 and 1995. It describes the spectrum of LN as the type and extent of renal lesion and provides information on the immunosuppression required and prognosis. There was a revision of this classification by the International Society of Nephrology (ISN) and Renal Pathology Society (RPS) Working Group after their consensus conference in 2002 in order to standardise definitions, emphasise clinically relevant lesions, and encourage uniform and reproducible reporting between centres (Table 26.5) [68, 69]. This classification facilitates clinical management by increased comprehension of the etiopathogenesis of SLE and guides the clinician with treatment decisions, protocols and clinical research. However, there is widespread variation of the timing, type and distribution of histological lesions, including immune-complex mediated vasculitis, fibrinoid necrosis, inflammatory cell infiltrate and collagen sclerosis.

Table 26.5 International Society of Nephrology and Renal Pathology Society Working Group (ISN/RPS) revised histopathological classification of lupus nephritis

1. **Minimal mesangial lupus nephritis (LN)**
Normal glomeruli by LM, but mesangial immune deposits by IF
2. **Mesangial proliferative lupus nephritis (LN)**
Purely mesangial hypercellularity of any degree or mesangial matrix expansion by LM with mesangial immune deposits, with none or few, isolated subepithelial or subendothelial deposits by IF or EM not visible by LM
3. **Focal lupus nephritis (LN)**
Active or inactive focal (<50% involved glomeruli), segmental or global endo- or extracapillary GN, typically with focal, subendothelial immune deposits, with or without focal or diffuse mesangial alterations
III (A) active focal proliferative LN
III (A/C) active and sclerotic focal proliferative LN
III (C) inactive sclerotic focal LN
* Indicate the proportion of glomeruli with active and with sclerotic lesions
* Indicate the proportion of glomeruli with fibrinoid necrosis and/or cellular crescents
4. **Diffuse segmental (IV- S) or global (IV- G) LN**
Active or inactive diffuse (50% or more involved glomeruli), segmental or global endo- or extracapillary GN with diffuse subendothelial immune deposits, with or without mesangial alterations. This class is divided into diffuse segmental (IV-S) when at least 50% of the involved glomeruli have segmental lesions, and diffuse global (IV-G) when at least 50% of the involved glomeruli have global lesions
IV (A) Active diffuse segmental or global proliferative LN
IV (A/C) Diffuse segmental or global proliferative and sclerotic LN
IV (C) Diffuse segmental or global sclerotic LN
* Indicate the proportion of glomeruli with active and with sclerotic lesions
* Indicate the proportion of glomeruli with fibrinoid necrosis and/or cellular crescents
5. **Membranous lupus nephritis**
Numerous global or segmental subepithelial immune deposits or their morphologic sequelae by LM and IF or EM with or without mesangial alterations
May occur in combination with III or IV in which case both will be diagnosed. May show advanced sclerosis
6. **Advanced sclerotic LN**
90% or more glomeruli globally sclerosed without residual activity

ISN/RPS Classes I and II LN denote purely mesangial involvement (I, mesangial immune deposits without mesangial hypercellularity; II, mesangial immune deposits with mesangial expansion and hypercellularity), ISN/RPS Class III LN denotes focal glomerulonephritis (involving less than 50% of total number of glomeruli), with subdivisions for active and chronic lesions. ISN/RPS Class IV LN denotes diffuse glomerulonephritis (involving at least 50% of total number of glomeruli with examples in Figs. 26.1, 26.2a–d, and 26.3a–d) either with segmental (ISN/RPS Class IV-S) or global (ISN/RPS Class IV-G) involvement, and also with subdivisions for active and chronic lesions, ISN/RPS Class V denotes membranous LN (combinations of membranous and proliferative glomerulonephritis (i.e., ISN/RPS Class III and V or Class IV

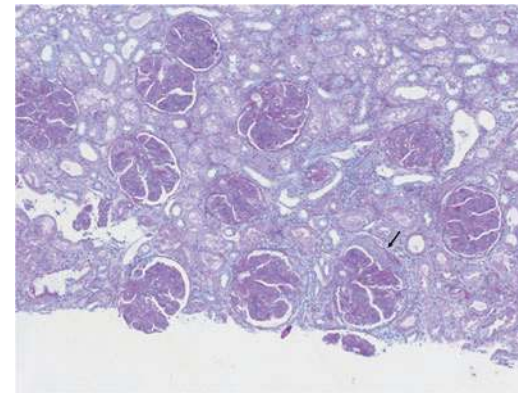

Fig. 26.1 Photomicrograph of a case of lupus nephritis demonstrating predominant diffuse endocapillary proliferative change with scattered superimposed extracapillary proliferative lesions (*Arrow*), Lupus nephritis Class IV-G (A/C) (PAS, original magnification ×100) (Used with permission of Taylor & Francis from Marks et al. [70])

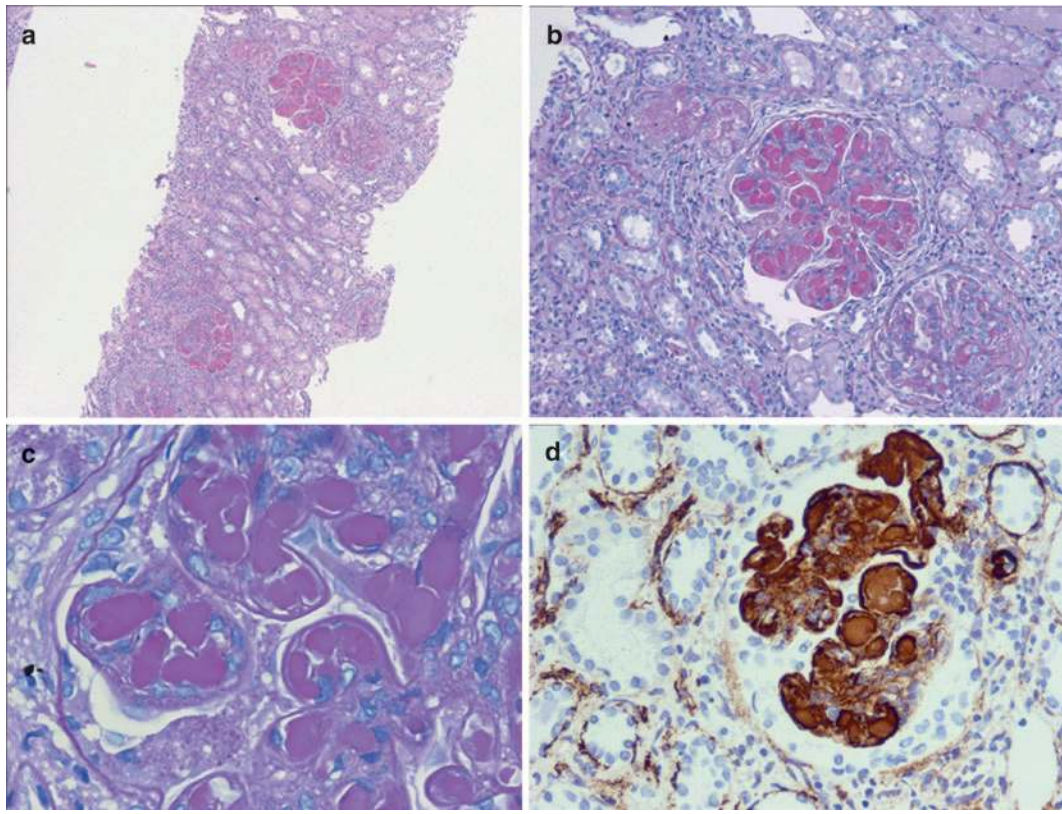

Fig. 26.2 Photomicrographs of a case of lupus nephritis presenting as apparent acute renal failure, demonstrating diffuse endocapillary proliferative change with scattered crescent formation (**a**, **b**) and extensive subendothelial deposits visualised as wire-loop and hyaline drop lesions (**b**, **c**). Immunostaining revealed a characteristic 'full-house' pattern of immunoglobulin and complement deposition. (**d**) Lupus nephritis Class IV-G (**a**) (PAS and immunostain, original magnifications ×40–400) (Used with permission of Taylor & Francis from Marks et al. [70])

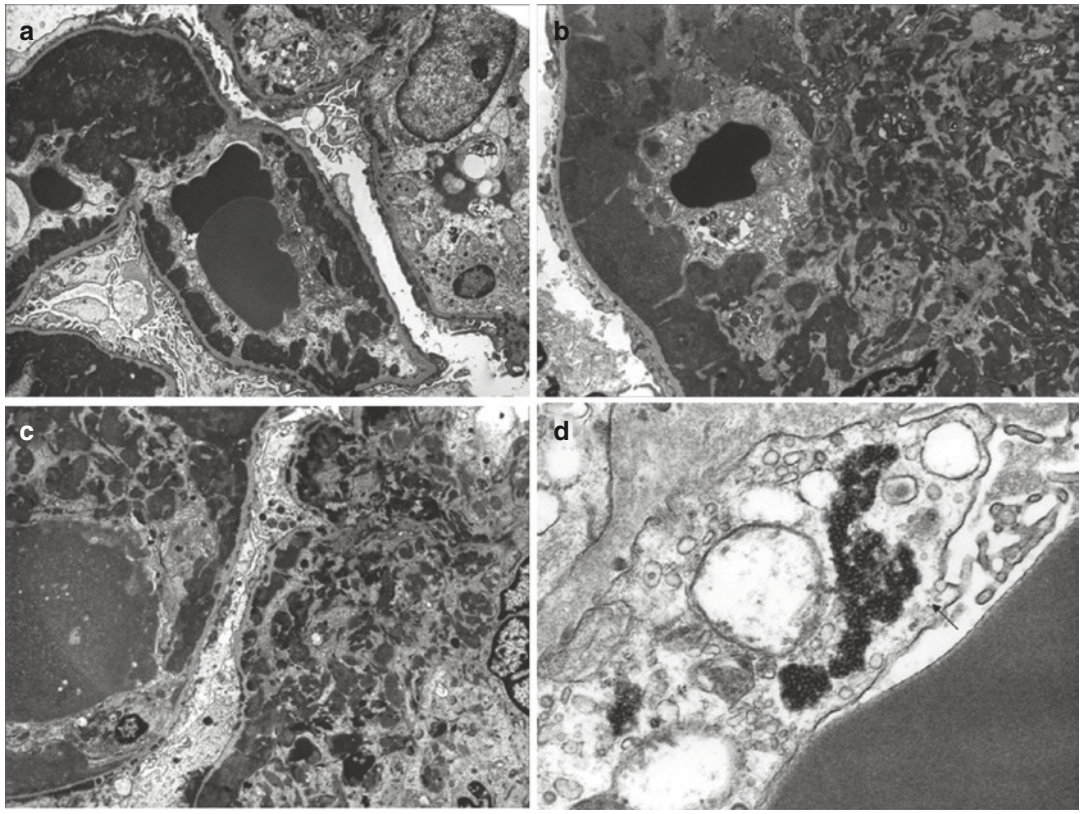

Fig. 26.3 Electron micrographs of lupus nephritis demonstrating extensive mesangial and paramesangial electron dense deposits in association with massive subendothelial deposits (**a**–**c**) (**a**) corresponds to the case in Fig. 26.1 and (**b**, **c**) correspond to the case in Fig. 26.2). In addition, some cases may demonstrate the presence of tubuloreticular inclusions. (**d**) (Used with permission of Taylor & Francis from Marks et al. [70])

and V) should be reported individually in the diagnostic line) and ISN/RPS Class VI for advanced sclerosing lesions (which now for the first time categorically states that at least 90% of glomeruli need to be globally sclerosed without residual activity). In addition, the ISN/RPS classification includes overlap cases (see Fig. 26.4a–d for an example of mixed ISN/RPS Class IV and Class V LN).

The histopathological features of LN includes the delineation of active and chronic histological lesions, which has been extensively reported in the various classification systems (Table 26.6) [71].

The active glomerular and tubulointerstitial lesions, which are potentially reversible and are scored up to 24 (with 12 denoting poor renal prognosis), include endocapillary hypercellularity, fibrinoid necrosis, karyorrhexis, cellular crescents, hyaline thrombi, wire loops (subendothelial deposits), haematoxylin bodies, leucocyte infiltration and tubulointerstitial disease with tubular atrophy and mononuclear cell infiltration. The chronic lesions are irreversible and include glomerular sclerosis, fibrous crescents, fibrous adhesions, extramembranous deposits, and tubulointerstitial disease with interstitial fibrosis and tubular atrophy.

The clinicopathological correlation of LN has been evaluated in both adults and children according to different histopathological classifications. The largest adult series investigating the clinicopathological outcomes according to the ISN/RPS classification of LN followed 60 Japanese subjects for 1–366 (mean 187) months (Fig. 26.5) [72]. The primary outcome was

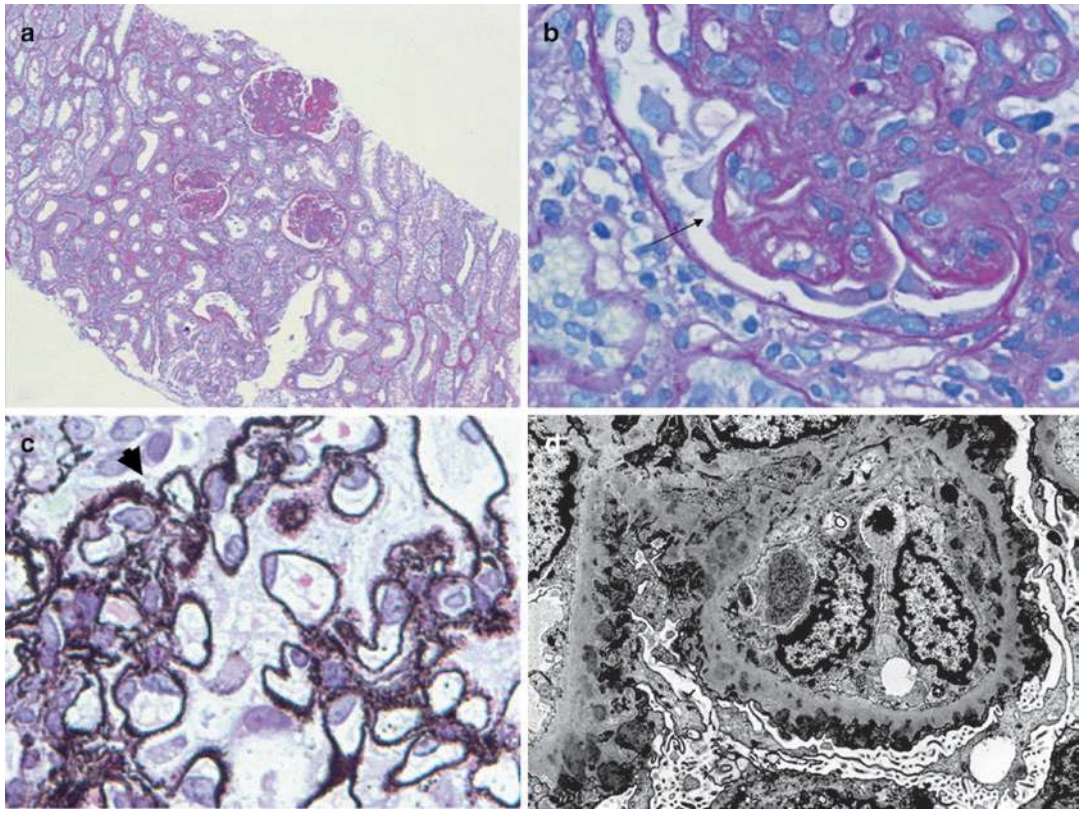

Fig. 26.4 Photomicrographs of a case of lupus nephritis presenting with nephrotic syndrome demonstrating diffuse endocapillary proliferative change with subendothelial deposits ((**a**, **b**) PAS, original magnifications ×40 and 400 respectively). In addition some glomeruli show florid 'spike' formation on silver staining (**c**) PAMS, original magnification ×400) with mesangial, subendothelial and subepithelial deposits on ultrastructural examination (**d**). Lupus nephritis, mixed Class IV and Class V changes (Used with permission of Taylor & Francis from Marks et al. [70])

Table 26.6 Activity and chronicity indices of lupus nephritis

	Activity index	Chronicity index
Glomerular	Endocapillary hypercellularity Fibrinoid necrosis Karyorrhexis Cellular crescents Hyaline thrombi Wire loops (subendothelial deposits) Haematoxylin bodies Leucocyte infiltration	Glomerular sclerosis Fibrous crescents Fibrous adhesions Extramembranous deposits
Tubulointerstitial	Mononuclear cell infiltration	Interstitial fibrosis
	Tubular necrosis	Tubular atrophy

defined as developing end-stage kidney disease (ESKD) with secondary outcome as patients' death and/or ESKD. The primary and secondary outcomes of all subjects were 82% and 78% at 10 years, and 80% and 73% at 20 years, respectively. The primary outcome of subjects with nephrotic syndrome (n = 21 versus 39 non-nephrotic) was statistically poorer (p = 0.0007) with hazard ratio of 3.39 as the mean time of 50% renal survival was 200 ± 29 months.

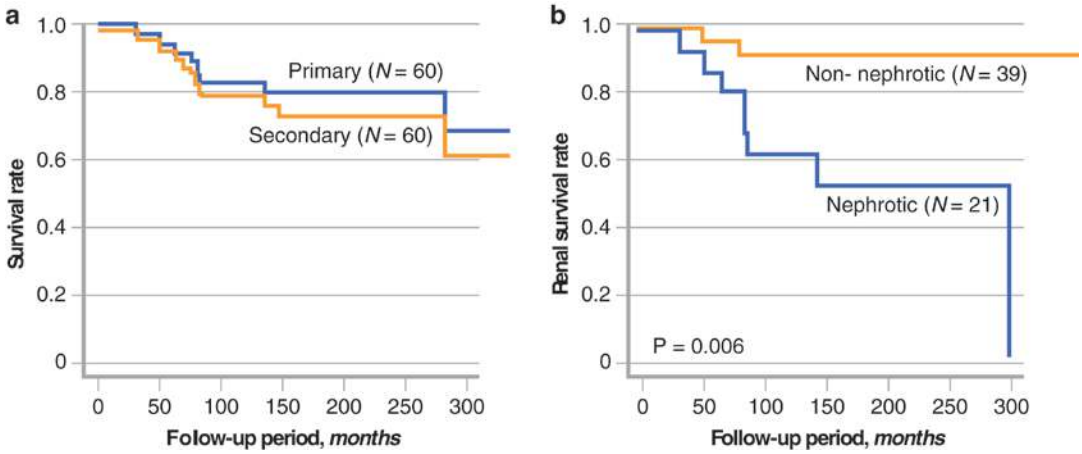

Fig. 26.5 Prognosis of lupus nephritis. The primary (ESKD) and secondary (patients' death and/or ESKD) outcomes (**a**) of 60 Japanese adult lupus nephritis subjects with and without nephrotic syndrome (**b**) at mean follow-up of 187 months (Used with permission of Nature Publishing Group from Yokoyama et al. [72])

In comparison with adult-onset SLE, there are usually less patients in the series of childhood cases of LN. There have been larger series investigating clinicopathological outcomes of 39–67 children according to the WHO classification [73] and the ISN/RPS classification of LN [74], which provide evidence that up to half of children with LN will have the most severe class (ISN/RPS Class IV or diffuse LN). The classification demonstrated that the subgroup of diffuse global sclerosing (ISN/RPS IV-G(C)) LN was associated with the worst clinical outcome [74].

Treatment

The optimal treatment of children and adolescents with SLE is provided by a multi-disciplinary team of health professionals, including a paediatric nephrologist, paediatric rheumatologist and other paediatric specialists, with a dedicated specialist nurse and members of a psychosocial team. Treatment should be guided by the most severe organ system involved.

Drug Treatment

The treatment of lupus with or without nephritis is based on evaluation of the severity of the disease. The treatment should be individually tailored depending on the presenting symptoms and severity of renal involvement, with emphasis on renal dysfunction and the degree of proteinuria. In all cases with suspected renal involvement, the histopathological grading of the renal biopsy is very helpful in deciding further treatment. Other potentially life-threatening symptoms, such as cerebral lupus should also be taken into consideration when deciding on the initial treatment. Most treatments have common or potential side-effects, which need to be considered for the individual child.

The treatment of JSLE is not based on large randomised controlled trials, but there is an increasing number of studies in adult and adolescent patients and published clinical experience in children. The recommendations below describe the most commonly used protocols for treating children with SLE. Consensus reports on the treatment of paediatric lupus have been published [75–79]. The armamentarium of immunosuppressive agents is presently developing quickly with new drugs being introduced [80], so guidelines may change in the not too distant future.

Traditionally treatment has been divided into induction therapy to gain control of acute disease and maintenance therapy to maintain control over the disease. This is a helpful approach, but it is not an uncommon clinical situation that a flare of disease activity is difficult to define.

Severe Multisystem Disease with or Without Nephritis (ISN/RPG Class III–V)

The treatment is divided into induction therapy to get control of acute disease and maintenance therapy to maintain control over the disease. Clinicians need to consider the severity of the disease but also the potential side-effects from treatment in considering which combination of immunosuppressive agents are used. At the onset of each treatment, it is important to discuss side effect profiles and risks of non-adherence. There is growing evidence to suggest the use of belimumab for adults as well as tacrolimus for induction, particularly in membranous (ISN/RPS Class V) LN. Evidence in adults has shown best results when using tacrolimus in combination with MMF and corticosteroids.

Induction Therapy

A common presentation to the nephrologist is a child that over a few weeks or months has developed generalised symptoms and at assessment is found to have an acute nephritic and/or nephrotic syndrome with a suspicion of SLE that is later confirmed by serology. It is important in cases with significant renal disease to commence treatment early without unnecessary delay to protect the kidneys from developing chronic damage.

The mainstay of induction treatment is based on corticosteroids to rapidly halt disease with rapid wean by addition of other immunosuppressive agent(s) (Table 26.7). Historically, this used to be cyclophosphamide (CyC), but is now more commonly mycophenolate mofetil (MMF). For children and young people with active disease, including severe renal dysfunction, B-lymphocyte depletion therapy with IV rituximab is often added to corticosteroids and MMF [44, 81]. In children with a crescentic glomerulonephritis, we and others have additionally tried plasma exchange.

Table 26.7 Induction therapy of ISN/RPS Class III, IV and V lupus nephritis

Methylprednisolone (intravenous) pulses × 3	600–1000 mg/m^2 (maximum 1 g)
Prednisolone (oral)	0.5–1 mg/kg/day (maximum 30–60 mg/day with rapid weaning)
Mycophenolate mofetil[a]	600 mg/m^2 twice daily (maximum of 2–3 g/day in two divided doses)
In severe cases or if not responding to the above:	
Plasma exchange	Daily for 5–10 days
Rituximab	See protocol in text

[a]Induction therapy with cyclophosphamide or azathioprine are alternatives in selected cases

Corticosteroids

IV pulses of methylprednisolone should be given during three consecutive days (600–1000 mg/m^2) with a maximum of 1 g, infused over at least 30 min. In severe cases, these pulses may need to be repeated. There is increasing evidence that low (and even no chronic oral) corticosteroid exposure can but utilised for some patients [82, 83].

The methylprednisolone pulses are followed by high doses of oral prednisolone (0.5–2 mg/kg/day to a maximum dose of 30–60 mg/day). This high dose is dictated by the severity of the clinical situation, but should be weaned rapidly. This treatment will inevitably result in cushingoid side-effects, which can be debilitating for adolescents (such as fluid and water retention with increased appetite and weight gain with rounded facies, striae and growth delay). Other important side effects include mood changes, hypertension, diabetes mellitus, osteoporosis and osteopenia. Therefore, in order to maximize long-term adherence to therapy, it is very important to reduce the corticosteroid dose as quickly as the clinical situation allows in order to minimise these side-effects.

Mycophenolate Mofetil

There is now increasing evidence from studies that MMF in adult patients has equivalent effectiveness to CyC for induction. The first study to

show this was in 42 adult patients from Hong Kong treated with 12 months of oral MMF therapy or 6 months of CyC followed by 6 months of azathioprine. Both groups responded equally well to the treatment, but there were significantly more side-effects in the CyC group [84].

The Aspreva Lupus Management Study (ALMS), the largest study on the treatment of SLE with MMF, included 370 patients with ISN/RPS Class III, IV and V LN [85]. It consisted of two treatment stages: one 24 week induction phase and subsequently, a 3 year study of the maintenance phase. In the induction study, the 370 patients were randomised to either MMF (target dose 3 g/day) or IV CyC given as monthly pulses of 500–1000 mg/m^2. All patients received prednisolone tapered from a maximum dose of 60 mg/day. The response to treatment was defined as achieving the primary efficacy endpoint, which was decrease in urine protein/creatinine ratio to below 3 mg/mg if baseline was ≥3 mg/mg or by 50% if baseline <3 mg/mg *and* stabilisation (±25%) or improvement of serum creatinine. The ALMS study showed no difference between MMF and CyC: 56% and 53% of patients responded in the two groups, respectively. There was also no significant difference in the rate of side effects.

In a further publication, it was shown that the therapeutic response varied with race, in that Black and Hispanic patients responded better to MMF (60%) compared to CyC (39%), p = 0.03 [86]. The authors have also looked separately at patients with ISN/RPS Class V LN (i.e., a membranous pattern on the kidney biopsy) and again no difference was found between the MMF and CyC treated groups [87].

A meta-analysis comparing MMF and CyC as induction treatment has been published [88]. Four trials with 668 patients were included and no difference in clinical efficacy was found between the two drugs with the overall RR (relative risk) for renal remission being 0.67 (95% CI 0.35–1.28). However, there was significantly less alopecia (RR 5.77; 95% CI 1.56–21.38) and amenorrhoea (RR 6.64; 95% CI 2.00–22.07) with MMF, but no other side effects were significantly different.

There are no randomised controlled studies on the use of MMF in juvenile-onset SLE. We published one of the largest case series with 31 children and adolescents that were treated with MMF either initially or converted from azathioprine [89]. Seventy-three per cent of the treated children showed a response to the drug without any recorded major side effects. MMF should now be regarded as part of the standard treatment for children with severe lupus.

The main problems with MMF are the gastrointestinal side effects with abdominal pain and diarrhoea. These can mostly be avoided by increasing the dose slowly (e.g. starting with a dose of 300 mg/m^2 twice daily and slowly increasing to the target dose of 600 mg/m^2 twice daily). If gastrointestinal side effects are a continuing problem then the daily dose may be divided three or even four times a day.

Cyclophosphamide

IV pulses of CyC have been the most important corticosteroid-sparing agents in achieving remission in severe SLE and LN. However, this treatment has substantial short and long-term side effects, including nausea (which can be alleviated with routine use of ondansetron), alopecia, haemorrhagic cystitis and infectious risk of septicaemia due to neutropenia. Many girls develop amenorrhoea and with high doses; there is a risk for developing infertility. The modified National Institute of Health protocol of IV CyC (500–1000 mg/m^2 with dose reduction in renal dysfunction) can be administered as monthly pulses for 6 months. It is important to keep the child well hydrated and to give MESNA to protect the child from developing haemorrhagic cystitis.

Controlled studies in adult patients show that IV CyC causes significantly more side-effects, including severe infections, compared to oral azathioprine or MMF [84, 90–92]. Therefore, it is important to closely monitor the total white cell, neutrophil and lymphocyte counts with the nadir usually occurring around 7–10 days after the infusion. Subsequent doses may need to be reduced based on haematological side effects.

There is a long-term increased risk for developing malignancies and for infertility. Up to 14% of CyC treated patients younger than 41 years

have premature ovarian failure, which is a common consequence of CyC treatment. CyC is thus a drug that still has a place in the treatment of LN, but it is gradually being replaced by other drugs.

Rituximab

Rituximab is a humanised anti-CD20 antibody that was designed for treatment of B-cell lymphoma and in adults has been increasingly used for B-cell depletion therapy in autoimmune diseases such as rheumatoid arthritis or SLE [43]. We have used IV rituximab in over 200 children with SLE or vasculitis and published the results of our first children [44, 93]. Our patients have shown very good responses to the treatment and we have not experienced a significant increase in severe side effects.

A relatively large randomised, placebo-controlled study of IV rituximab, called the LUNAR trial, was performed in 144 adult patients with ISN/RPS Class III or IV LN [94]. The primary endpoint was the renal response at week 52. A complete response was normalisation of serum creatinine, no red blood cells (<5/hpf) in the urine and a urine protein/creatinine ratio of <1.0 mg/mg.

Only 31% of patients in the placebo group and 25% of the rituximab treated patients fulfilled these very strict criteria for a complete response. The patients that showed no response to the treatment were 43% in the rituximab and 54% in the placebo group ($p = 0.18$). There were more serious side effects in the placebo group: 74/100 patient years vs. 43/100 patient years in the rituximab group. Greater improvements of complement levels ($p = 0.025$) and antibodies to dsDNA ($p = 0.007$) were recorded in the rituximab group compared to placebo [95].

The negative result of this study was surprising, but several potential reasons have been postulated. Rituximab was given in addition to other treatments that in normal circumstances would have been regarded as sufficient. In addition, the endpoints were quite strict and difficult to fulfil. There are plans for further studies on rituximab with different designs. This study, along with another randomised study in lupus patients without renal involvement (the EXPLORER trial) [96], have not shown any severe side-effect profiles.

A further large case series of 164 adult patients with biopsy proven LN showed a complete response, partial response and no response in about a third of the treated patients [97]. These results, together with the paediatric case series that show a good therapeutic response, means that we continue to recommend the use of rituximab in many situations, as is becoming standard of care in ISN RPS Class III, IV and V LN as well as those with severe life-threatening disease and those patients with active disease despite standard treatment.

Different protocols have been used and our protocol involves two administrations of IV rituximab as an infusion of 750 mg/m^2 (rounded up to the nearest 100 mg with a maximum dose of 1 g) with 14 days in between. In addition, an IV dose of 100 mg methylprednisolone is given immediately prior to each rituximab infusion.

Plasma Exchange

We consider the use of plasmapheresis in very severe and refractory cases of SLE with cerebral lupus and/or crescentic glomerulonephritis, with five to ten plasma exchanges as an adjunct in some patients [81]. However, this is a controversial area and a controlled trial in adult patients with severe LN could not confirm any benefits from adding plasma exchange to the standard treatment of methylprednisolone and CyC [98], which was confirmed in a meta-analysis [99].

Intravenous Immunoglobulin

IV immunoglobulin (at a dose of 2 g/kg to a maximum of 70 g) can be useful, particularly in children with severe haematological disease. Some children benefit from regular infusions (such as every 6 weeks) (Table 26.8).

Table 26.8 Maintenance therapy of lupus nephritis

Prednisolone (oral)	0–5/10 mg alternate or every day
Mycophenolate mofetil (oral)	600 mg/m^2 twice daily (maximum of 2–3 g/day in two divided doses)
Alternatively,	
Azathioprine (oral)	2–2.5 mg/kg once daily
Hydroxychloroquine (oral)	4–6 mg/kg/day Normal dose 200 mg once daily

Maintenance Therapy

All cases of severe lupus require maintenance therapy for a long period, although the length of treatment is not well defined. We advocate maintenance therapy for at least 2–3 years and possibly indefinitely in many cases, especially those with active LN.

Corticosteroids

After the initial rather rapid reduction of the oral corticosteroid, the prednisolone dose should be continuously weaned; many patients may not require long-term oral corticosteroids if adequate immunosuppression is given (e.g., MMF and IV rituximab). The clinical response will decide how quickly the dose can be reduced. Long-term treatment with corticosteroids for several years is often required, although there are quite different approaches on preferred maintenance dose. Our opinion is that it is very important to reduce the dose as quickly as possible. This is important to reduce side effects, but also to improve adherence. Teenagers do not like the cushingoid appearance they develop with corticosteroids so some stop taking their medications when the acute symptoms have resolved. This can lead to non-adherence with other treatments. Our goal is to wean corticosteroids, and either t discontinue or utilize alternate day treatment at a dose in the order of 5–10 mg every other day. These doses of prednisolone should allow the child to grow normally [100].

Mycophenolate Mofetil

MMF is most likely the best choice for long-term maintenance therapy. After the 24 week induction part of the ALMS trial, 227 patients were randomised to 3 years of maintenance therapy with MMF (2 g/day) or azathioprine (2 mg/kg/day) [101]. In this study, MMF was superior to azathioprine with respect to the primary end-point, time to treatment failure; hazard ratio was 0.44 (95% CI 0.25–0.77; p = 0.003). Treatment failure occurred in 16% of the patients in the MMF group and 32% in the azathioprine group. Serious adverse events occurred in 24% of patients treated with MMF compared to 33% treated with azathioprine and the withdrawal due to adverse events was significantly higher in the azathioprine group (40% versus 25%; p = 0.02) [102].

MMF was also compared to azathioprine in the MAINTAIN trial [103]. In this study, 105 patients were treated with similar doses of azathioprine and MMF as in the maintenance phase of ALMS. There was a tendency to fewer renal flares in the MMF group, but the number of patients was not high enough for this to achieve statistical significance.

Azathioprine

Azathioprine at a dose of 2–2.5 mg/kg/day has historically been first line maintenance therapy and has a generally favourable side-effect profile. In the comparison of azathioprine or MMF used as maintenance therapy, MMF was shown to be better than azathioprine (which is the opposite of maintenance treatment of vasculitis) [102, 103]. Some centers advocate testing of thiopurine methyltransferase activity before commencement of azathioprine, but in our practice we advocate close monitoring of full blood counts [104].

Hydroxychloroquine

The use of antimalarial drugs (such as hydroxychloroquine at a dose of 4–6 mg/kg/day) should be considered for all lupus patients. It is especially helpful in children with marked skin or lung disease, lethargy and arthritis. Hydroxychloroquine also seems to reduce blood lipids and possibly the risk for later atherosclerosis. Some clinicians advocate a high dose of 10 mg/kg (up to 400 mg/day) for patients with severe lung disease. Ophthalmology referral and monitoring should take place, as there is a significant risk of retinopathy, particularly with long-term hydroxychloroquine use [105]. In the UK, national guidance suggests at least annual ophthalmology monitoring after 5 years of therapy.

Emerging Therapeutic Options

A number of new therapeutic options for SLE have emerged, with many more currently in development. Belimumab is a fully humanized monoclonal antibody targeting B-lymphocyte stimulator (BLyS) which has been licensed in a number of countries for adults and children with SLE. Adult studies have shown efficacy in SLE [106] and a recent randomized, controlled trial of belimumab in adults with LN showed an improved renal response and a lower risk of renal-related events or death when belimumab was given in addition to standard therapy alone [107]. Belimumab has also been approved for JSLE in a number of countries; the results of the ongoing Phase 2 PLUTO trial in children aged 5–17 years with SLE has shown a favorable side-effect profile with improved efficacy of belimumab compared to placebo [108].

In addition to the use of rituximab for induction therapy, there is increasing interest in its use as maintenance therapy for SLE. Currently, data are limited; a recent observational study in adults did not find any difference in relapse risk in those receiving maintenance rituximab compared to those who had a single course [109]. There may, however, be a role for maintenance rituximab particularly in adolescents or young adults with difficult to treat disease or medication adherence issues on oral treatment.

There is ongoing research into the use of other B-cell depleting monoclonal antibodies. There are reports of the use of other humanized B-cell depleting antibodies such as ofatumumab in patients with SLE. These are currently limited to case series and may be useful in certain situations such as infusion reactions or allergy to rituximab [110, 111].

There is increasing interest in the use of tacrolimus as therapy for SLE, particularly in patients with membranous (Class V) LN or with proteinuria as a predominant feature [112]. Adult studies have shown it is non-inferior to MMF, but the highest efficacy seems to come from combined tacrolimus and MMF, although many studies to date have been conducted in Asian patients so further work is needed to confirm this in different ethnic groups [113–115]. Although there are no studies beyond case reports of tacrolimus for JSLE, its use can be considered in difficult cases and in particular with a membranous appearance on histology. A recent study on voclosporin in combination with MMF showed a superior renal response, but also higher rates of adverse events [116].

The type 1 interferon pathway has also been targeted in SLE, and the monoclonal antibody anifrolumab has recently completed phase 3 RCTs in adults, showing some efficacy in reducing composite disease activity endpoints [117]. Many other therapeutic targets are currently under investigation for SLE, and while these are currently not likely to reach children and adolescents with JSLE for some years, there is hope for many more treatment options in the future.

Treatment of Antiphospholipid Syndrome (APS)

Treatment for APS includes reducing lupus disease activity and appropriate anticoagulation treatment (such as life-long aspirin or warfarin if severe thromboembolic disease).

General Renal Management

As outlined in other parts of this book, general renal care is important in all children with renal impairment. This includes monitoring and treatment of hypertension and proteinuria; blood pressure targets are around 50th centile for age, sex and height centile and albuminuria and proteinuria should be minimised (which may be due to active disease, which requires increasing immunosuppression or chronic damage,which requires treatment with angiotensin converting enzyme inhibition or angiotensin receptor blockers). It is important to continuously evaluate renal function of these children with estimated GFR and, if appropriate, a formal GFR measurement. Supportive treatment of chronic kidney disease and ESKD is required in some of these children.

General Management

Sun Protection

All children with SLE and especially those with active skin disease should be advised to always use appropriate sunscreen and protect themselves from the sun, with regular monitoring of 25-hydroxyvitamin D levels and appropriate vitamin D supplementation.

Immunisations

Children with SLE, treated with immunosuppressive drugs, should avoid immunisation with live vaccines. Vaccination with killed vaccines should be carefully considered as they might induce a flare of the disease and also might have decreased efficacy. In the United Kingdom and in other countries, pneumococcal vaccinations are recommended for all patients likely to be on corticosteroids for more than a month.

Management of Infection

Children on immunosuppressive treatment are more susceptible to severe infections than other children and it should be emphasised to the parents and the children that they should seek medical advice early in the case of fever or symptoms of an infection.

Prognosis

In the era before treatment became available, the prognosis of patients with SLE was very poor with very few patients with a severe nephritis surviving more than 2 years [118]. The introduction of corticosteroids and immunosuppressive treatment with CyC and azathioprine made a huge impact on the long-term prognosis. Cameron showed already more than 25 years ago a general and renal survival of about 20% after 20 years [56]. He could also show that between the years 1965 and 1991 the 10-year patient survival improved from 10 to 20%.

The proportion of death from SLE improved very rapidly in high-income countries from the 1960s to the 1970s, with slower improvement thereafter. In contrast, in low and middle-income countries there was a pronounced increase in survival between 1970 and 1990 followed by a plateau or a decreased survival. There is now a significant difference in both 5- and 10-year survival between high and middle/low income countries. Five year survival estimates of 0.99 and 0.85, respectively and 10-year survival estimates of 0.97 and 0.79, respectively [119].

The proportion of children dying from their renal failure is now lower, although the prognostic factors for developing renal failure remain the same (male gender, non-Caucasian race, nephrotic syndrome at onset and severity of disease on renal biopsy [73]).

Another important measurement of success in the treatment of LN is the proportion of children achieving full renal remission. A recent German study in 79 pediatric lupus patients showed a rate at one year of complete remission of 38%, and 41% partial remission [120]. An Italian study found a rate of a complete remission of about 50% while children from South Korea showed the highest rate of complete remission at 59.7% [121, 122]. Remission was defined as normal kidney function and no proteinuria. There is thus still a need to improve long-term kidney survival. In recent years, it has also become evident that patients with SLE confront another important threat to their long-term survival due to an increased risk of atherosclerosis.

Treatment Related Complications and Mortality

In recent studies, infections have been the main cause of death, therefore suggesting that the most important short-term goal is to find therapies which are as good as current treatments but with fewer side effects. Recent comparative studies in adults showed that CyC treatment was associated with significantly more severe infections compared to treatment with azathioprine or MMF [84, 90–92]. This emphasises the importance of monitoring white cell and neutrophil counts during therapy and early treatment of infectious complications. However, infections can sometimes be difficult to differentiate from a flare of

disease activity, especially in children who are in ESKD on renal replacement therapy. CRP can be used as a helpful tool in these situations as very few patients even with active lupus have raised CRP levels [56] while most children with septicaemia do.

Severe viral infections, in particular with varicella zoster virus are seen, and children exposed to the virus or with early symptoms of varicella (or more often herpes zoster virus) should be treated with aciclovir therapy to reduce the risk of generalised infection.

Growth failure is a major problem in children with lupus, which can be related to the inflammatory disease or a complication of corticosteroid treatment. Sometimes it can be difficult to differentiate between them and this is a controversial area, but it is our opinion that ongoing inflammation more often causes the growth failure than the treatment with low corticosteroid doses. Therefore, increasing immunosuppression rather than reducing treatment is often more beneficial for the growth of these children.

SLE patients have an increased risk of osteoporosis, partly caused by their long-term treatment with steroids [123]. Treatment with calcium and vitamin D supplementation is advocated by some centres, but unfortunately it seems as if the beneficial effects from that treatment only persist during the treatment [124]. However, an increased nutritional calcium intake does seem to be able to improve bone accretion over a longer time [125]. It is recommended that children with SLE should be monitored with regular bone density scans.

Children on long-term immunosuppressive treatment have an increased risk for developing malignancies, in particular skin cancers and lymphoma, and should be advised to use sun protection. Bladder cancer has been associated with the use of CyC in children with SLE [126]. A 10 year follow-up of a large cohort of 1000 adult lupus patients found that 23 developed malignancies, with breast and uterine cancers being the most common [127]. Therefore, the risk of malignancy does not seem to be excessive.

Active lupus often causes amenorrhoea and delayed puberty, whereas treatment with CyC can also reduce fertility. A study of 39 women younger than 40 years old showed that 12.5% (2 out of 16) receiving seven IV doses of CyC developed sustained amenorrhoea compared to 39% (9 out of 23) receiving 15 or more doses (with a higher risk in women older than 25 years) [128].

Thrombosis

In the aforementioned 10-year follow up of 1000 adult patients, 9.2% [129] developed thrombosis, and of the 68 patients who died, 26.5% had a thrombotic event. Thrombosis is more common in children with antiphospholipid syndrome. A 10-year follow-up of 149 children with SLE from Toronto showed that 24 were positive for lupus anticoagulant and that 13 of them experienced 21 thromboembolic events [130]. The authors emphasised the need to treat this subgroup of children with life-long anticoagulation.

Cardiovascular Disease

A Swedish registry study on 4737 patients with SLE from 1964 to 1994 showed a 16-fold increased risk of death from cardiovascular diseases [129]. Therefore, it is our new challenge to try to prevent atherosclerosis and to improve our patients' long-term survival. This increased risk for cardiovascular death is multifactorial and includes classical risk factors, such as hypertension, hyperlipidaemia and corticosteroid treatment and disease-related risk factors such as proteinuria, vasculitis, low-grade systemic inflammation, antiphospholipid syndrome and elevated levels of homocysteine [131].

Carotid plaque and coronary artery calcifications are significantly increased in lupus patients [132, 133]. Efforts to decrease atherosclerosis include good control of inflammation, aggressive treatment of hypertension and proteinuria and prevention of corticosteroid-induced obesity. A randomised placebo controlled study on treatment with atorvastatin did not significantly reduce progression of the surrogate marker for atherosclerosis, carotid intima-media thickness [134]. The active treatment did, however, reduce levels of high sensitivity CRP and total cholesterol and low-density lipoprotein. A secondary analysis suggested that atorvastatin reduced atherosclerosis progression in paediatric lupus patients with higher CRP values

[135]. Hydroxychloroquine has been shown to have a beneficial effect on the cardiovascular risk profile [136].

Adherence to Treatment

One important prognostic factor in children with LN, as in all children with chronic kidney disease, is adherence with treatment, especially during puberty. Non-adherence in adolescents is a common reason for relapse of symptoms, and may cause acute kidney injury after initially successful treatment. In such serious clinical situations, we would advocate the use of IV therapies (with rituximab treatment now our preferred option, which can be given at six-monthly intervals as maintenance treatment irrespective of peripheral CD19 counts) instead of oral treatments to ensure adherence and disease control.

Renal Replacement Therapy

The optimal therapy for patients with ESKD due to LN is renal transplantation, especially as patients seem to do equally well compared to matched controls, with less than 10% recurrence of LN and similar long-term patient and renal allograft survival [137, 138]. Patients should be in remission for 6 months prior to transplantation, and the transplant immunosuppression regimen should be standard, unless there are immunological concerns. However, it should be noted that the risk for thromboembolic complications was higher in the SLE group. Patients with SLE can have peritoneal or haemodialysis prior to transplantation, but in view of hypocomplementaemia and immunosuppression, clinicians should be wary of clinical presentation of flare of disease activity (which can be difficult to diagnose) versus infectious complications. Patients on peritoneal dialysis presenting with peritonitis should be treated with IV and intraperitoneal antibiotics.

Conclusion

The prognosis for children with SLE has improved over recent decades, with improved survival and fewer debilitating symptoms. There is, however, still a major challenge to obtain full remission of LN, which is essential for optimizing long-term kidney function. Another important challenge is to minimise treatment related mortality and morbidity. This has led to less CyC use, which has been replaced by other less toxic drugs, mainly MMF. Rituximab is now also routinely used in some centers, but further studies are needed to fully delineate its role. It is also important to find ways to reduce the burden of premature cardiovascular disease in these children.

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27 IgA Vasculitis Nephritis (Henoch-Schönlein Purpura Nephritis)

Jae Il Shin

Introduction

Immunoglobulin A (IgA) vasculitis, formerly called Henoch-Schönlein purpura (HSP), was first described by William Heberden [1], a London physician, in 1801 and was named after the description of the clinical entity characterized by purpura and joint pain by Johann Schönlein in 1837 [2] and of the frequent association of gastrointestinal symptoms and kidney involvement by Edouard Henoch in 1874 [3]. IgA vasculitis is the most common form of systemic vasculitis in children; it mainly affects the skin, joints, gastrointestinal tract and kidneys. IgA vasculitis is usually self-limited to 1–4 weeks, but relapses can occur. The overall prognosis of IgA vasculitis is favorable, but the long-term prognosis is dependent on the degree of renal involvement [4, 5].

Diagnostic Criteria

The American College of Rheumatology (ACR) proposed in 1990 that the presence of any 2 or more of the following criteria was required for the diagnosis of IgA vasculitis: (1) an age of less than 20 years at disease onset, (2) palpable purpura, (3) acute abdominal pain, and (4) biopsy showing granulocytes in the walls of small arterioles or venules [6]. In 2005, the diagnostic criteria for IgA vasculitis were modified by the European League Against Rheumatism/Paediatric Rheumatology European Society (EULAR/PRES) [7]. In this new diagnostic system, the age criterion was deleted, 'predominant IgA deposition' was included in the definition of the 'biopsy' criterion, and arthritis and renal involvement were added as independent criteria [7]. Therefore, IgA vasculitis is diagnosed as follows: palpable purpura (mandatory criterion) in the presence of at least one of the following four features: (1) diffuse abdominal pain, (2) any biopsy showing predominant IgA deposition, (3) arthritis or arthralgia (acute, any joint), and (4) renal involvement (any hematuria and/or proteinuria) [7]. Skin biopsy is rarely performed to diagnose IgA vasculitis, but may be necessary in doubtful cases such as isolated purpura or atypical characteristics to differentiate from leukocytoclastic vasculitis. The latter will show no IgA deposits [7, 8].

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Epidemiology

The prevalence of IgA vasculitis varies from 3.0/100,000 to 26.7/100,000 children [9, 10]. IgA vasculitis is more common in preschool aged children and in males, and there seems to be an increased frequency in autumn and winter. The pathogenesis of IgA vasculitis is unclear, but it is considered as a complex disease caused by various genetic and triggering environmental factors [9]. It is often preceded by an upper respiratory tract infection 1–3 weeks prior to the onset of symptoms. Various infectious agents, such as parvovirus B19, hepatitis B and C virus, adenovirus, Group A β-hemolytic streptococcus, staphylococcus aureus, and mycoplasma, and various drugs, vaccinations, cancers, insect bites or exposure to cold weather have been reported as triggering factors for IgA vasculitis [9, 10]. Familial cases of IgA vasculitis have also been reported [11].

Clinical Findings

Skin

Palpable purpura is the most common finding, but petechiae, maculae, papulae, urticaria, ecchymosis or bullae can also occur. Skin lesions are generally distributed symmetrically over the extensor surfaces of the lower legs (gravity or pressure-dependent areas), arms and buttocks, but trunk, face, eyelids, earlobes and genitalia can also be involved [12]. In young children, edema of the scalp, hands and feet can be observed. The skin findings usually resolve within 1–2 weeks, but persistent (>4 weeks) or relapsing skin manifestations are observed in about 25% of children with IgA vasculitis, and may be associated with the occurrence and severity of renal involvement [13–15].

Joints

Joint symptoms occur in about 80% of children with IgA vasculitis, and large joints of the lower extremities, such as the ankles and knees, are usually affected [12]. Joint pain is a frequent finding and periarticular swelling and tenderness, usually without synovial fluid effusion, may be present. Joint symptoms resolve with time without any deformity or erosions [12].

Gastrointestinal Tract

Gastrointestinal involvement is reported in about 50–70% of children with IgA vasculitis and usually presents as diffuse abdominal pain, which can be increased after meals, nausea, emesis, and bloody stools such as melaena and hematochezia [12, 16]. Gastrointestinal symptoms are caused by bowel wall edema and submucosal hemorrhage due to vasculitis. Severe gastrointestinal complications can occur, including intussusception, bowel infarction, gangrene or perforation, duodenal obstruction, massive gastrointestinal hemorrhage requiring blood transfusion, appendicitis, pancreatitis, hydrops of the gall bladder, protein losing enteropathy and formation of fistulas or strictures.

Other Nonrenal Organ Involvement

Although rare, IgA vasculitis can be complicated by various other manifestations, such as neurologic findings (obtundation, seizure, paresis, cortical blindness, chorea, ataxia and cranial or peripheral neuropathy), urologic complications (orchitis, epididymitis, and stenosing ureteritis, presenting as renal colic), carditis, myositis or intramuscular bleeding, pulmonary hemorrhage, and anterior uveitis [17]. Because some of these manifestations can be rapidly fatal, close observation and monitoring of affected patients is important.

Kidney

The incidence of renal involvement in IgA vasculitis varies from 20 to 80% in published case series [13–15]. Renal involvement includes iso-

lated haematuria (14%), isolated proteinuria (9%), both hematuria and proteinuria (56%), nephrotic-range proteinuria (20%) and nephrotic-nephritic syndrome (1%) [15]. Hypertension may develop at the onset of disease or during recovery, even with minimal or no urinary abnormalities [18]. In a large UK primary care database of 10,405 patients with childhood-onset IgA vasculitis, there was a significantly increased risk of hypertension and stage 3–5 chronic kidney disease (CKD) compared with age-matched and sex-matched controls, although there was no evidence of association with ischaemic heart disease, cerebrovascular disease or venous thromboembolism [19]. Therefore, appropriate surveillance for hypertension and CKD in children with IgA vasculitis may be necessary, and risk factor modification could improve long-term outcomes in these patients [19].

The first urinary abnormalities are detected within 4 weeks of disease onset in 80% of children with IgA vasculitis and within the next 2 months in the remainder, although a small number of patients present with urinary abnormalities several months later or as the initial feature [20]. Minor urinary abnormalities usually resolve with time, whereas severe renal involvement such as nephrotic syndrome and acute nephritic syndrome, can progress to CKD. In a systematic review of 1133 children with IgA vasculitis observed in 12 studies, the overall incidence of long-term renal impairment (defined as persistent nephrotic syndrome, nephritis, or hypertension) was 1.8%. Permanent renal impairment occurred in none of the 65.8% children with normal urinalysis during the acute disease, in 1.6% of the 26.9% with isolated haematuria and/or proteinuria, and in 19.5% of the 7.2% patients initially presenting with nephritic or nephrotic syndrome [20].

Risk Factors for Renal Involvement

Some authors reported that an older age at onset, persistent purpura (>4 weeks), severe abdominal symptoms, decreased factor XIII (fibrin stabilizing factor) activity, and a relapsing disease pattern are significant risk factors for renal involvement in children with IgA vasculitis, and these factors were linked to each other and all indicated a severe disease course [13–15]. In addition, persistent purpura, severe abdominal symptoms, and relapse were associated with the development of significant proteinuria [14]. A recent meta-analysis showed that significant risk factors associated with renal involvement in IgA vasculitis were male gender, abdominal pain, gastrointestinal bleeding, severe abdominal pain, persistent purpura, relapse, a high white blood cell count ($>15 \times 10^9$/L), a high platelet count ($>500 \times 10^9$/L), elevated anti-streptolysin O (ASO) titer and decreased complement component 3 (C3) [13].

Atypical Presentation

IgA vasculitis is diagnosed clinically, but atypical presentation often causes difficulties in the diagnosis. Gastrointestinal symptoms precede the cutaneous rash in about 25% of children with IgA vasculitis, [16] and joint symptoms or scrotal pain may precede the skin rash [16]. In addition, some diseases such as systemic lupus erythematosus, microscopic polyangiitis or Crohn disease can mimic IgA vasculitis [21]. Since atypical presentation of IgA vasculitis can lead to an incorrect diagnosis, causing unnecessary therapies and procedures (e.g., appendectomy) and unfavorable outcomes, it is important to include IgA vasculitis in the differential diagnoses, although diagnosis can be very difficult in those settings.

Laboratory and Radiologic Investigations

Because IgA vasculitis is diagnosed clinically, there is no specific diagnostic test for IgA vasculitis. Recommended initial investigations are complete blood count to evaluate for anemia or leukocytosis, erythrocyte sedimentation rate to evaluate for inflammation, coagulation profile to exclude bleeding disorders, biochemical profile

to screen for acute kidney injury or hypoalbuminemia, ASO titer, urine dipstick and protein/creatinine ratio to evaluate for renal involvement and tests to screen for sepsis if the diagnosis is unclear and purpura is present (e.g., blood culture for meningococcemia) [22]. Antinuclear antibody titer, anti-double stranded DNA antibody, antineutrophil cytoplasmic antibody (ANCA), complement levels (C3 and C4) and immunoglobulins (IgG, IgA and IgM) may also be necessary to differentiate IgA vasculitis from other vasculitides or overlapping diseases.

IgA vasculitis has a bleeding tendency due to abnormal platelet aggregation, decreased factor XIII levels by vasculitic process and increased von Willebrand factor (vWF) levels by endothelial injury despite normal platelet count and clotting factors [23, 24]. Plasma D-dimer levels can be increased [24]. A stool test for occult blood can be used to detect gastrointestinal hemorrhage.

Serum IgA levels (mostly IgA1) are increased in 50% of children with IgA vasculitis, and IgA-rheumatoid factor, IgA-containing immune complexes, IgA-fibronectin aggregates and cryoglobulins have been found [25, 26]. Serum IgE and eosinophil cationic protein (ECP) levels can be elevated, [27, 28] and C3, C4, and CH50 levels are occasionally decreased in the acute stage of disease [29]. ANCAs (c-ANCA and p-ANCA) are generally negative in IgA vasculitis except in rare cases [30, 31], but IgA-ANCA has been detected in the acute stage of disease [32, 33]. Antiphospholipid antibodies may be positive [34].

Imaging studies may be necessary to detect complications of IgA vasculitis, with selection based on the clinical picture [22]. Chest X-ray can detect pulmonary involvement; abdominal X-ray can identify ileus or perforation of the gastrointestinal tract. Renal ultrasound can detect increased echogenicity of the kidneys or hydronephrosis; abdominal ultrasound can identify thickened bowel wall or intussusception [22].

Renal Histopathologic Findings

Light Microscopy Findings

The light microscopic findings of IgA vasculitis nephritis (IgAVN) are characterized by mesangial proliferative glomerulonephritis with varying degrees of mesangial hypercellularity (Fig. 27.1a), segmental sclerosis (Fig. 27.1b) and crescents (Fig. 27.1c), similar to the predominant findings in IgA nephropathy (IgAN) [35, 36]. These glomerular changes are usually graded according to the International Study of Kidney Disease in Children (ISKDC) classification system (Table 27.1) [37]. Crescents are reportedly much more common in IgAVN than in IgAN [25] and are frequently seen in association with capillary wall destruction and endocapillary cell proliferation with subendothelial immune deposits of IgA and complement [38]. Crescents are classified as cellular, fibrocellular or fibrous. Crescents are cellular at the onset of the disease and evolve with time towards fibrous, causing global glomerulosclerosis (Fig. 27.1d).

Because the ISKDC classification does not include tubulointerstitial changes and other histologic features in IgAVN, some authors have used histopathologic scoring systems, such as activity and chronicity scores [39, 40]. Acute changes included mesangial matrix increase, mesangial hypercellularity, endothelial swelling, hyalinosis, basement membrane adhesion to Bowman's capsule, glomerular lobulations, glomerular neutrophils, fibrinoid necrosis, nuclear debris, interstitial vasculitis with leukocytoclastic reaction, tubular damage, interstitial edema and interstitial mononuclear infiltrate [39]. Chronic changes include interstitial fibrosis, tubular atrophy, fibrous crescents, global sclerosis, vascular hyalinosis and intimal hyperplasia [39]. The degree of tubulointerstitial lesions is correlated with the glomerular pathology [35].

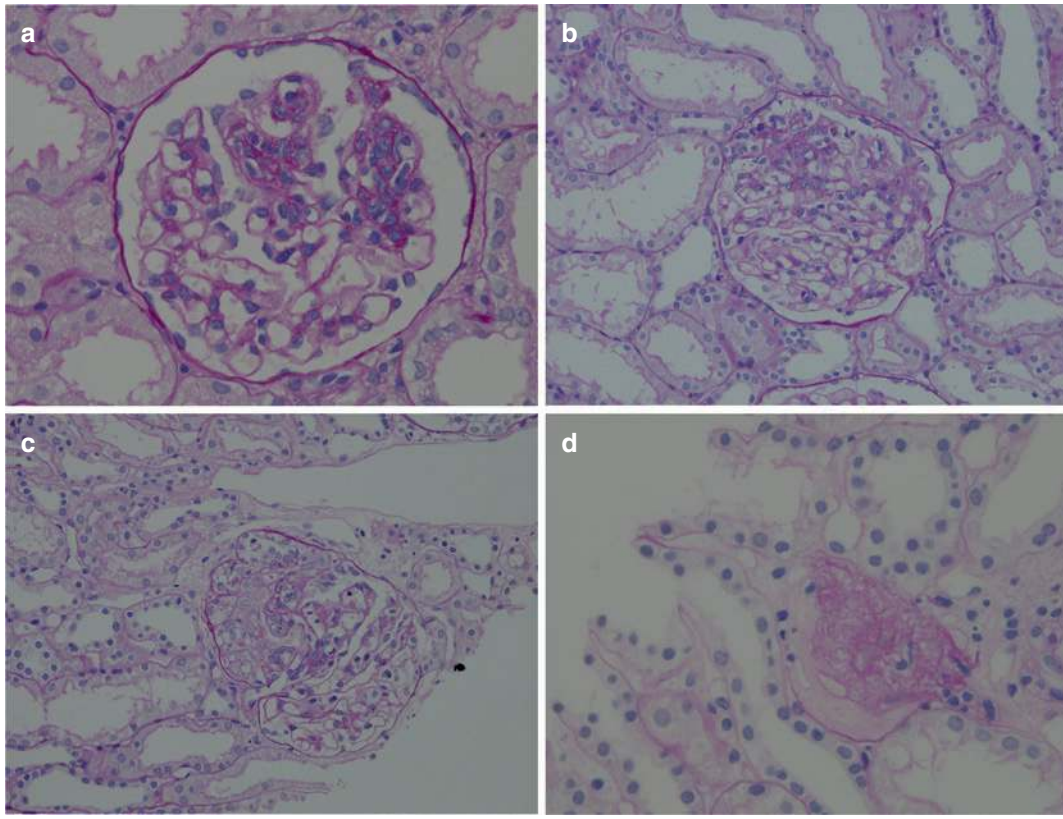

Fig. 27.1 Light microscopy findings in IgA vasculitis nephritis. (**a**) Mild mesangial cell proliferation (PAS, ×400). (**b**) Glomerulus with segmental sclerosis (PAS, ×200). (**c**) Glomerulus with cellular crescent (PAS, ×200). (**d**) Glomerulus with global sclerosis (PAS, ×400)

Table 27.1 The classification of IgA vasculitis nephritis in the International Study of Kidney Disease in Children

Grade I		Minimal alterations
Grade II		Mesangial proliferation without crescents
Grade III	IIIa	Focal mesangial proliferation or sclerosis with <50% crescents
	IIIb	Diffuse mesangial proliferation or sclerosis with <50% crescents
Grade IV	IVa	Focal mesangial proliferation or sclerosis with 50–75% crescents
	IVb	Diffuse mesangial proliferation or sclerosis with 50–75% crescents
Grade V	Va	Focal mesangial proliferation or sclerosis with >75% crescents
	Vb	Diffuse mesangial proliferation or sclerosis with >75% crescents
Grade VI		Membranoproliferative glomerulonephritis

Immunofluorescence Findings

Granular deposits of IgA (predominantly IgA1) in mesangial areas are characteristic of IgAVN (Fig. 27.2a). The IgA deposits are diffuse, in contrast to the frequent focal and segmental changes of glomeruli. C3 (Fig. 27.2b) and the alternative complement pathway components are frequently found, but the components of the classic complement pathway, such as C1q and C4, are rarely detected [38, 41]. IgA and C3 can be deposited in arterioles and capillary walls [25, 36, 38]. IgG and IgM are less frequently detected [38, 42]. Glomerular fibrin deposits (Fig. 27.2c) are more frequently found in IgAVN than in IgAN, [25, 38] and are associated with the formation of crescents.

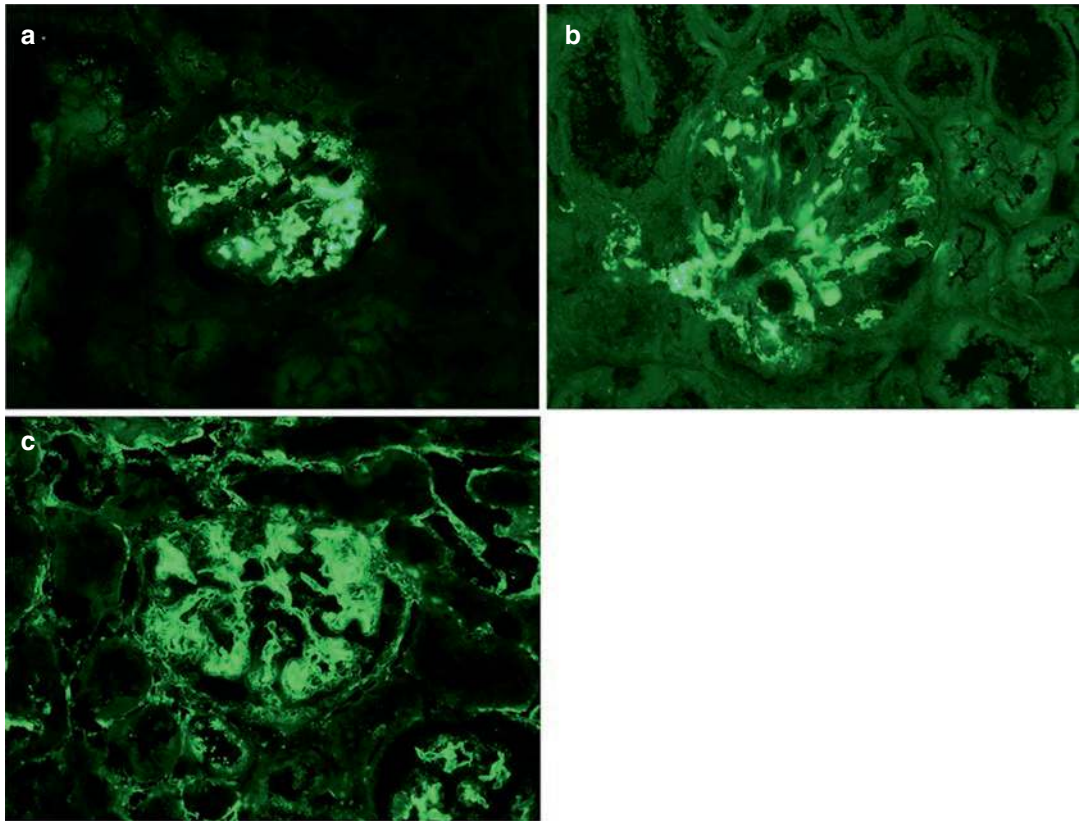

Fig. 27.2 Immunofluorescence findings in IgA vasculitis nephritis. (**a**) Mesangial IgA deposition (×100). (**b**) Mesangial C3 deposition (×200). (**c**) Mesangial fibrinogen deposition (×100)

Electron Microscopy Findings

Electron-dense deposits are mainly found in mesangial areas (Fig. 27.3a), but the deposits can be detected in subendothelial areas (Fig. 27.3b); rarely, hump-like deposits are found in subepithelial areas [25, 35, 41]. There are varying degrees of foot process effacement of visceral epithelial cells, depending on the degree of glomerular injury [25, 35, 41].

Clinicopathologic Correlations

In general, a good correlation between the severity of renal involvement and pathologic grading is observed in IgAVN and the severity of proteinuria at onset is a significant determinant of renal pathologic findings such as crescent formation, endocapillary proliferation, and tubular atrophy [43]. Proteinuria is also correlated with the ISKDC grade [44]; however, even mild to moderate proteinuria may be associated with severe morphological changes [44]. Hence, renal biopsy may be indicated, not only in patients with nephrotic syndrome, but also in those with mild proteinuria.

Although there are few reports on follow-up renal biopsies in IgAVN, there is also a generally good correlation between the clinical course and histopathologic changes [45]. IgAVN children who achieve clinical remission show a decrease in IgA deposits and regression of mesangial proliferation or crescents on follow-up renal biopsy, while those who have persistent nephritis demonstrate severe histologic findings with chronic lesions [45]. However, some reports emphasized that abnormal renal histologic findings can per-

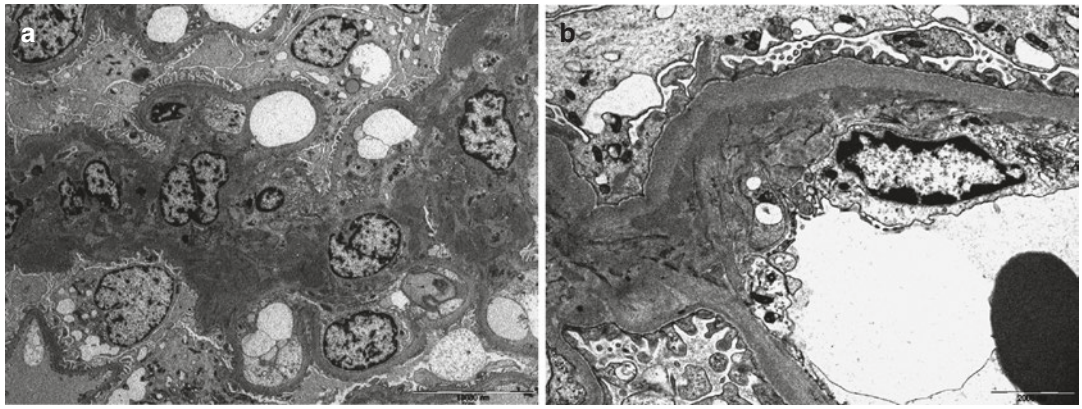

Fig. 27.3 Electron microscopy findings in IgA vasculitis nephritis. (**a**) Electron dense deposits in mesangial areas (×3000). (**b**) Electron-dense deposits in subendothelial areas (×10,000)

sist despite clinical remission [46, 47]. Algoet et al. reported that renal histologic findings were normal only in 25% of patients who had achieved complete clinical remission 5–9 years after the onset of IgA vasculitis [46]. Shin et al. also showed persistent histologic abnormalities in all patients of a IgAVN cohort after immunosuppressive treatment regardless of clinical improvement, suggesting the kidneys were not completely healed even in those with clinical remission [47].

Risk Factors for Poor Prognosis

The clinical presentation at the onset of IgAVN is predictive of long-term outcome [20]. The risk of progression to CKD was highest in IgA vasculitis children who presented with nephritic-nephrotic syndrome (45–50%) followed by those with nephrotic syndrome (up to 40%), acute nephritic syndrome (up to 15%), hematuria and non-nephrotic proteinuria (5–15%) and microscopic hematuria with or without minimal proteinuria (<5%) [20, 48]. In a systematic review, the risk of long-term renal impairment was 12 times higher in IgAVN patients with nephritic or nephrotic syndrome than in those with only abnormal urinalysis, and was 2.5 times higher in females than males [20]. Additional information is provided by very long-term outcome studies of IgAVN [49, 50]. Revisiting a cohort of IgA vasculitis patients at an average of 23 years after first manifestation, Goldstein et al. observed highly unpredictable late outcomes; 7 of 78 patients with normal urinalysis or apparent complete recovery showed active renal disease or ESRD [49]. Ronkainen et al. also showed that even patients with mild renal symptoms at the onset of IgA vasculitis have a risk for severe long-term complications [50].

Also, the clinical course during follow-up may be important to predict the prognosis [48, 51, 52]. Bunchman et al. showed that a creatinine clearance <70 mL/min/1.73 m^2 3 years after onset predicted progression to ESRD, whereas a clearance >125 mL/min/1.73 m^2 predicted normal renal function at 10-year follow-up [51]. Coppo et al. demonstrated that the risk for progression was related to increasing mean proteinuria levels during follow-up in both children and adults with IgAVN [52].

Histopathological lesions related to poor prognosis are a high grade by ISKDC, crescents in >50% of glomeruli, glomerular sclerosis or tubulointerstitial changes [48, 53, 54]. However, the initial renal biopsy may not predict the outcome of IgAVN since patients with mild histopathological disease activity (ISKDC II-III) usually receive less aggressive immunosuppressive therapy [55]. Therefore, some investigators suggested that serial biopsies might be helpful to establish the ultimate outcome in IgAVN patients with renal exacerbations [46]. A younger age at onset was an independent determinant of histo-

logical regression. The activity index at the follow-up biopsy correlated positively with changes in mesangial IgA deposits and the chronicity index at the follow-up biopsy correlated positively with the time immunosuppressive therapy was started [47]. One report suggested that the serum IgA/C3 ratio might be a useful marker for predicting serial histologic lesions of IgAVN because it correlated with the severity of renal pathology and clinical outcome in children with severe IgAVN [56].

Schärer et al., in a comprehensive multivariate analysis, demonstrated that initial renal insufficiency, nephrotic syndrome, and the severity of histological alterations (as defined by the percentage of glomeruli with crescents) are significant independent predictors of progressive renal failure in patients with IgAVN [54].

From a systematic review of 12 unselected IgA vasculitis populations, it was recommended that if the urinalysis is normal at presentation, monitoring can be limited to 6 months in patients with persistently normal urine findings [20]. However, the recommended duration of follow-up remains controversial since in individual children with normal urinalysis at onset renal involvement may still develop after several years [49, 50].

Pathogenesis

While the complete pathogenesis of IgA vasculitis remains to be elucidated, abnormalities of IgA have an important role. IgA vasculitis develops as a consequence of leukocytoclastic vasculitis due to IgA deposition in the wall of capillaries and post-capillary venules of various organs, including skin, gastrointestinal tract and the mesangium of the kidney [10, 12, 21]. Genetic predisposition, activation of complement, cytokines, autoantibodies and coagulation abnormalities are also involved in the pathogenesis of IgA vasculitis [21].

Genetic Predisposition

Although IgA vasculitis occurs mostly as sporadic cases, familial clustering has been reported [11]. Various candidate genes and genetic polymorphisms have been associated with the risk for IgA vasculitis or IgAVN [21]. These include human leukocyte antigen (HLA-A, B, B35, DRB1, DQA1), cytokines (interleukin (IL)-1ß,IL-1 receptor antagonist, IL-18, transforming growth factor (TGF)-ß), adhesion molecules (P-selectin, intracellular adhesion molecule-1), cytotoxic T-lymphocyte antigen 4, the MEFV gene (encoding pyrin, an important active member of the inflammasome), the renin-angiotensin system genes (angiotensin converting enzyme, angiotensinogen) and the C1GALT1 gene (encoding ß 1,3-galactosyltransferase, an important role in the glycosylation of the IgA1 hinge region).

Abnormalities of IgA

Elevated serum levels of IgA, principally IgA1, and circulating IgA-containing immune complexes are observed in patients with IgAVN [57, 58]. One study reported that the number of IgA-producing cells was increased in IgA vasculitis, but not in other forms of leukocytoclastic vasculitis [59]. Both increased IgA synthesis and decreased clearance have been reported as contributors to the pathogenesis of IgAVN [25]. It has been hypothesized that production of polymeric IgA by the mucosal immune system in response to various mucosally presented antigens [60] may be increased, and the reticuloendothelial system function impaired [61]. In addition, all patients with IgA vasculitis have IgA1-circulating immune complexes of small molecular mass, while only those with nephritis have additional large-molecular-mass IgA1-IgG-containing circulating immune complexes [58].

There are two subclasses of IgA (IgA1 and IgA2) and ~90% of serum IgA is IgA1. IgA1 and

IgA2 differ structurally in the hinge region of the heavy chain. IgA1, unlike IgA2, has a proline-rich hinge region composed of 5–6 O-linked glycosylation sites. An abnormal glycosylation of the IgA1 hinge region occurs in the context of a deficiency of galactose and/or sialic acid [21, 57]. Such an aberrantly glycosylated IgA1 is prone to cause IgA aggregation and may change IgA1 structure, modifying interactions with IgA receptors and matrix proteins, leading to mesangial deposition of IgA1 [21, 57]. Although no confirmed genetic loci for IgA vasculitis have been identified to date, it was recently reported that aberrant glycosylation of IgA1 is inherited in both pediatric IgAN and IgAVN [62]. However, Kiryluk et al. reported that an increase of the poorly galactosylated IgA1 *O*-glycoforms levels may be insufficient to cause IgAN or IgAVN because first-degree relatives had high serum levels of poorly galactosylated IgA1 *O*-glycoforms without any signs of either IgAN or IgAVN [62]. They suggested that a 'second hit,' such as the formation of glycan-specific IgG (and IgA) antibodies, which could form large circulating immune complexes prone to deposition, might be required to develop overt disease [62].

Activation of Complement

Activation of complement, mainly via the alternative pathway, is an important mechanism of tissue injury in IgA vasculitis. Complement components are found in skin and glomeruli, and breakdown products of complement in plasma [29, 63, 64]. C4A and C4B deficiencies have been described in patients with IgAVN, [65] and glomerular deposition of C3 and properdin has been reported in 75–100% of patients with IgAVN [66]. Activation of the lectin pathway of complement has also been demonstrated in patients with IgAVN, which might contribute to the development of advanced glomerular injury and long-term urinary abnormalities in IgAVN [64]. The lectin pathway is initiated by mannose-binding lectin (MBL). MBL also forms complexes with MBL-associated serine proteases (MASP-1, MASP-2 and MASP-3) [64]. Hisano et al. reported that MBL/MASP-1 might be associated with glomerular deposition of fibrinogen [64].

Activation of Eosinophils

Activation of eosinophils has also been proposed to contribute to the pathogenesis of IgAVN [27, 28]. Elevated plasma IgE levels were more common [27] and serum eosinophil cationic protein (ECP) levels were significantly higher in children with IgA vasculitis than in those with IgAN or healthy controls [28]. Davin et al. speculated that the IgA-containing immune complexes could enhance IgE production locally by stimulation of the dermal and intestinal mast cells and deposition of IgA immune complexes was further enhanced by a subsequent increase in local capillary permeability [27].

Cytokines and Coagulation Abnormalities

Several proinflammatory cytokines, including tumor necrosis factor (TNF)-α, IL-1β, IL-2, IL-6, IL-8, IL-17, TGF-β, and vascular endothelial growth factor, have been reported to be involved in the development of IgA vasculitis. They are likely secreted by vascular endothelial cells, thus initiating and propagating the inflammatory reaction [21].

Circulating immune complexes can cause vascular endothelial injury and coagulation abnormalities in IgA vasculitis; plasma vWF levels are elevated at the acute stage of IgA vasculitis [24]. Factor XIII activity can also be decreased during the acute stage of IgA vasculitis, possibly due to degradation of factor XIII by proteolytic enzymes from inflammatory cells [23].

Mesangial Proliferation and Crescent Formation

Once IgA-containing complexes are deposited in glomerular mesangium, various components of IgA-containing complexes, such as Fcα and Fcγ

fragments, fibronectin, or C3b, can bind to their receptors on the surface of mesangial cells and trigger proliferation of mesangial cells, production of extracellular matrix and synthesis of cytokines, such as monocyte chemoattractant protein-1 and IL-8, recruiting neutrophils and monocytes [25, 36, 67–71]. Other cytokines (TNF-α, IL-1, IL-6 and TGF-β) involved in the pathogenesis of IgA vasculitis can also stimulate mesangial cells [69–71].

In addition, local complement activation and intraglomerular coagulation by mesangial fibrin deposition can destroy the glomerular basement membrane. Attraction of macrophages and proliferation of cytokine-induced epithelial cells in Bowman's space can disrupt capsular integrity, leading to interstitial fibroblast infiltration into Bowman's space, causing crescent formation [25, 36, 38, 72].

Treatment

Prevention of Nephritis in IgA Vasculitis

Corticosteroids are commonly administered in the acute stage of IgA vasculitis to reduce the severity and duration of abdominal pain or arthralgia [73]. A number of retrospective studies, randomized controlled trials (RCTs), systematic review and meta-analyses have addressed the use of corticosteroids for preventing nephritis, with conflicting results [48, 73–76]. A well-conducted, randomized, double-blind, placebo-controlled trial showed no benefit of prednisone (4-week treatment) in preventing the development of nephritis in IgA vasculitis, but observed more rapid resolution of nephritis [73]. These results might be due to the reduction of mesangial proliferation and crescent formation by prednisone [77]. In addition, another randomized, double-blind, placebo-controlled trial reported that early 2-week treatment with prednisolone did not reduce the prevalence of proteinuria 12 months after disease onset in children with IgA vasculitis [74]. Based on this evidence, the Kidney Disease Improving Global Outcome (KDIGO) guidelines recommend not using corticosteroids to prevent nephritis in children with IgA vasculitis [78].

Although the general use of prednisone to prevent nephritis is not supported, IgA vasculitis patients with extrarenal symptoms might benefit from early treatment. Some IgA vasculitis patients cannot tolerate oral medications due to abdominal pain; in these, initial intravenous followed by oral steroid therapy could be a useful and effective therapeutic strategy [79].

Treatment of IgAVN

Treatment of IgAVN should be aimed to prevent long-term renal morbidity in patients at risk, but there have been few RCTs to establish the optimal treatment due to the rarity of severe IgAVN. Therefore, the treatment of IgAVN remains controversial.

The KDIGO Guidelines and Therapeutic Considerations in Pediatric IgAVN

The KDIGO initiative recently published guidelines on the treatment of IgAVN [78]. The guidelines suggest that IgAVN children with persistent proteinuria of >0.5–1 g/day/1.73 m^2 should be treated with angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARB), and children with GFR >50 mL/min/1.73 m^2 and persistent proteinuria >1 g/day/1.73 m^2, after a trial of renin–angiotensin system (RAS) blockade, should receive a 6-month course of corticosteroids (same as for IgAN). For cases with >50% crescents on biopsy, the guidelines recommend steroids and cyclophosphamide. If plasma creatinine is >500 μmol/L, oral prednisone should be preceded by 3 methylprednisolone (MP) pulses, and the patient should also receive plasma exchange. These guidelines are the same as for crescentic IgAN and ANCA vasculitis, and are independent of the patient's age [78]. However, there was concern by some pediatric nephrologists that the KDIGO guidelines might delay the initiation of effective treatment and increase the long-term risk of CKD due to undertreatment of IgAVN [80]. They argued

that (1) the guidelines suggested for adults and children with IgAVN are based on randomized, controlled trials performed in adults with IgAN. However, IgAVN and IgAN are different diseases and have different outcomes despite similarities; (2) it is very important to treat initial episodes adequately without delay in IgAVN, and following the KDIGO guidelines might delay a potentially more effective treatment, increase the risk of CKD progression in patients with ISKDC grade IIIa, and cause a higher cumulative exposure to immunosuppressive therapy; (3) cyclophosphamide was ineffective in the treatment of IgAVN in the randomized controlled pediatric trials of the ISKDC; and (4) the KDIGO guidelines do not suggest addition of immunosuppressive drugs to steroids in patients with <50% crescentic glomeruli, even in the presence of nephrotic syndrome and/or deterioration of GFR, but more aggressive treatments, such as MP pulses, other immunosuppressive drugs or plasma exchange, may be required in these patients [80].

There are three aspects regarding the treatment of IgAVN that warrant additional commentary. First, the highly heterogenous spontaneous evolution of IgAVN should be considered. Many children with severe proteinuria at onset achieve spontaneous remission, but some children presenting with mild proteinuria develop severe renal pathology and progress to renal failure in the long-term [44, 81]. Second, the choice of therapy should be driven by the purpose of treatment. In most glomerular diseases, proteinuria reduction is accepted as an adequate surrogate marker of renal disease remission [82]. In IgAVN, however, some long-term studies have shown that clinical remission may not uniformly translate into a favorable long-term outcome [49, 50]. One study showed a discrepancy between clinical remission and histological improvement [47]. Therefore, it may be important to induce histological regression in addition to reduction of proteinuria in treating severe IgAVN. Third, it should be considered whether treatment should be based on clinical presentation or renal pathology in IgAVN. Ronkainen et al. reported that the first renal biopsy did not predict the outcome of IgAVN. The outcome of patients with ISKDC grades II–III was worse than of those with grades IV–V, probably because the latter received more aggressive immunosuppressive treatment. The authors suggested that the treatment of IgAVN should be based on clinical presentation rather than biopsy findings [55].

Indications for Renal Biopsy

Although clear outcome-based indications for renal biopsy in IgAVN have not been established, biopsy is usually recommended in patients with (1) nephrotic syndrome, (2) nephritic syndrome, (3) decreased renal function, (4) nephrotic-range proteinuria, and (5) non-nephrotic proteinuria persisting for more than 3 months [22]. Although the utility of renal biopsy in IgAVN with persistent mild to moderate proteinuria may be controversial, Halling et al. argued for biopsy since severe morphological changes are found in some of these patients [44]. It is also important to recognize that the interval between disease onset and the time of renal biopsy may affect histopathological findings; the percentage of crescents may increase markedly within days in patients with active disease [39, 80]. Repeat renal biopsies should be considered in patients showing worsening of renal symptoms or poor response to treatment [80].

Treatment of Mild or Moderately Severe IgAVN

Patients with mild IgAVN, such as microscopic hematuria or gross hematuria of short duration, generally do not require any medications.

In IgAVN children with persistent proteinuria of 0.5–1 g/day/1.73 m^2, the KDIGO guidelines recommend the use of ACE inhibitors or ARB [78]. In a study of 31 patients with moderately severe IgAVN (ISKDC grade I–III and serum albumin >2.5 g/dL), proteinuria was reduced efficiently by RAS blockers, except a single case of a clinical relapse at one year [83]. Davin and Coppo suggested that the use of RAS blockers is appropriate in cases lacking acute inflammation and crescentic lesions, whereas delaying effective anti-inflammatory treatment may be detrimental in patients with acute inflammatory

glomerular lesions [80]. This is supported by two studies: one showed that 9 of 13 patients with mild to moderate proteinuria had severe morphological changes; the second showed that 18% of patients with mild proteinuria at onset had a poor prognosis [44, 81]. Hence, a rational therapeutic approach could be as follows: in patients with non-nephrotic proteinuria and a normal serum albumin early in the course of IgAVN, monotherapy with a RAS blocker may be used for 1–2 months and the further course observed. In patients with non-nephrotic proteinuria and mildly decreased serum albumin levels at onset of IgAVN, combined oral steroids and RAS blockade may be used for 1–2 months. In patients with persistent (>2–3 months) non-nephrotic proteinuria and/or decreased serum albumin levels during the course of IgAVN despite these treatments, renal biopsy should be performed and more potent anti-inflammatory treatments considered guided by the histopathological findings. These treatments may include MP pulses, azathioprine, mycophenolate mofetil (MMF) or a calcineurin inhibitor, combined with oral steroids and RAS blockade. The beneficial effect of alternative treatments such as fish oil, rifampin or tonsillectomy on moderately severe IgAVN has not been established [48, 84].

Treatment of Severe IgAVN

Although the definition of severe nephritis in IgAVN differs among studies, it generally includes nephrotic syndrome, acute nephritic syndrome, nephrotic range proteinuria (>40 mg/m^2/h) or proteinuria >1 g/day and histopathological lesions exceeding ISKDC grade IIIa [50]. Treatment of severe IgAVN remains controversial due to a paucity of RCTs [48, 84, 85]. Most published work relies on retrospective analyses of small cohorts with heterogenous disease severity. However, there is consensus that intense initial therapy is indicated in severe IgAVN, considering a 15% overall long-term CKD risk and well-documented unfavorable outcomes of untreated patients, reduced CKD risk following intense treatment, and worse outcomes with delayed treatment [86–89].

Oral steroids have been shown to be ineffective in severe nephritis [5, 87, 88]. Niaudet et al. suggested that intravenous MP pulses should be started early in the course of severe IgAVN before crescents become fibrous, because renal scarring by extensive glomerular damage during the acute episode may be irreversible and lead to progressive CKD [88]. Hence, in patients with very severe IgAVN, intravenous MP pulses should be initiated immediately. Oral steroids can be utilized and tapered following the MP pulses. In addition, RAS blockers can be used as an add-on therapy concurrently as a nephroprotective, proteinuria-minimizing therapy [80].

An RCT performed by the ISKDC showed no differences in outcome between oral cyclophosphamide (90 mg/m^2/day for 42 days) and supportive therapy in children with severe IgAVN [90], although retrospective case series had suggested a beneficial effect [91, 92]. A placebo-controlled, prospective study comparing cyclophosphamide plus prednisone to prednisone alone demonstrated the lack of efficacy of cyclophosphamide in adults with IgAVN [93]. In view of these negative results and the potential side effects, cyclophosphamide is not recommended in severe IgAVN [80].

Another randomized clinical trial in a limited number of children suggested that cyclosporin A was non-inferior to intravenous MP pulses in children with severe IgAVN. Resolution of nephrotic-range proteinuria was achieved within 3 months in all 11 cyclosporin-treated patients, while it was not achieved with the initial treatment of MP pulses in 6 of the 13 due to slower response [94]. Additional immunosuppressive treatment was not necessary in any of the cyclosporin-treated patients, but was needed in 6 patients treated with MP pulses [94]. Repeat renal biopsy findings performed after 2-year follow-up showed similar improvement in both treatment arms [94].

Hence, calcineurin inhibitors are an alternative first choice or follow-up therapy in patients who do not respond rapidly to intravenous MP pulses. Azathioprine, MMF or other immunosuppressive drugs can also be used, although the

claim of efficacy for these drugs is based exclusively on non-randomized or uncontrolled studies [39, 40, 95–102]. In a recent meta-analysis of 9 articles, IgAVN patients treated with combined therapy (immunosuppressive agents plus steroids) demonstrated a significant increase in complete remission rates when compared with steroids alone and children seemed to benefit more from combined treatment than adults [99]. Administration of azathioprine and cyclosporin was associated with histological regression with reduced IgA deposits in severe IgAVN [40, 97, 98]. In one study, rituximab was shown to be effective for induction and maintenance of long-lasting remission in adults with severe refractory IgAV with biopsy proven crescentic nephritis; 10 of the 12 patients had a complete response [100]. However, the role of rituximab in children with severe IgAVN requires additional study.

Persistence of urinary abnormalities is an ominous sign in severe IgAVN [40, 52]. Hence, in patients with persistent proteinuria after initial intensive therapy, a follow-up renal biopsy may be needed to assess the histopathological effects of initial treatment. Subsequent therapy may be guided by the extent and acute vs. chronic nature of the remaining renal lesions. If persistent active histological lesions are found, adjustment of the immunosuppressive therapy should be considered. Although rare, in persistent, severe IgAVN resistant to azathioprine late remission was induced by switching to cyclosporin or MP pulses [103, 104]. RAS blocker monotherapy is a valid approach in cases of persistent proteinuria with a high chronicity index at the follow-up renal biopsy [80]. Conversely, it should be emphasized that proteinuria can resolve spontaneously years after discontinuation of immunosuppression in patients with severe IgAVN [40, 98].

In patients presenting with impaired renal function with rapidly progressive course or crescentic IgAVN affecting more than 50% of glomeruli (ISKDC IV and V), several intensive therapies have been suggested, emphasizing that early treatment is important in achieving a successful outcome [54, 87–89, 105–112]. Plasmapheresis has been utilized for rapidly progressive IgAVN to remove circulating immune complexes, immunoglobulins and mediators of inflammation; it has been used either alone or with other immunosuppressive drugs [54, 89, 105–108]. Hattori et al. reported that plasmapheresis as the sole therapy was effective in improving the outcome of patients with rapidly progressive IgAVN, particularly if instituted early in the course of the disease [89]. Schärer et al. suggested that plasmapheresis might delay progression, albeit not prevent eventual ESRD in children with crescentic IgAVN [54]. Therefore, plasmapheresis can be utilized promptly in patients who have nephritic and nephrotic syndrome and progressive decline in kidney function associated with ISKDC IV or V, and in those who are resistant to steroids and other immunosuppressive drugs.

In addition, combinations of several drugs, including cyclophosphamide, have been tried in the setting of rapidly progressive IgAVN, although cyclophosphamide alone was not effective in treating severe IgAVN [109–112]. Öner et al. reported a beneficial effect of triple therapy (MP pulses, cyclophosphamide, dipyridamole) in 12 patients with rapidly progressive IgAVN [109]. Iijima et al. also suggested the efficacy of combined therapies (MP pulses, cyclophosphamide, heparin/warfarin, dipyridamole) in 14 patients with rapidly progressive IgAVN (ISKDC IV or V) [110].

It has been speculated that fibrinolytic urokinase treatment might decrease crescent formation by reducing glomerular fibrin deposition [72, 111, 112]. Kawasaki et al. found methylprednisolone and urokinase pulse therapy (MUPT) which includes methylprednisolone (MP) pulses, urokinase, warfarin, dipyridamole was effective in patients with rapidly progressive IgAVN [111]. Addition of cyclophosphamide to MUPT was more effective than MUPT alone in the treatment of rapidly progressive IgAVN [112].

The experimental nature of these therapeutic approaches should be emphasized. The relative efficacy of individual and combined treatments in severe and rapidly progressive IgAVN awaits demonstration in RCTs with strict inclusion

criteria and standardized treatment protocols. In addition, a prospective cohort study, Cure Glomerulonephropathy (CureGN), includes patients with IgAVN and is expected to provide insights on the course and prognosis of the disease and efficacy of various therapies [113].

Special Conditions

Renal Transplantation

IgAVN may recur after renal transplantation. In 1994, the risk of clinical recurrence was 35% and the risk of graft loss due to recurrence 11% at 5 years after renal transplantation [114]. A study published in 2011 showed lower post-transplant IgAVN recurrence rates and the risk of graft loss related to recurrence was 2.5% and 7.5% at 5 and 10 years, respectively [115]. Although the risk of graft failure due to recurrence is increased in IgAVN, the diagnosis of IgA vasculitis does not affect overall renal allograft survival [116–118]. Graft survival of IgA vasculitis was similar to that of renal hypoplasia or dysplasia [117]. Histologic recurrence is frequently observed on routine renal biopsies, but often does not cause clinical consequences [117]. The severity of IgAVN at presentation and the types of immunosuppression after renal transplantation did not affect the risk of IgAVN recurrence [115, 118].

Pregnancy

Women with a history of IgA vasculitis during childhood may be at increased risk for renal complications during pregnancy [22, 50, 119]. Ronkainen et al. reported that 16 (70%) of 23 pregnancies were complicated by hypertension, proteinuria, or both and 5 (56%) of the 9 women with complicated pregnancies had a poor renal outcome [50]. Therefore, close monitoring during pregnancy is recommended in women with a history of childhood IgA vasculitis.

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Metabolic Disorders Affecting the Kidney

28

Aude Servais, Olivia Boyer, Myriam Dao, and Friederike Hörster

Introduction

Inherited diseases of metabolism are a heterogeneous group of rare diseases, most often of autosomal recessive inheritance. These include energy metabolism disorders, intoxication diseases, and abnormalities in the synthesis or catabolism of complex molecules involved in intracellular maturation (lysosomal, peroxisomal, glycosylation abnormalities, etc.).

Kidney involvement may be inaugural, or more often complicate the evolution of an already diagnosed metabolic disorder. Improved survival in patients with metabolic diseases has led to the discovery of renal features that were not apparent at the time of the initial description of the condition (e.g., methylmalonic acidemia), and extrarenal manifestations in renal or urologic diseases that were thought to be primary and isolated (e.g., cystinosis). Metabolic disorders should be considered in the presence of kidney involvement, especially when children present with extrarenal symptoms.

The renal clinical spectrum of inherited diseases of metabolism is wide:

- Fanconi syndrome (proximal tubulopathy): the proximal renal tubule is most often affected in metabolic disorders due to a very high energy expenditure.
- chronic tubulointerstitial nephropathy, resulting from acute or chronic toxic tubular epithelium injury (e.g., myoglobinuria in fatty acid oxidation defects, methylmalonic acid in methylmalonic acidemia).
- glomerular damage with proteinuria, nephrotic syndrome, hematuria and/or hypertension, due to the abnormal deposition of storage material (e.g., Fabry disease), or structural defects (e.g., glycogenosis type 1, respiratory chain defects).
- hemolytic uremic syndrome, as a result of toxic damage to endothelial cells (e.g., in methyl-malonic acidemia vitamin B12-sensitive, or cobalamin C deficiency).
- nephrocalcinosis and urinary lithiasis, resulting from a defect in the reabsorption of a specific solute (e.g., cystine in cystinuria) or from the urinary excretion of solutes accumulated

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in the plasma (e.g., oxalate in primary hyperoxaluria).
– renal cysts as a result of developmental defects.
– urine colour abnormalities (e.g. dark or reddish in porphyria, black in alkaptonuria);
– renal consequences of rhabdomyolysis.

These pathologies can lead to chronic kidney disease (CKD) and sometimes to kidney failure requiring renal replacement therapy and kidney transplantation.

When the cause of the inherited metabolic disorder is unclear, the type of renal involvement can be suggestive (Table 28.1).

Table 28.1 Renal involvement in inherited diseases of metabolism

Clinical features	Etiologies
Proximal tubulopathy (Fanconi syndrome)	Cystinosis Mitochondriopathies Tyrosinemia type 1 CDG syndrome Fructosemia Galactosemia Glycogenosis type 1 Bickel-Fanconi syndrome Wilson disease Lowe disease Dent disease Lysinuric Protein Intolerance
Other tubular defects, chronic TIN	MMA Mitochondriopathies Glycogenosis type 1 Pyruvate carboxylase deficiency Carnitine palmitoyl transferase (CPT) type 1 deficiency Adenosine deaminase deficiency Transaldolase deficiency Fatty acid Beta-oxidation disorders Imerslund-Gräsbeck syndrome
Glomerulopathy Proteinuria, nephrotic syndrome nephrotic syndrome, haematuria and/or hypertension	Fabry disease Mitochondriopathies Glycogenosis type 1 Mucopolysaccharidosis (MPS) type 1 (Hurler) Lysinuric protein intolerance Gaucher disease LCAT (lecithine cholesterolacyl transferase) deficiency
Kidney cysts	Mitochondriopathies: Pearson syndrome Zellweger syndrome CDG syndrome Glutaric acidemia type II Smith-Lemli-Opitz syndrome

Methylmalonic Acidemia and Propionic Acidemia

Methylmalonic acidemias comprise a family of diseases which share the common feature of elevated concentration of methylmalonic acid in blood, urine and other body fluids. There are secondary forms related to vitamin B12 deficiency and different primary diseases of cobalamin metabolism and methylmalonyl-CoA mutase deficiency leading to this biochemical phenotype. It is useful to differentiate between isolated methylmalonic acidurias and combined methylmalonic acidurias, which additionally show elevated concentrations of homocysteine (Table 28.2) [1–3].

Methylmalonyl-CoA mutase is located within the metabolic pathway linking degradation of certain aminoacids (isoleucine, methionine, threonine and valine), odd-chain fatty acids and cholesterol side chains to the citric acid cycle. Propionic acidemia is caused by deficiency of propionyl-CoA carboxylase, an enzyme upstream

Table 28.2 Methylmalonic acidemia and propionic acidemia

	Gene	MIM number
Methylmalonic acidemia: isolated forms		
mut type (*mut*^{0}, *mut*$^-$)	*MUT*	#25100
cblA type	*MMAA*	#251100
cblB type	*MMAB*	#251110
cblD variant 2	*MMADHC*	#277410
Methylmalonic acidemia: combined forms with homocystinuria		
cblC type	*MMACHC*	#277400
cblD-variant 1	*MMADHC*	#277410
cblF type	*LMBRD1*	#277380
cblJ type	*ABCD4*	#614857
Propionic acidemia	*PCCA* *PCCB*	#606054

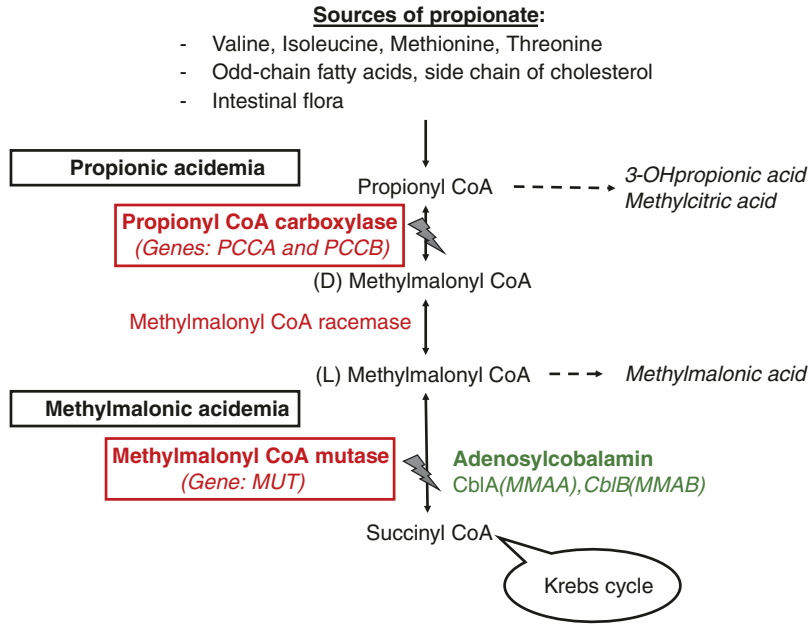

Fig. 28.1 Methylmalonic acidemia and propionic acidemia

of methylmalonyl-CoA mutase (Fig. 28.1). These diseases are the most frequent classical organoacidemias with an estimated incidence of 1:50,000 (isolated methylmalonic acidemias) and 1:150,000 (propionic acidemia). Within the group of isolated methylmalonic acidemias, most patients are affected by a complete deficiency of methylmalonyl-CoA mutase (mut^0) [1].

Clinical Picture of Isolated Methylmalonic Acidemias

Acute manifestation is a life-threatening metabolic decompensation characterized by profound metabolic acidosis, ketosis and hyperammonemia leading to deep coma and death, if not treated adequately. Most patients manifest within the neonatal period, the others later in life. Metabolic crises are often preceded by a catabolic state, typically induced by minor infections. A severe crisis can lead to irreversible neurologic sequelae such as basal ganglia necrosis, epilepsy or mental retardation, but a growing number of patients show normal psycho-motor development. Apart from the development of chronic renal failure (see below), failure to thrive and recurrent vomiting often complicate the clinical course. Acute or chronic pancreatitis are rarer but potentially dangerous complications.

If suggestive clinical symptoms occur, the diagnostic work-up of a patient can be started by measurement of acylcarnitines in dried blood spots or directly by measurement of organic acids in urine [4]. The differential diagnosis of the several genetic defects is actually performed by molecular genetic testing, but several different genes have to be considered [4].

Disease severity differs considerably between subtypes: late onset patients who are responsive to hydroxocobalamin tend to have a better outcome if treated adequately, in contrast to patients affected by a complete deficiency of methylmalonyl-CoA mutase (mut^0) [5–7]. Synergistic impairment of different targets within mitochondrial energy metabolism by different metabolites is the key to understand the pathophysiology of these diseases as multisystem disorders [8–10].

Clinical Picture of Combined Methylmalonic Acidurias

The most common disorder in this group is *cblC* disease (more than 500 reported cases). The majority of patients has an early disease onset, which is defined as disease onset before 12 months of age. In addition to acute metabolic crisis, patients may show signs of prenatal damage (intrauterine growth retardation and/or congenital heart disease) and thrombotic microangiopathy (atypical hemolytic uremic syndrome). Visual impairment and nystagmus due to optical nerve atrophy and retinopathy are typical complications within the disease course. Patients develop a complex neurological phenotype with brain atrophy and white matter disease leading to developmental delay, psychiatric problems and peripheral neuropathy.

Elevated concentration of plasma homocysteine levels is the biochemical hallmark of the disease: usually homocysteine levels are below 50 μmol/L (depending on the individual laboratory cut-offs), but in untreated patients levels above 100 μmol/L are found. It is recommended to measure total homocysteine in blood [3]. For further diagnostic work-up, investigation of plasma amino acids to identify low levels of methionine and organic acids in urine to identify elevated levels of methylmalonic acid are recommended. To rule out deficiency, plasma levels of vitamin B12 and folate should also be investigated [3]. Molecular genetic analysis of the *cblC* gene is possible and the three most common mutations show a genotype-phenotype correlation according to early or late disease onset [3].

Kidney Involvement

Chronic kidney disease (CKD) is a common complication of MMA, manifesting in childhood in half of the patients [5, 7, 11]. It is most frequently observed in mut° and cblB phenotypes, less frequently in patients with cblA and mut- phenotypes [12]. Median age at onset varies from 6.5 to 11.9 years (range: 1.5–33 years). Twelve to fourteen percent of patients evolve to end-stage renal disease (ESRD) requiring renal replacement therapy [5, 7]. The mechanisms responsible for renal failure in MMA remain poorly understood [5, 8, 13, 14]. It has been suggested that CKD in MMA is the consequence of tubular dysfunction. The hypothesis of chronic tubulopathy is supported by experimental studies [8, 13, 14].

A recent experimental study demonstrated a link between MMA, diseased mitochondria, mitophagy dysfunction and epithelial stress in tubular renal cells [15]. However, only few clinical case reports found proximal tubulopathy or distal tubular acidosis type 2 [16–18]. Kidney biopsy studies showed severe interstitial fibrosis and tubular atrophy with ultrastructural (enlarged mitochondria in proximal tubules) and functional (loss of cytochrome C, decrease in NADPH activity) alterations [7, 13, 16, 19] (Fig. 28.2). A recent study investigated tubular functions in 13 adolescent and adult MMA patients who did not previously receive kidney and/or liver transplantation [20]. The authors confirmed the high prevalence of CKD (54% had mGFR below 60 mL/min/1.73 m^2) but they failed to demonstrate tubular involvement. No patient had complete proximal tubular syndrome. Only one patient had biological signs suggestive of incomplete proximal tubulopathy with increased β2-microglobulin excretion and renal loss of magnesium [20].

As all patients with isolated MMA, even in the moderate forms, are considered to be at risk of developing chronic kidney disease (CKD), renal function should be closely monitored. There is a risk of developing progressive kidney failure, requiring renal replacement therapy and kidney transplantation. The estimated glomerular filtration rate (eGFR) based on serum creatinine levels

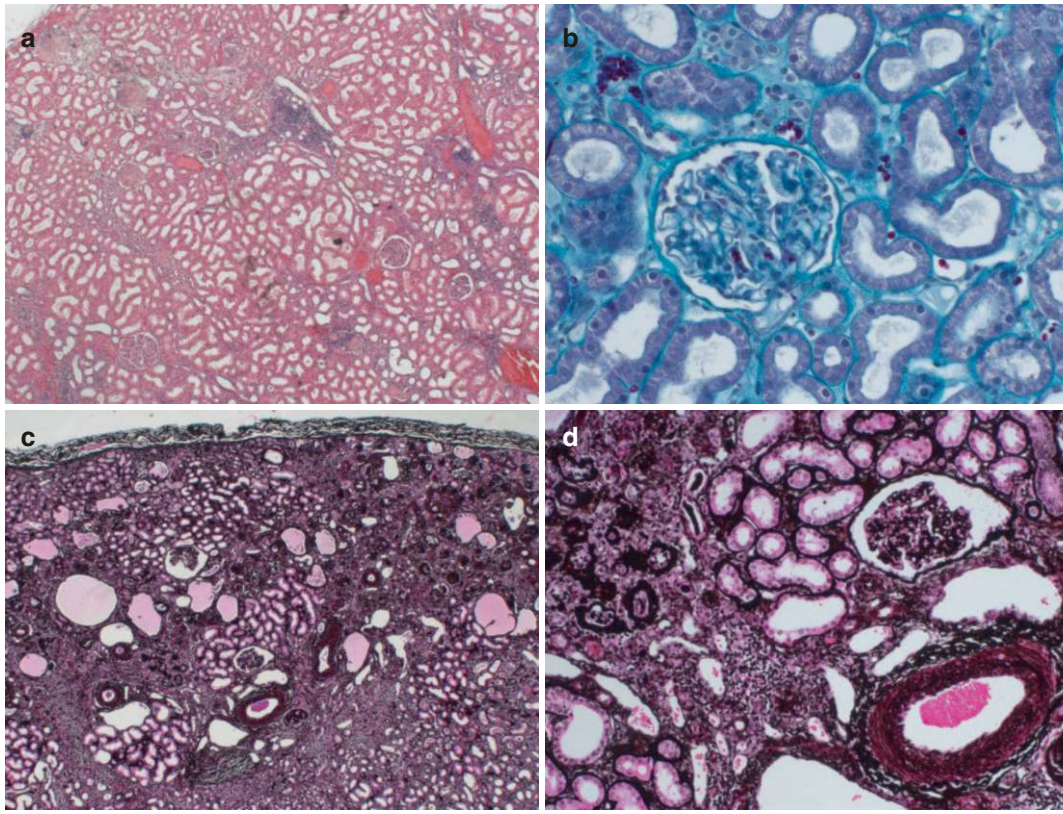

Fig. 28.2 Methylmalonic acidemia (MMA) renal biopsy. Two nephrectomy specimen procured at the time of transplantation in children with MMA at different stages of chronic kidney disease. The first specimen (**a** and **b**) shows non-specific mild tubular atrophy with interstitial fibrosis (IF/TA) and moderate inflammation within fibrotic areas (**a**) (HES staining, ×250). (**b**) Glomeruli are unremarkable and proximal tubules shows vacuoles in epithelial cells (Masson Trichrome, ×500). The second specimen (**c** and **d**) shows severe IF/TA with ischemic glomeruli (**c**) and tubular dilatations (Jones staining, ×250). Arteries display thickening of the media (**d**) (Jones staining, ×500). (Picture from M Rabant and MC Gubler)

may overestimate the actual GFR due to the low muscle mass and protein intake of patients [20]. Therefore, it is recommended to measure the GFR using "gold standard" techniques such as the inulin or iohexol clearances, when therapeutic decisions such as dialysis or transplantation are discussed.

The cumulative urinary excretion of methylmalonic acid over time (measured in repeated urine samples) correlates with the risk of CKD [5]. However, as kidney function declines, urinary methylmalonic acid is no longer a reliable marker and must be replaced by measurement of plasma methylmalonic acid. Standard medical management and follow-up of CKD follows the general therapeutic principles established in patients without inborn errors of metabolism, including monitoring of blood pressure, electrolytes, prevention and management of anemia, growth and bone disease. The experience with nephroprotective measures in the paediatric population has not been extensively studied.

In PA, a few cases of kidney failure have been reported [21].

Conservative Treatment

Isolated Methylmalonic Acidemias

The classical treatment consists of protein restricted diet (limited intake of precursor amino acids), which has to be tailored to the patients' needs and carefully monitored. L-carnitine (100 mg/kg/day) is applied to restore carnitine levels and CoA levels, bind and eliminate propionyl-CoA molecules, which are assumed to be responsible for some of the toxic metabolite effects in MMA [4]. Hydroxocobalamin is a powerful therapeutic tool in responsive patients; therefore this option has to be carefully evaluated [6]. In the recent guideline, a protocol for patients' monitoring has been proposed (Table 28.3) [4].

Table 28.3 Suggested monitoring in isolated methylmalonic acidemia and propionic acidemia according to guideline (adapted from [4])

Assessment	Frequency
Metabolic follow-up	
NH3, blood gas, lactate	Each clinic visit
Quantitative plasma amino acids (3–4 h of fasting prior to sample collection)	3–6 monthly
Methylmalonic acid in plasma (and urine if available)	3–6 monthly
Acylcarnitine profile in dried blood or plasma (propionylcarnitine and free carnitine)	3–6 monthly
Diet and nutritional status	
Diet history	Each clinic visit
Growth (weight, length or height, head circumference)	Each clinic visit
Full clinical examination	Each clinic visit
Albumin, total protein	6-monthly
Bone health (Ca, P, ALP, Mg, PTH, 25-OH vitamin D in blood; Ca, P in urine)	12-monthly
Full blood count, ferritin, folic acid, vitamin B12	12-monthly
Long-term complications	
Neurological examination with assessment of developmental milestones	Each clinic visit
Kidney function (serum creatinine, electrolytes, cystatin C, uric acid; urinary electrolytes and protein loss; GFR)	6-monthly
Pancreas function (lipase, pancreatic amylase)	6-monthly
Cardiac assessment (ECG, echocardiography)	12-monthly
Formal developmental/cognitive assessment	When clinically indicated
EEG, cerebral MRI	When clinically indicated
Ophthalmologic assessment	12-monthly
Formal hearing test	When clinically indicated

Combined Methylmalonic Acidurias

Experience on treatment mostly derives from cblC patients, the most common disorder. Treatment aims at normalizing plasma methionine and methylmalonate levels and reducing plasma total homocysteine. In severe cases levels of total homocysteine cannot be normalized, but levels between 40 and 60 μmol/L can be reached [3].

Treatment consists of hydroxocobalamine parenterally and betaine [3]. This treatment reduces homocysteine and methylmalonate levels and prevents metabolic crisis, but visual and cognitive impairment may not improve [3, 22].

Kidney and Liver Transplantation

The long-term outcome of MMA remains poor with medical treatment. Organ transplantation is an alternative, especially in case of renal failure but also as an enzyme replacement therapy. However, it remains unanswered exactly which patients should be transplanted. Liver transplantation (LT), kidney transplantation (KT), or combined liver and kidney transplantation (LKT) have been proposed for renal failure or as an enzyme replacement therapy in case of frequent metabolic decompensations, but also to prevent long-term complications [23]. Several reports emphasized the improved quality of life after LT [23].

The metabolic results after LKT are dramatically better than after KT, reducing protein restriction and improving quality of life. However, it is associated with an increased frequency of adverse events. Global patient survival after transplantation is calculated at 86–87% [23, 24], similar to non-transplanted patients and to patients receiving transplantation for other indications [24]. Mortality risk is highest within 14 days after transplantation. To decrease the mortality risk, it is recommended to ensure a stable metabolic state at time of transplantation and to have transplantation performed by an experienced transplantation team [24]. Some patients experience post-LKT neurological disorders, mainly reported in Mut0 type MMA [23, 24]. This might be due to calcineurin inhibitor toxicity [25].

Early LT may be considered to prevent years of protein deprivation and MMA toxicity and to delay the need for KT. LKT should be proposed when the measured GFR is lower than 60 mL/min/1.73 m^2 [23]. However, an isolated KT can be individually discussed.

Lysinuric Protein Intolerance

Lysinuric protein intolerance or cationic aminoaciduria is a rare autosomal recessive disorder. Affected children come to medical attention soon after weaning with failure to thrive and episodes of altered consciousness caused by hyperammonemia. This clinical picture is similar to urea cycle disorders, which are an important differential diagnosis. The disease may also present with chronic digestive symptoms and failure to thrive. Hepatosplenomegaly is often present [26]. Later, patients develop hematologic complications and bone marrow abnormalities reminding of lymphohistiocytosis or biological macrophage activation syndrome. This is accompanied by immunologic abnormalities and auto-immune manifestation. The respiratory system may also be involved by pulmonary alveolar proteinosis, which is a severe life-threatening complication.

The defect in the transporter leads to a characteristic pattern in urine amino acids, which show elevated concentrations of arginine, lysine and ornithine and low levels of these amino acids in plasma. Orotic acid in urine may also be elevated. Classically ferritin and LDH levels are elevated in plasma. Molecular genetic analysis of the *SLC7A7* gene is used to confirm the diagnosis, but there is no clear genotype-phenotype correlation [26].

Kidney Involvement

Renal involvement is described in LPI patients with both tubular and glomerular abnormalities [27]. In the first large clinical series describing kidney involvement in LPI, 74% of patients presented with proteinuria, 38% with microscopic or macroscopic hematuria and 38% with renal failure [28]. Patients may progress to ESKD [26–28]. Severe anemia and increased bleeding may be observed in these patients [28].

The majority of patients with kidney involvement have tubular dysfunction [27–29]. The most common observation is nephrocalcinosis and chronic tubulointerstitial nephritis [27, 30]. This

is probably due to the accumulation of calcium phosphate in the kidney secondary to hypercalciuria and possibly to proximal tubular acidosis [27]. Hyperechogenic kidneys may be observed by renal ultrasound. Fanconi syndrome has been reported in two independent cases [31, 32].

Glomerular involvement is quite variable. Mesangial thickening can be observed [27]. Focal glomerulosclerosis, mesangial, membranous and lupus-like proliferative glomerulonephritis can also be associated with LPI [33–36]. Another striking observation is the finding of amyloidosis in the liver and in kidneys of some patients.

Treatment

To maintain metabolic control age-adapted dietary protein restriction is applied and patients have to be carefully monitored for nutritional deficiencies. Citrulline is supplemented and ammonia scavenging drugs, which are used in other urea cycle disorders, are also applied [37].

Kidney Transplantation

The disease does not prohibit treatment by transplantation. Better metabolic control is observed after transplantation.

Fabry Disease

Fabry disease is an X-linked lysosomal storage disorder caused by mutations in *GAL*, the gene that encodes the lysosomal enzyme α-galactosidase A, leading to deficient activity of this enzyme. The disease is characterized by a progressive accumulation of globotriaosylceramide (Gb-3) and related glycosphingolipids in the plasma and different cell types. Male individuals are primarily affected, but female heterozygotes may display moderate or severe disease, which is likely related to the pattern of X-chromosome inactivation [38]. The disease can be divided into a severe, classical phenotype, most often seen in men without residual enzyme activity, and a generally milder non-classical phenotype. Patients with classical Fabry disease initially present with characteristic Fabry disease symptoms, such as neuropathic pain, cornea verticillata, and angiokeratoma.

Once suspected, men are diagnosed with Fabry disease if enzyme activity is below 35% of the mean and gene sequencing. The gold standard of diagnosis for females is α -Gal gene sequencing only.

The Gb3 degradation product, globotriaosylsphingosine (lysoGb3), is currently used in disease screening, but also in the determination of pathogenicity of a mutation. LysoGb3 has also been accepted as an accurate marker of disease activity [39].

Fabry Disease in Children and Adolescents

Children may present with characteristic neuropathic pain, which may lead to the diagnosis. Overt kidney involvement is rare in children, but renal histologic lesions can be demonstrated in children even before the onset of overt proteinuria and CKD [40, 41]. The natural history of Fabry nephropathy has not yet been thoroughly assessed in children [42]. Initially, glomerular hyperfiltration may mask impairment of kidney function [43]. Patients may present with microalbuminuria and proteinuria during the second or third decades, and CKD has been scarcely reported in adolescents [40–42, 44, 45].

Renal Biopsy

Renal biopsy may be performed for diagnosis in case of presentation with glomerular disease without extrarenal manifestation or for therapeutic discussion. On renal biopsy, vacuolization of podocytes and epithelial cells is a

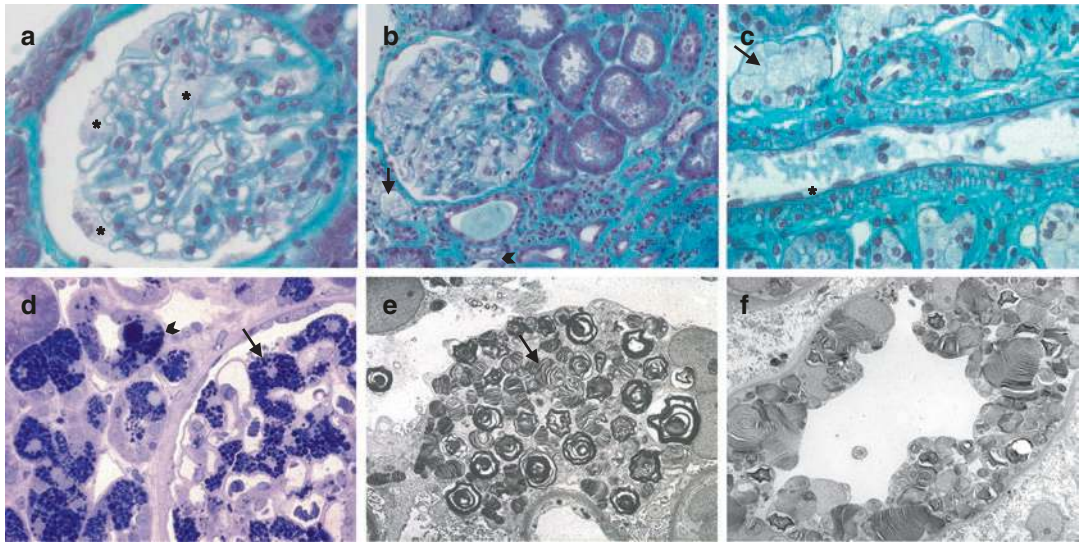

Fig. 28.3 Fabry disease renal biopsy. (**a**) Glomerulus with typical vacuolizations of podocytes (star) (Masson Trichrome ×400). (**b**) Masson Trichrome (×200) showing tubular inclusions (arrow) in distal tubules as well as peritubular capillaries inclusions (short arrow). (**c**) Vascular section showing endothelial cells inclusions (star) associated with tubules inclusions (arrow) (Masson Trichrome ×400). (**d**) Toluidine blue-stained semi-thin section showing darkly stained round inclusions in podocytes (arrow) and distal tubular cells (short arrow). (**e** and **f**) Electron microscopy showing glycosphingolipid inclusions shaped as multilamellated myelin figures in podocytes (**e**) and tubular cells (**f**). (Picture from M Rabant and MC Gubler)

characteristic histologic finding (Fig. 28.3) [46]. Tubular as well as peritubular capillaries inclusions, and endothelial cells inclusions are also observed. By electron microscopy, glycosphingolipid inclusions shaped as multilamellated myelin are found in podocytes (Fig. 28.3). Mesangial expansion, segmental and global glomerulosclerosis, tubular atrophy and interstitial fibrosis are also seen, even in early stages of the disease.

Long Term Follow-Up: Renal and Extra-Renal Complications

Long–term disease manifestations include hypertrophic cardiomyopathy, cardiac rhythm disturbances, progressive renal failure, and stroke. Non-classical Fabry disease, also referred to as late-onset or atypical Fabry disease, is characterized by a more variable disease course, in which patients are generally less severely affected and disease manifestations may be limited to a single organ.

Therapy

Treatment of Fabry disease consists of enzyme replacement therapy or chaperone molecule and adjunctive treatment, including angiotensin converting enzyme inhibitors or angiotensin receptor blockers, and analgesics. Studies have shown that enzyme therapy can delay but not always prevent some of the clinical complications of the disease. Enzyme therapy has been shown to provide the greatest benefit to patients if started early on. Antibody formation possibly also contributes to reduced therapy effect. Migalastat is a small-molecule chaperone which facilitates enzyme trafficking to lysosomes in certain mutant enzymes and can be administered orally [47, 48].

Glycogen Storage Disease (GSD) Type 1a and 1b

Glycogen Storage disease type 1 is caused by a mutation of the *G6PC* gene (GSD 1a) or *SLC37A4* gene (GSD 1b) leading to two distinct clinical subtypes. The first manifestations mostly leading to further medical work-up are hypoglycemic episodes occurring at the age of 3–6 months 3–4 h after feeding, hepatomegaly and failure to thrive. The laboratory findings (combination of hypoglycemia, metabolic acidosis, hyperuricemia, elevated lactate and triglycerides) are suggestive. The diagnosis is confirmed by molecular genetics. In GSD 1b additional neutropenia and leucocyte dysfunction render the patients susceptible to bacterial infections.

In patients with GSD I, several renal complications have been reported. Enlargement of the kidneys is the earliest finding, caused by accumulation of glycogen in the kidneys. Because of hyperuricemia, uric acid lithiasis may occur. These complications can be prevented by an optimal metabolic control with diet and by xanthine oxidase inhibitor. Another cause of lithiasis may be the decreased urinary citrate excretion together with an increased urinary calcium excretion in GSD I patients. Proximal tubular dysfunction has also been described in patients with GSD I. Hyperphosphaturia and loss of bicarbonate in urine may lead to tubular acidosis. The main renal complication is glomerular hyperfiltration and persistent proteinuria. Renal biopsies performed in three GSD I patients with persistent proteinuria showed focal segmental glomerulosclerosis [49]. These findings suggest an etiology of glomerular hyperfiltration and proteinuria similar to diabetic nephropathy.

The treatment aims at avoiding hypoglycemia by frequent carbohydrate intake. Optimal metabolic control has a renoprotective effect on the development of microalbuminuria and proteinuria. Treatment with an angiotensin converting enzyme inhibitor significantly decreases the GFR in GSD I patients with glomerular hyperfiltration and reduces proteinuria [50].

In GSD 1b bone marrow stimulation by G-CSF is often necessary [51].

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Primary Hyperoxaluria

29

Bodo B. Beck, Cristina Martin-Higueras, and Bernd Hoppe

Introduction

The **primary hyperoxalurias** (PH) are a group of rare disorders in hepatic glyoxylate metabolism that result in excessive endogenous oxalate generation, their biochemical hallmark [1–4]. The PH's *per se* are not renal diseases, but inborn errors of metabolism that usually first manifest within the kidney and genitourinary tract as recurrent urolithiasis and/or nephrocalcinosis (Fig. 29.1).

Currently, three distinctive types of PH are known. They were termed PH type 1 to 3 in the order of their identification. All are inherited in an autosomal-recessive mode and each type is caused by a single enzymatic defect in the hepatic glyoxylate metabolism that induces overproduction of oxalate [1, 2]. The level of urinary oxalate excretion describing the primary range is commonly defined as greater ≥1 mmol/1.73 m^2/day (normal is <0.5 mmol/1.73 m^2/day) (Tables 29.1 and 29.2).

PH type 1 (PH1) is the most frequent and most devastating PH subtype that regularly leads to end stage kidney disease (ESKD) from infancy to late adulthood. PH type 2 (PH2), which is much less frequent in Europe and North America, bears a considerable risk of chronic kidney disease (CKD) (50%) and ESKD (25%) in adulthood [6]. The third type of PH (PH3) is the second most common and, according to recent literature, also shows a certain risk of CKD (20% of patients ≥CKD stage 2), while ESKD has been reported in few patients [2–4, 7–10]. While PH is rare, idiopathic calcium-oxalate urolithiasis is a frequent condition that bears in comparison only a modest risk of renal impairment. However, patients with severe secondary hyperoxaluria, especially those with Crohn's disease and status post ileocecal resection, are also prone to CKD and subsequently ESKD [11].

Humans, like all mammals, cannot metabolize oxalate. Whatever amount of oxalate is produced in the organism (physiologically or at increased levels in states of primary hyperoxaluria) or absorbed from the gastrointestinal tract (physio-

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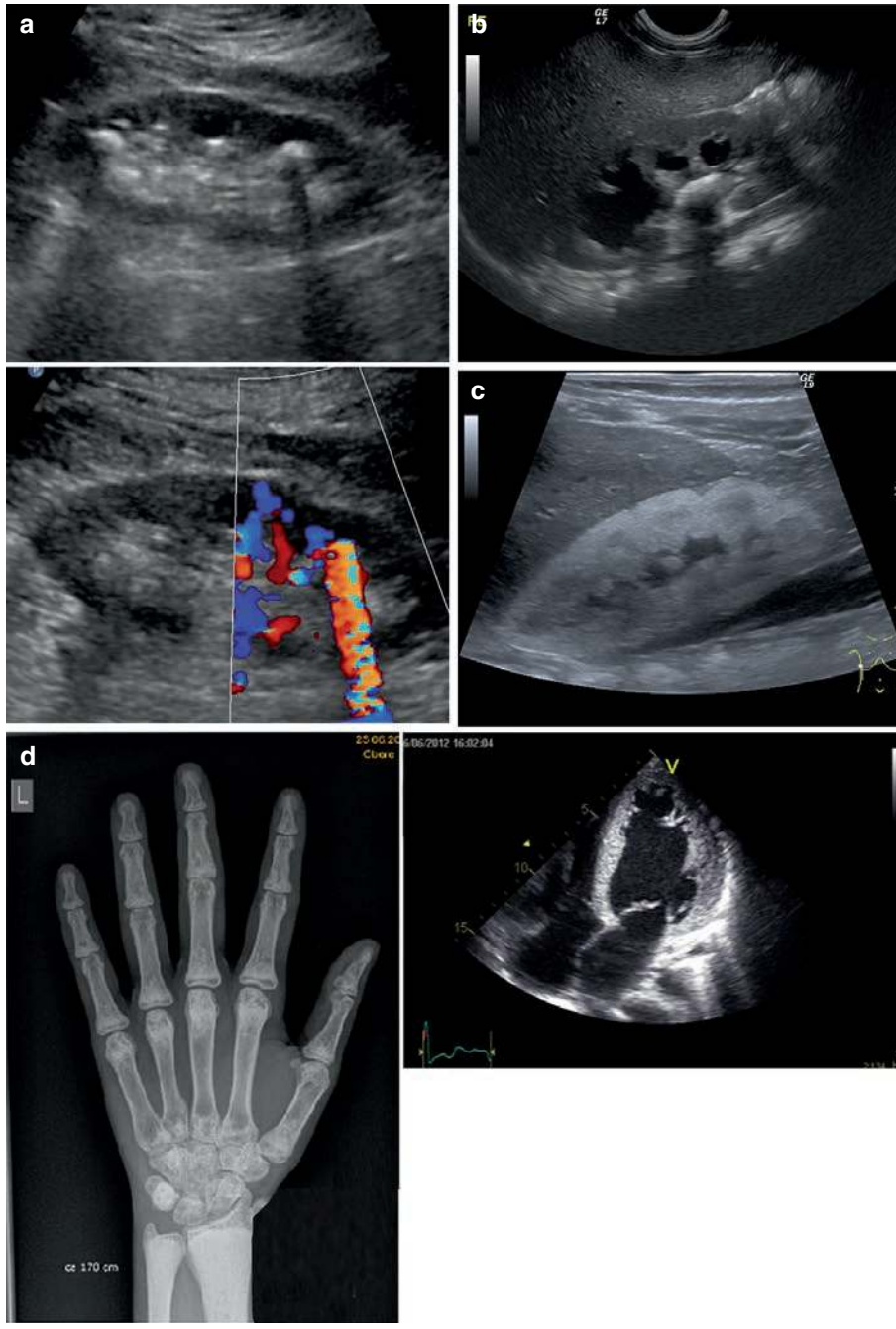


Fig. 29.1 Hallmarks of the primary hyperoxalurias. (**a**) Multiple kidney stones (with positive twinkling sign) in a 21-year-old patient with primary hyperoxaluria type 1 and recurrent stone passages. Stable kidney function with hyperhydration, vitamin B6 and alkaline citrate medication (eGFR = 80.9 mL/min). (**b**) Staghorn calculus in a 7-month-old boy with primary hyperoxaluria type 3. Repeated stone passages in the following months, now, aged 6 years, one not growing stone in the right kidney, no further stones passage. (**c**) Generalized nephrocalcinosis in a 16-month-old girl with primary hyperoxaluria type 1. (**d**) Oxalate osteopathy and cardiac manifestation of systemic oxalosis in a 24-year-old female PH1 patient. Renal replacement therapy since 5 years, plasma oxalate levels >100 μmol/L before hemodialysis. Extreme hyperechogenicity of left ventricular mass depicting severe calcium-oxalate deposition

Table 29.1 Synopsis of clinical features and characteristics of PH1–3

PH type	Clinical presentation	ESKD risk	Infantile oxalosis	Systemic oxalosis	Clinical remission
1	UL, NC, UTI, hematuria, failure to thrive	>50–100%	10–20% of cases	+++ in case of advanced CKD/ESKD	None
2	UL, UTI, hematuria NC↓	ca. 25%	Not reported	+ reported	None
3	UL, UTI, hematuria NC↓↓ is reported	CKD <20%, ESKD reported	Not reported	(+) uncommon	Stones also in adulthood

UL Urolithiasis, *NC* nephrocalcinosis (NC↓ indicates less frequent plus less severe NC in PH2; NC↓↓ indicates that (severe) nephrocalcinosis is a rare finding in PH3), *UTI* Urinary tract infection, *CCR* complete clinical remission, *ESKD* end-stage renal disease

Table 29.2 Synopsis of biochemical features in PH1–3

PH type	Degree of hyperoxaluria	Concomitant/ intermittent hypercalciuria	L-glycerate excretion	HOG/ DHG/4OHGlu excretion	Persistence of hyperoxaluria	Glycolate excretion
1	+++	−−− (rare)	−−− (normal)	−− (normal)	+++ (HO)	+++ (high)
2	++	−−− (rare)	+++ (high)	−−− (normal)	+++ (HO)	−− (normal)
3	++	Rare	−−− (normal)	+++ (high)[a]	++ (HO; 80% cases)	−− (normal)

HOG 4 Hydroxy -2- oxoglutarate, *HO* Hyperoxaluria

[a] It was recently shown by Pitt at all that individuals with PH3 excreted increased levels of HOG (2,4 dihydroxyglutarate, DHG, the reduced form of HOG) and 4-hydroxyglutamate (4OHGlu) the immediate HOG precursor [5]

logically or at increased levels in conditions associated with secondary forms of hyperoxaluria) has to be excreted via the kidneys in order to avoid the potentially deleterious accumulation of this metabolic end product [1]. Oxalic acid is found in virtually all dietary products in variable quantities depending on the source and processing of the product. Particularly high amounts can be found in some leafy green vegetables and legumes (beetroot, rhubarb, spinach, black tea, black chocolate, peanuts, sweat potatoes etc.) [12]. The oxalate content of a balanced Western diet constitutes in general no major medical issue unless in conditions prone to secondary hyperoxaluria (SH) such as malabsorption states in inflammatory bowel disease, short bowel syndrome, cystic fibrosis, celiac disease and many more [11, 13]. SH can also result from selective overconsumption of oxalate rich food or intoxications with precursor compounds (e.g. ascorbic acid, ethylene-glycol, glycolate, hydroxyproline) that are further metabolized into oxalate [14].

PH (rare, usually not amenable to dietary restrictions) must be differentiated from SH (more frequent and diverse, potentially/partially treatable by dietary restriction of glyoxylate precursors) for diagnostic and therapeutic reasons, which can sometimes be challenging [15]. Here, 24-h urine collections under different dietary regimens (low oxalate and high oxalate diets) can help to better distinguish PH from SH [16].

In principle, the main physiologic route of excretion via the kidneys allows the excretion of large quantities of this metabolic end-product. However, the poor solubility of oxalate and the concentration of the urine along the tubular system factually limit the amount of calcium oxalate that can be excreted without causing harm to the kidney [1, 2]. In primary or secondary hyperoxaluria oxalate levels are increased to 1.5-fold to >10-fold the upper level of normal, which may lead to the precipitation of insoluble calcium oxalate (CaOx) salts in the tubular lumen and interstitial tissue [17]. Interstitial deposition of CaOx induces a strong inflammatory reaction that leads to a progressive decline in kidney function and finally ESKD [18, 19].

Once renal function has declined below a certain glomerular filtration rate (GFR) threshold of approximately 30–40 mL/min/1.73 m^2, continuous systemic accumulation of oxalate occurs. This is indicated by a rise in plasma oxalate concentration (POx; normal <7.4 μmol/L, depending on the analytical method) [20] that can vastly

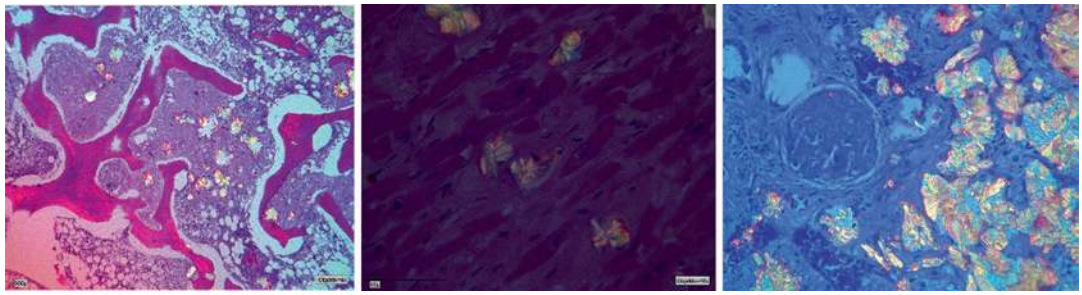


Fig. 29.2 Systemic oxalosis in a female PH type 1 patient with end stage renal failure since 3 years, chronic hemodialysis treatment and delayed diagnosis until skin biopsy revealed massive calcium-oxalate crystal deposition at age 33 years. Here, birefringent calcium-oxalate crystals are shown in polarized microscopy (from left) in bone marrow (leading to treatment resistant anemia), heart (leading to arrhythmias), and kidney (leading to end stage renal failure [21, 22]). Patient died due to severe systemic oxalosis shortly after diagnosis. It is to mention, that diagnosis was primarily not considered although her sister had received a combined liver-kidney transplantation because of PH1

exceed plasma saturation (about 30 μmol/L) and lead to a second phase of severe multisystem injury characterized by CaOx deposition in virtually all tissues (bone, retina, myocardium, vessel walls, skin etc.), termed systemic oxalosis (Figs. 29.1 and 29.2) [1, 2, 23]. Particularly bone deposition can be massive, which results in debilitating pain and fractures, as well as treatment resistant anemia. After successful kidney transplantation large amounts of oxalate can be mobilized from the bone compartment, which can pose a serious threat to the kidney graft [1, 2]. Severe systemic oxalosis in a strict sense is mainly observed in PH1, but it also has been reported in a few individuals with PH2 [6, 24]. So far it has not been reported for PH3, but this group is less well studied regarding the long-term consequences of hyperoxaluria and risk for CKD.

Interestingly, in non-PH ESKD patients a substantial contribution of elevated POx to the pro-inflammatory state and increased cardiovascular risk of the latter population has recently been suggested [23, 25]. In view of the vascular consequences of longstanding hyperoxalemia in PH1, further research addressing the role of POx for cardiovascular outcomes in the general ESKD population appears warranted.

A novel therapeutic concept is based on RNA interference (RNAi). This alternative approach focuses on depleting the messenger RNA of key enzymes in hepatic glyoxylate metabolism that are responsible for generating oxalate precursors or substrate for the defective AGT. The first approved RNAi drug is Lumasiran (Oxlumo©), which silences liver-specific glycolate-oxidase (gene symbol *HAO1*). Depletion of glycolate oxidase mRNA results in a reduction of glyoxylate in the peroxisomal compartment. Another RNAi therapeutic, Nedosiran, targets the liver-specific isoform of L-lactate dehydrogenase (LDHA) and therefore blocks the final step of oxalate production in the liver.

Epidemiology of Primary Hyperoxaluria

The PHs are a spectrum of rare, but most likely underreported diseases, as evident by the high rate of late diagnosis in PH1 in many countries. The true prevalence of PH1–3 is unknown. The estimated prevalence of PH1, the most commonly diagnosed PH form worldwide, is 1 to 3 cases per million population and the incidence rate is approximately 1 case per 100,000 live births in Europe and North America [26–33]. Extrapolation from the allelic frequencies of pathogenic PH1–3 sequence variants found in gene bank data yields much higher prevalence estimates for all primary hyperoxaluria subtypes than reported in the registries. For example, the allelic frequency of the most prevalent patho-

genic PH3 variants would predict about 2000 patients in Germany (estimated prevalence ca. 1:40 000), which is about 20 times the number currently identified throughout Europe (n = 95, in the latest report on PH3 by the OxalEurope registry) [10]. The low number of adult patients identified with PH3 points towards a markedly reduced penetrance and/or high rate of clinical remission with age in this incompletely understood form.

PH1 accounts for roughly 1% of childhood ESKD in many industrialized countries with a low rate of consanguineous marriages. A higher prevalence is observed in countries in which parental consanguinity is more common. PH1 is reported as the cause of pediatric ESKD in up to 10% of cases in some Middle Eastern and North African countries [34, 35]. The two largest PH registries (OxalEurope and the Rare Kidney Stone Consortium (RKSC)) have identified >1500 patients with proven type 1 disease [28, 30].

Most data discussed in this chapter is derived from populations of European ancestry. It should be kept in mind that the distribution of PH types, causative mutations and their allelic frequency might vary widely among different populations.

Primary Hyperoxaluria Type 1

Biology and Molecular Basis

The first clinical description of PH1 is attributed to the French physician Lepoutre who in 1925 reported extensive CaOx crystal tissue depositions in the kidney of an infant [36]. Later, the biochemical hallmark of massive oxalate excretion in the urine was monitored by various methods that became more and more refined over time [37], but it took until 1986 for Danpure and Watts to discover deficiency of hepatic alanine-glyoxylate aminotransferase (AGT) as the cause of PH1 [38]. Shortly afterwards the cloning of the corresponding *AGXT* gene (OMIM 604285) allowed the identification of the first causative mutations [39]. Those landmark discoveries paved the way to the development of routine molecular diagnostic testing and led to a rational transplantation strategy for PH1.

PH1 (OMIM 259900) is caused by absent (nonsense/frameshift mutations, large deletions), deficient (missense/splice site mutations) or mistargeted activity (specific missense mutations) of liver-specific, pyridoxal dependent alanine:glyoxylate aminotransferase (AGT) [40, 41]. AGT catalyzes the transamination of the immediate oxalate precursor glyoxylate to glycine in the peroxisomal compartment. In the absence of catalytically active AGT in the peroxisome glyoxylate is converted to oxalate and glycolate via the cytoplasmatic lactate dehydrogenase A (LDHA). A synopsis of hepatic glyoxylate metabolism is given in Fig. 29.3.

The single copy *AGXT* gene is located on chromosome 2p37.3. Its genomic sequence consists of 11 exons spanning ~10 kb, resulting in a 1.7 kb cDNA with an open reading frame of 1176 base pairs. The gene product AGT is a homo-dimeric protein exclusively expressed in human hepatocytes, each 43 kDa subunit containing 392 amino acids and holding one molecule of pyridoxal-phosphate as cofactor [42]. The large N-terminal domain contains most of the catalytic active site, the cofactor binding site, and the dimerization interface. The smaller C-terminal domain contains the atypical peroxisomal targeting sequence (PTS1, LysLysLeu) and a presumed ancillary sequence, PTS1A, required for proper peroxisomal transport [43]. The crystal structure of normal AGT has been solved and provided valuable knowledge of AGT folding and how specific mutations and polymorphisms effect protein folding, dimerization and catalytic activity [44].

At least 220 causative mutations of all types have been reported throughout the *AGXT* gene [2]. Approximately 50% of the identified changes are missense mutations, including the most frequent mutation in Caucasians, p.G170R, and other recurring mutations (p.I224T, p.F152I). Hence, a substantial fraction of patients should carry at least one missense variant. Those point mutations will generally not result in protein truncation with total absence of AGT, but rather predispose to local changes in the polypeptide

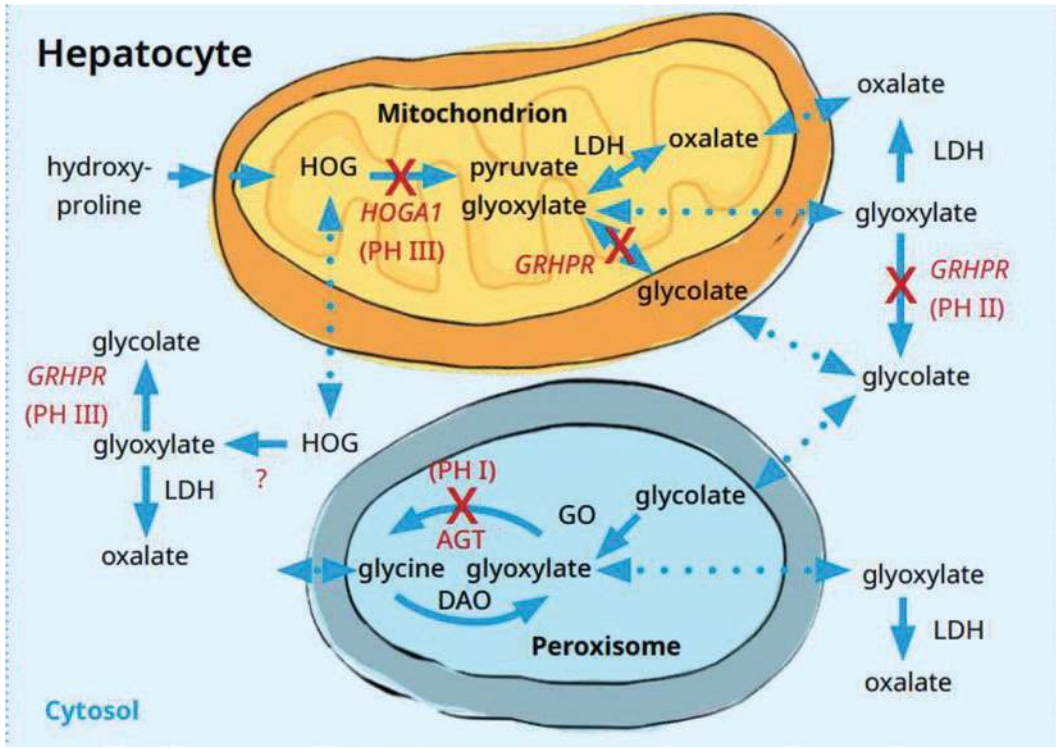


Fig. 29.3 Currently known disturbances in oxalate metabolism. *AGT* alanine:glyoxylate aminotransferase, *GRHPR* glyoxylate reductase/hydroxypyruvate reductase, *HOG* hydroxy-2-oxo-glutarate, *HOGA* hydroxy-2-oxo-glutarate aldolase, *LDH* lactate dehydrogenase, *PH (1–3)* primary hyperoxaluria, *GO* glycolyate oxidase, *DAO* diamino-oxidase, *PLP* pyridoxal phosphate

chain that will affect its enzymatic activity and/or stability. Moreover, as most mutations are not localized within the active or binding sites of the enzyme but rather interfere with protein folding and dimerization, potentially salvageable residual activity could be expected for the majority of (non-consanguineous) patients displaying at least one misfolding mutation. Missense point mutations impair AGT enzymatic function by: (1) abrogating the catalytic activity (rare), (2) forming insoluble AGT aggregates or impaired AGT homodimers, (3) disrupting peroxisomal import or iv) a combination of all [39].

In Caucasians the *AGXT* gene contains a number of common variants forming two main haplotypes, named AGT major allele (80% frequency) and AGT minor allele (20% frequency). In Asia the minor allele frequency is much lower, around 2% [40]. The two haplotypes differ in a number of polymorphisms occurring on the minor allele, within which a single base-pair change on position c.32 C>T (p.P11L) is the functionally most significant one. The leucine variant on position 11 (p.11L) influences the function of AGT in several ways. It reduces AGT activity by 30–50%, impairs dimerization of AGT subunits, and introduces a weak mitochondrial mistargeting sequence to the protein leading to dislocation of about 5% of the enzyme to the mitochondria. These changes are insufficient to cause a PH1 phenotype [39].

The most frequent causative mutation, c.508G>A (p.G170R), occurs at an allelic frequency of 25–40% among Caucasians, always on the background of the minor allele (p.G170R-Mi). This mutation is associated with aberrant localization of AGT within the mitochondria instead of peroxisomal targeting. AGT mistargeting is

caused by the synergistic interaction between the p.P11L polymorphism and the p.G170R mutation. This phenomenon is attributed to the principal difference between mitochondrial and peroxisomal protein-import machineries [45]. While peroxisomes import their matrix enzymes in a folded and even oligomerized conformation [46], mitochondria-targeted proteins are associated with chaperones which maintain them in a translocation-competent unfolded conformation. The unfolded mutant protein unmasks the N-terminal mitochondrial targeting sequence encoded by the p.P11L polymorphism, leading to its translocation into the mitochondrial matrix. Further folding of the mutated AGT is facilitated by means of mitochondrial chaperones, resulting in the formation of active dimeric molecules. Despite being essentially active, mitochondrial AGT cannot prevent oxalate overproduction [47]. In addition to p.G170R, the recurrent mutations p.I244T, p.F152I, and p.G41R also result in mitochondrial mistargeting [48].

The frameshift mutation c.33dupC (formerly known as c33_34insC; pK12Qfs*156) leads to a protein truncation and is the most common mutation found on the major allele (~12% allelic frequency in Caucasians) [41]. This variant is commonly found in patients suffering from infantile oxalosis.

Another frequently encountered missense mutation, c.731T>C (p.I244T, 6–9% allelic frequency), is especially common in patients from Spanish-African descent. Since it represents the causative mutation in about 90% of PH1 patients from the Canary Islands it is believed to result from a founder effect in this population [49, 50].

Besides aberrant compartmentalization, protein aggregation, accelerated degradation and other mechanisms contribute to AGT deficiency [51, 52]. The p.G41R, p.F152I, p.G170R, and p.I244T variants are all soluble and catalytically active in the absence of the p.P11L polymorphism, whereas in its presence all lead to protein destabilization and aggregation. Since the dimer structure can be stabilized by the interaction with pyridoxal phosphate (PLP, a vitamin B6 component), some of these missense mutations (p.G170R, p.F152I) have been associated with variable pyridoxine-responsiveness in patients. Responsiveness to vitamin B6 therapy may be affected via improved protein stability, activity and peroxisomal import. Renal survival is prolonged in patients with biallelic B6 responsive mutations, best documented for the group of p.G170R homozygotes. The latter group has a more favorable long-term outcome and many p.G170R homozygotes can be managed with isolated kidney transplantation in case of ESKD. However, early ESKD and infantile oxalosis can even occur in p.G170R homozygotes and patients with other missense mutations which are rated beneficial on molecular grounds. There is no established genotype-phenotype correlation for other important clinical aspects like onset of disease or degree of hyperoxaluria [53–55].

Chemical and physical actors that assist protein folding may decrease the negative effect of AGT missense mutants [21]. Further to the effects of vitamin B6, some *in vitro* evidence supports beneficial effects of amino-oxy-acetic acid (inhibitor), chemical chaperons (betaine, glycerol, DMSO, trimethylamine oxide and phenylbutyric acid), low temperature and pH. From this extended list, only vitamin B6 is an accepted therapeutic agent and even for this treatment only a single controlled small prospective clinical trial has been performed [56]. Therefore, therapeutic recommendations mostly rely on case reports, small retrospective case series and *in vitro* data [57].

Clinical Spectrum

Apart from being the most frequent type, PH1 is of particular importance as ESKD is the typical long-term outcome for this group.

The typical clinical presentation includes (recurrent/familial) calcium-oxalate urolithiasis and/or nephrocalcinosis, hematuria, urinary tract infection, and (chronic) renal failure (Fig. 29.1). Clinical presentation is highly variable and encompasses fulminant infantile oxalosis with

diffuse nephrocalcinosis (white kidneys) and failure to thrive within the first year of life, occasional stone formation at adult age, as well as elderly adult patients presenting with isolated renal failure without any past medical history compatible with metabolic stone disease. While the disease can first manifest at any age, median age of manifestation is 6 years. About 10–20% of the cohort will experience ESKD within the first few years of life, the condition termed infantile oxalosis [1, 2].

Contrary to the common misconception that monogenic diseases should result in a rather uniform and childhood onset presentation this is not the case in PH1 as in many Mendelian disorders[1, 2, 53]. PH1 shows marked inter- and intra-familial phenotype variability despite identical genotypes [21, 56].

With advanced renal insufficiency and the failure to excrete oxalic acid, the disease turns from a kidney and urinary tract limited phenotype into a potentially lethal multi-systemic condition [57–61]. In case of ESKD, plasma oxalate (Pox) levels will exceed the supersaturation threshold for calcium oxalate. Pox levels typically are >80–200 μmol/L pre-dialysis, with calcium oxalate crystal precipitation occurring at Pox >30 μmol/L [23]. This inevitably results in systemic deposition of calcium-oxalate salts (oxalosis) in virtually all tissues, with a predilection of retina (most often in the infantile cases) [24], myocardium, vessel walls, skin, bone, bone marrow and the central nervous system (Figs. 29.1 and 29.2) [21]. Systemic oxalosis has devastating consequences that include cardiomyopathy, cardiac conduction disturbances/heart block, vasculopathy, treatment resistant anemia, oxalate osteopathy resulting in debilitating bone and joint deformation and pain, retinopathy, skin infection and if untreated untimely death.

The multitude and systemic nature of symptoms can obscure a correct diagnosis for years [62, 63]. About 20–35% of adults with PH1 in developed countries are diagnosed only when having reached ESKD or even after kidney graft failure [28–31].

Primary Hyperoxaluria Type 2

Biology and Molecular Basis

PH2 (OMIM 260000) is a result of deficient glyoxylate reductase/hydroxypyruvate reductase (GRHPR) enzyme activity [64, 65]. *GRHPR* was first cloned from a human liver library in 1999.

The single copy *GRHPR* gene (OMIM 604296) located on chromosome 9p13.2 is the only gene to result in PH2. Its genomic sequence consists of 9 exons spanning ca. 14 kb, resulting in a 1.2 kb cDNA with an open reading frame of 984 base-pairs. The gene product is a 328 amino acid protein with a molecular weight of 35.4 kDa. The normal protein forms a homodimer that, unlike AGT, shows widespread tissue expression in cytoplasm and mitochondria outside the liver, indicating a role in metabolism. GRHPR, like LDH, utilizes pyridine nucleotides (NADPH, NADH) as cofactors. GRHPR has a 100-fold higher affinity towards NADPH than for NADH, which strongly favors the reaction from hydroxypyruvate to D-glycerate in the cytosol [66]. Under physiologic conditions cellular glyoxylate is mainly restricted to the mitochondria and peroxisomes [47, 67]. Flux between the two compartments is provided by mitochondrial GRHPR (glyoxylate → glycolate) and reverse oxidation (glycolate → glyoxylate) by peroxisomal glycolate oxidase (GO). This largely prevents glyoxylate from being converted to oxalate. If present in the cytosol glyoxylate forms the substrate for 3 reactions catalyzed by the enzymes lactate dehydrogenase A (LDHA) and GRHPR: i) GRHPR catalyzes the reduction to glycolate using NADPH and NADH as cofactors; ii) in the presence of NAD^+ oxidation to oxalate by LDHA; or reduction to glycolate with NADH as cofactor [68]. The competition between glyoxylate oxidation (LDH) versus reduction (GRHPR) is largely determined by the NAD^+/NADPH ratio and the LDHA/GRHPR ratio available in the cytosol, which if determined by sheer quantities alone would strongly favor oxidation [47, 68]. Fortunately, the GRHPR route of mitochondrial

conversion of glyoxylate to glycolate and subsequent peroxisomal transamination to glycine seems to be largely preferred over oxidation by cytosolic LDHA in physiologic states.

In PH2 hepatic glyoxylate metabolism is predominantly affected and results in increased amounts of cytosolic glyoxylate and hydroxypyruvate, now available for conversion by (LDHA) to both oxalate and L-glyceric acid (L-glycerate, Fig. 29.3) [69, 70]. Excessive amounts of both metabolites are excreted by the kidney that led to the initial description of PH2 as "L-glyceric aciduria" [71].

To date, 48 causative mutations have been identified in patients with PH2. While the c.103delG (p.D35Tfs*11) frameshift mutation is predominant in Caucasians with an allelic frequency between 30 to 50%, the mis-splicing variant c.403_404+2delAAGT is the primary mutation found in individuals of Asian descent. Interestingly all identified causative *GRHPR* mutations including the missense variants are functionally null alleles resulting in loss of protein expression and/or of catalytic activity [65].

Clinical Spectrum

At least in Europe and North America, PH2 appears to be much less frequent than PH1 (<10% of PH patients), although it cannot be excluded that PH2, due a less severe clinical course, is more underdiagnosed than PH1 (Table 29.1). With increasing awareness and the availability of genetic screening this entity might be recognized more often in the future.

The disease typically manifests in childhood and presentation can overlap largely with PH1 (recurrent urolithiasis, hematuria, UTI etc.), but nephrocalcinosis is less commonly seen. An asymptomatic clinical course for years or late stone disease does not preclude a diagnosis of PH2 [72, 73]. In our experience, ESKD in PH2 is often found in patients who underwent unilateral nephrectomy in childhood in the absence of a clear diagnosis.

In general, infantile oxalosis is not regularly observed in PH2. However, systemic oxalosis has been reported, and CKD or ESKD occurs more frequently with this type than previously thought [6, 72]. Once ESKD is reached there is some risk of systemic oxalosis. One adult patient with PH2 developed cardiomyopathy while on hemodialysis [74–77]. Retinal deposits were found in another PH2 patient with ESKD [24].

Apart from hyperoxaluria, elevated urinary L-glyceric acid excretion is a characteristic feature in most patients with this type [71, 72, 78], although cases with genetically proven PH2 and normal urinary L-glyceric acid excretion are known, one of those was published [78].

The scarcity of PH2 makes any prognostic and therapeutic recommendations difficult. A recent report on long term outcomes of PH2 did not observe a genotype-phenotype correlation. The significant rate of CKD and ESKD and the necessity of combined liver kidney transplantation in some PH2 patients indicate that PH2 may be a more problematic clinical condition than initially anticipated.

Primary Hyperoxaluria Type 3

Biology and Molecular Basis

PH3 (OMIM 613616) was first associated with mutations in the *HOGA1* gene (4-hydroxy-2-oxoglutarate aldolase) on chromosome 10q24.1 in 2010 [3]. The gene product catalyzes the final step of mitochondrial hydroxyproline metabolism from 4-hydroxy-2-oxoglutarate to glyoxylate and pyruvate (Fig. 29.3) [3, 47, 79]. To date at least 50 different mutations in *HOGA1* (OMIM 613597) have been identified that cause loss of enzymatic activity in liver and kidney tissue [3, 4, 7, 80]. The enzymatic block leads to accumulation of the glyoxylate precursor 4-hydroxy-2-oxoglutarate (HOG), its reduced form 2,4 dihydroxyglutarate (DHG) and the HOG precursor 4-hydroxyglutamate (4OHGlu). The latter was recently proposed as a new potential biomarker for PH3 [5, 47]. Next to oxalate all three of the latter compounds can be found at elevated levels in the urine and liver tissue samples, but not in plasma, of PH3 patients [5,

47, 81]. The precise mechanisms underlying hyperoxaluria are not fully understood, but the following model has been proposed: In non-PH3 states almost all HOG is converted to glyoxylate which is in turn reduced to glycolate by mitochondrial GRHPR (see PH2 paragraph above). In HOGA1-deficiency, accumulation of HOG (reduced HOG and 4OHGlu) leads to leakage/transport from the mitochondrial compartment into the cytosol. Cytosolic HOG, its reduced form, and 4OHGlu are partially excreted in the urine; to some extent HOG is converted into the reactive oxalate precursor glyoxylate by a yet to be identified HOG aldolase. This aldolase, potentially N-acetylneuraminate lyase, is an enzyme with high homology to HOGA1 [5, 47]. Another factor that may contribute to increased oxalate generation could be the reported inhibition of GRHPR, the cytosolic counterpart of LDH, by excess HOG [81].

Currently 50 causative mutations have been identified in patients with PH3. While the splicing mutation c.700+5G>T constitutes the most frequent mutation in patients from European descent (AF 45%), the in frame deletion c.944-46delAGG (p.Glu135del) is the most frequently encountered in patients of Askenazi Jewish descent, and the splicing mutation c.834+1G>T mutation is commonly found in Chinese patients. So far, no relevant genotype-phenotype correlation could be established for PH3 [10].

Further unraveling of the metabolic consequences in PH3 is of paramount importance for our understanding in general as they may lead to the discovery of novel therapeutic options not restricted to PH3. Hydroxyproline turnover is almost exclusively derived from dietary collagen intake and endogenous metabolism of collagen. Modulation of hydroxyproline metabolism either by dietary measures or pharmacological interventions may become feasible in the future. Subsequent detailed studies will help to further clarify the impact of nutrition and dietary interventions for the spectrum of hyperoxaluria and associated stone disease [15, 82].

Clinical Spectrum

PH3 seems to be more frequent than PH2 in some populations and according to the allelic frequency of pathogenic mutations in large gene banks (about 15% of PH patients in the German PH registry; high carrier rate in the Ashkenazi Jewish population). The scarcity of adult patients identified with symptomatic PH3 suggests a reduced penetrance and possibly an increasing rate of clinical and/or biochemical remission with age. The latter is a matter of controversy, which to resolve will require the identification and phenotyping of more adult individuals with biallelic mutations.

Based on the limited available data PH3 represents the least severe form of primary hyperoxaluria, but is not a benign condition [3, 4, 7, 80]. The most severe clinical phenotype of PH3 is observed in infancy and early childhood, with recurrent kidney stones and the necessity of repeated stone removal procedures. However, new stones may still be formed at adult age. Approximately 20% of PH3 patients will develop chronic kidney disease [10].

Previous biochemical findings of persisting hypercalciuria and intermittent hyperuricosuria have been questioned by new studies [5]. Compared to PH1, in which urinary citrate excretion is clearly subnormal at diagnosis, normal citrate excretion has been found in PH3 patients even before treatment was inititated [10].

Diagnosis and Differential Diagnosis

General Remarks and Clinical Diagnosis

Any child presenting with a calcium oxalate stone, (progressive) nephrocalcinosis or renal failure of unknown cause should be evaluated for primary hyperoxaluria [1, 2, 13, 83] (Tables 29.1 and 29.2). The same applies to adult recurrent

stone formers with or without renal impairment. Non-PH1 renal failure per se produces a multitude of symptoms not confined to the kidney (e.g. osteopathy, anemia, cardiovascular disease). However, in patients on chronic dialysis without an established diagnosis, especially those who do deteriorate on an adequate dialysis regimen and/or those who display features of a presumed collagenosis, autoimmune disorder, or vasculopathy, it is prudent to rule out PH1. Pathologists should be explicitly asked to look for potential calcium oxalate deposition (birefringent crystals under polarized light) in all patients undergoing kidney biopsy for unexpected and/or early graft dysfunction [2, 21].

Biochemical Screening and Diagnosis

Repeated measurement of oxalate excretion in 24-h urine collections (UOx) constitutes an excellent screening tool for detection of PH1–3 as long as renal function is within normal limits (Table 29.2). Unfortunately oxalate determination is not routinely available in many clinical laboratories, which often leads to its omission from screens. Urinary screening should be performed on repeated samples as incomplete collection, shorter sampling intervals than 24 h, diurnal variation (up to 15–20%) [84], and active stone growth may lead to false positive or false negative findings [2, 21, 85]. When oxalate/creatinine ratio in spot urine samples must be used, i.e. in infants and toddlers, interpretation requires repeated sampling, the use of age-related reference values, and extra caution with regard to interpretation of findings. A low urinary creatinine concentration frequently leads to falsely elevated molar ratios. Especially in preterm babies higher ratios are common, in particular under parenteral nutrition containing amino acids [13].

All other glyoxylate metabolites should also be determined in urine samples from patients with suspected hyperoxaluria, as this already allows a differentiation of PH subtype. Glycolate is elevated in urine and plasma in patients with PH1, L-glyceric acid in urine from patients with PH2 and HOG, DHG and 4OHGlu in patients with PH3. If these laboratory parameters are available, the diagnosis of PH and the subclassification of the PH subtype can be made already on biochemical grounds and later proofed by genetics. In this setting, personalized genetic testing will provide definitive diagnostic confirmation. PH can be differentiated from SH by collecting urines under different dietary conditions (high vs. low oxalate contents) [16].

Although the absolute amount of oxalate excretion allows a reasonable statistical discrimination between the three groups, biochemical subtyping at the individual patient level is compromised by intra-individual variation of oxalate excretion [21]. In patients with advanced chronic kidney disease (glomerular filtration rate, GFR < 30 mL/min*1.73 m^2) urinary oxalate excretion might be well within normal limits due to reduced glomerular filtration. As CKD progresses towards ESKD, urinary oxalate measurement becomes even less useful or even impossible and determination of Pox becomes more important, which has no diagnostic role in early CKD stages. Pox levels remain within the normal range (< 10 μmol/L) or increase only slightly for a long period of time, but correlate inversely with decreasing GFR at higher grades of CKD. Marked elevations of both Pox (to >80 μmol/L) and glycolate prior to dialysis are commonly observed and may give a high index of suspicion to a diagnosis of PH1, while selective hyperoxalemia occurs in PH2 and PH3 [86, 87]. While the specificity of elevated Pox as a diagnostic marker of PH is limited by the global accumulation of oxalate in ESKD patients irrespective of the underlying disease, Pox values are typically much lower (30–50 μmol/L) and rarely exceed the 80 μmol/L threshold in non-PH patients [23].

Confirmation of Diagnosis by Genetic Testing

A clinically and biochemically based diagnosis of PH1–3 always requires confirmation by a definite test, which today is done by targeted or NGS based mutational analysis of the three causative

Table 29.3 Synopsis of most common genetic findings in PH1–3

PH type	Gene (exon)	Min. no of identified causative mutations	Frequent/founder mutations	AF	Reported cases for PH type
1	***AGXT*** (11)	220	c.508G>A (p.R170G-Mi)[a] c.33dupC (p.K12Qfs*156-Ma)[a] c.731T>C (p.I244T- Mi/(Ma))[b]	~25–40%[a] ~10–12%[a] ~7–9%[b] (>90%[CI])	>>>1000
2	***GRHPR*** (9)	50	c.103delG (p.D35Tfs*11)[a] c.403_404+2 delAAGT (missplicing)[c]	~37–60%[a] ~16%[c]	>100
3	***HOGA1*** (7)	50	c.700+5 G>T (missplicing)[a] c.944_946delAGG (p.E315del)[d]	~50%[a] >90%[d]	>>100

Mi AGT minor allele, *Ma AGT* major allele; Mi/(Ma) indicates that c.731C>T occurs both with the minor and major allele, but is mostly segregating in cis with the minor allele

Mutations are given according to allelic frequency =AF, in different populations: [a]Caucasians, [b]Spain/North Africa [CI]Canary Islands, [c]Asians, [d]Ashkenazi Jewish

genes: *AGXT*, *GRHPR,* and *HOGA1* (Table 29.3). Genetic testing can be considered the gold standard for diagnosis since it provides fast and reliable distinction of the precise PH type. Exact genotype information is clinically relevant since some missense mutations in the *AGXT* gene are more likely to respond to a specific medication (vitamin B6 treatment) and correlate with better long-term renal survival. Moreover, genotype data will become even more important with the personalized medicine approaches that are being developed or may become available in the future [53–55, 83, 88, 89].

Clinical and biochemical data can be used to prioritize and guide stepwise genetic analysis in order to make the molecular analysis even faster and more cost effective (Fig. 29.3 and Table 29.4).

In case sequencing only identifies a single causative mutation in heterozygous state in line with a high clinical suspicion of PH, copy number variations (CNV; larger heterozygous deletions/amplifications) should be considered. CNVs cannot be detected by Sanger sequencing. NGS based bioinformatic CNV detection is available but less sensitive than multiplex ligation-dependent probe amplification (MLPA), the gold standard for CNV detection. a commercial MLPA assay (MLPA; MRC Holland) allows simultaneous detection of abnormal CNVs in *AGXT* and *GRHPR* [90]. However, CNVs seem to be very rare in primary hyperoxaluria (<1%) [91].

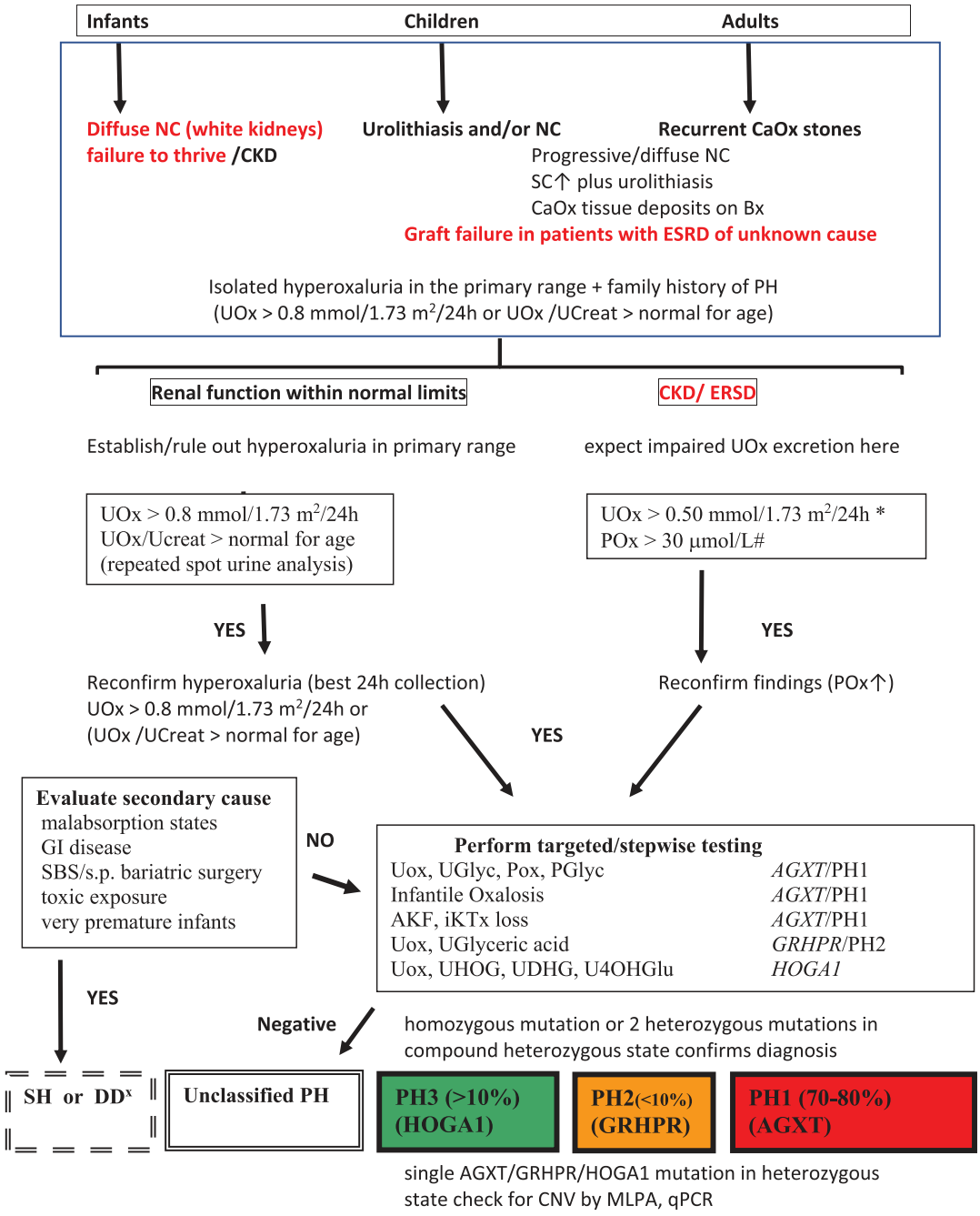
Historically, enzyme activity/expression used to be assayed by liver biopsy to confirm the diagnosis. This invasive and elaborate procedure has been abandoned and replaced by genetic screening.

Differential Diagnosis and Secondary Hyperoxaluria

In principle, a wide spectrum of disorders resulting in recurrent urolithiasis with calcium oxalate stones, nephrocalcinosis and/or CKD/ESKD can mimic primary hyperoxaluria. Careful clinical and biochemical assessment including repeated measurement of urinary oxalate excretion is effective in excluding most relevant differential diagnoses, at least in patients with normal kidney function (Table 29.4).

PH needs to be differentiated from secondary forms of hyperoxaluria (SH), where dietary or toxic exposure to oxalate or its precursors (hydroxyproline, ascorbic acid) leads to increased adsorption from the gastrointestinal tract [13]. In contrast to SH, patients with PH show oxalate absorption within the normal range (often <5–10% [15]). Oxalate excretion is usually <1 mmol/1.73 m^2/day in SH, but in some cases maybe as high as in patients with PH [11]. However, secondary causes attributable to gastrointestinal disease, which are usually characterized by fat malabsorption (e.g. inflammatory bowel diseases (IBD), cystic fibrosis, status post bariatric surgery, short bowel syndrome (SBS)) can lead to constant hyperoxaluria due to

Table 29.4 Diagnostic algorithm for the evaluation of primary hyperoxaluria



NC nephrocalcinosis, *CKD* chronic kidney disease, *SC* serum creatinine, *CaOx* calciumoxalate, *UOx* urinary oxalate excretion, *creat* creatinine, *ESKD* end stage renal disease, *POx* plasma oxalate, *GI* gastro intestinal, *SBS* short bowel syndrome, *NRF* normal renal function, *SH* secondary hyperoxaluria, *LBx* liver biopsy, *AGT* alanine:glyoxylate aminotransferase, *GRHPR* glyoxylate reducatase/hydroxypyruvate reductase, *HOGA1* hydroxyl-oxo-glutarate aldolase 1, *HOG* Hydroxy-oxo-glutarate

enhanced absorption of oxalate from the gastrointestinal tract and in exceptional cases may ultimately result in ESKD. In severe cases of ESKD from SBS or IBD, systemic oxalosis states similar to that seen in PH1 can occur [11].

Three 24-h urine oxalate collections should be performed under varying dietary conditions to obtain first evidence on whether hyperoxaluria may be primary or secondary. Patients collect 24-h urines under diets with normal, low and high oxalate content. Patients without hyperoxaluria and those with the typical pattern of secondary hyperoxaluria (low UOx under low Ox diet, high UOx under high Ox diet) are easy to diagnose [16].

Genetic Counselling in PH

Since all three types of PH are inherited in autosomal recessive manner, siblings of an affected individual have an *a priori* chance of 25% to be affected, a 25% chance of being unaffected and not a carrier, and a 50% chance of being an asymptomatic carrier of the familial mutation. All heterozygous carriers are asymptomatic with regards to primary hyperoxaluria, although heterozygosity for *HOGA1* mutations has been claimed to be a potential risk factor for calcium oxalate stone disease in a single report [4]. Since heterozygous carriers of an *AGXT*/*GRHPR*/*HOGA1* mutation are asymptomatic with respect to primary hyperoxaluria, they are acceptable liver and/or kidney donors.

In principle, prenatal testing is possible by DNA analysis from fetal cells (amniotic fluid, chorionic villus) and carrier testing for at-risk family members by testing in peripheral blood if the causative mutation(s) have been identified. Requests for prenatal testing (PD) in PH2 and particular for PH3, conditions that do not cause childhood ESKD and do not affect the intellectual capabilities, are uncommon. Preimplantation genetic diagnosis (PID) is a valuable alternative for families considering prenatal diagnosis for the purpose of pregnancy termination in PH1. With regards to family members at risk, it is recommended not to disclose PH1–3 carrier status to asymptomatic minors.

Treatment

Current Treatment Options

Conservative Treatment

Fluid Intake

Hyperhydration is the backbone of conservative treatment for stone disease in general and this is particularly true for all types of primary hyperoxaluria. High fluid intake (>3 liters per 1.73 m^2 per day) is essential. In infants and toddlers this is frequently problematic, so placement of a gastrostomy tube should be considered that will also ensure adequate fluid administration during nighttime. Situations of fluid losses (fever, diarrhea/vomiting, urinary tract infections etc.) or with compromised oral hydration (status post-surgery) have to be anticipated and there should be a low threshold to hospitalizing for intravenous fluid supplementation.

Dietary Considerations

At least in patients with PH1 and PH2 only a small proportion of the oxalate excreted in the urine is derived from the gut, so dietary oxalate restriction is of limited benefit and generally not recommended [15]. Conversely, restriction of oxalate intake is a necessity in SH. Patients with PH1 and PH2 absorb even less oxalate than healthy individuals, with a low fraction of dietary oxalate excreted in the urine (<5%) [15]. This phenomenon may be due to a concentration dependent flux (higher plasma, lower intestinal oxalate concentration) of oxalate into the intestinal lumen [92, 93]. However, it is reasonable to avoid large amounts of nutrients rich in oxalate and an excessive intake of ascorbic acid (vitamin C, oxalate precursor) and vitamin D (to avoid an increase in calcium excretion).

Calcium intake must not be restricted as this maneuver is likely to result in higher oxalate absorption from the gut because less intestinal calcium will lead to more free, absorbable oxalate [14, 15].

In PH3 the situation might be more complex since catabolism of 4-hydroxyproline derived from collagen turnover and dietary sources

results in significant glyoxylate and oxalate generation, which links the PH spectrum to the gastrointestinal tract, sometimes referred to as the "gut-kidney axis" [15].

Vitamin B6

A treatment option specific to PH1 is the oral administration of vitamin B6. The vitamin B6 component pyridoxal-phosphate (PLP) is the cofactor of all body transaminases and hence also of the AGT enzyme deficient in PH1. PLP was first described in 1961 by McLaurin and colleagues as a treatment option in two patients with PH1, long before AGT deficiency was known to be the cause of the disease [94]. Pharmacological doses of PLP supposedly help 30–50% of patients with PH1 with residual AGT activity to reduce or even normalize endogenous oxalate production [95–97]. Treated patients progressed to ESKD later and were older at the time of death [32, 98].

However, current evidence is mostly based on case series, single case reports and retrospective trials, while prospective trials are still pending [99–104]. There is an ongoing debate about the mechanisms of action and response patterns [105–107]. Some authors even concluded that pyridoxine treatment is not efficient in reducing oxalate excretion [108, 109]. The only prospective B6 treatment study showed that in about 50% of PH1 patients, urine oxalate excretion was reduced by >30% [88]. However, complete normalization of UOx was not observed, not even in patients with a homozygous p.G170R mutation. Also, no correlation was found between serum B6 levels and the reduction of urinary oxalate excretion [88].

Vitamin B6 comprises six compounds (vitamers) with vitamin B6 activity: pyridoxine, pyridoxal, pyridoxamine, and the activated phosphate esters pyridoxine 5' phosphate, pyridoxal 5'-phosphate and pyridoxamine 5'-phosphate [88]. Vitamin B6 is usually administered as pyridoxine-hydrochloride and resorption in the jejunum is passive. In the liver, vitamin B6 vitamers are enzymatically interconverted, and finally deliver pyridoxal 5'-phosphate, the active form that interacts with AGT [110].

As explained in detailed above, in PH1 AGT is misfolded or mistargeted to the mitochondria due to missense mutations in *AGXT* [111]. There are different hypotheses how the vitamin B6 ester PLP might reduce endogenous oxalate production and UOx excretion. Possible mechanisms include an increase in AGT expression, AGT enzyme activity, or, most likely, facilitated targeting of AGT into the peroxisomes [112]. All mechanisms would lead to an increased metabolic efficiency of AGT. Since mutant AGT protein is not as stable as wild type AGT, stabilizing compounds such as PLP acting as a pharmacological chaperone might lead to re-stabilization of the AGT protein with an increase of enzymatic function [113].

The responsiveness to vitamin B6 treatment depends on the underlying mutation. Mutations which lead to residues at the active site directly interacting with PLP (e.g. p.W108R, p.S158L and p.D183N) are considered to be associated with PLP responsiveness [114]. In addition, missense mutations that result in misfolding and impaired import (e.g. p.G170R or p.F152I) are also likely to benefit from PLP [34, 115, 116]. as confirmed in a prospective study [117]. Responsiveness was, however, not seen in all patients and there also was intrafamilial heterogeneity in response to B6 despite the same genotype [88].

Notably, pyridoxine absorption and metabolism has not been studied *in vivo*, so efficacy of B6 may not only depend on the *AGXT* genotype, but also be affected by individual variation in absorption and metabolism of Vitamin B6.

Responsiveness is currently defined by a decrease in urinary oxalate excretion after a test period of a minimum of 3 months at maximum dose [1, 88]. It is recommended to administer vitamin B6 (pyridoxine) in all patients with proven PH1, starting at a dose of 5 mg/kg per day and not exceeding 20 mg/kg per day, aiming to decrease urine oxalate excretion by >30% [88, 118]. If responsive, patients should remain on medication even if commencing dialysis [56, 103, 116]. In case of suspected infantile oxalosis, PLP should be given immediately as renal failure can sometimes be reversed in patients with a susceptible genotype (e.g. p.G170R homozygotes) (Fig. 29.4).

From a mechanistic point of view, one would expect the effect of RNAi treatment (which

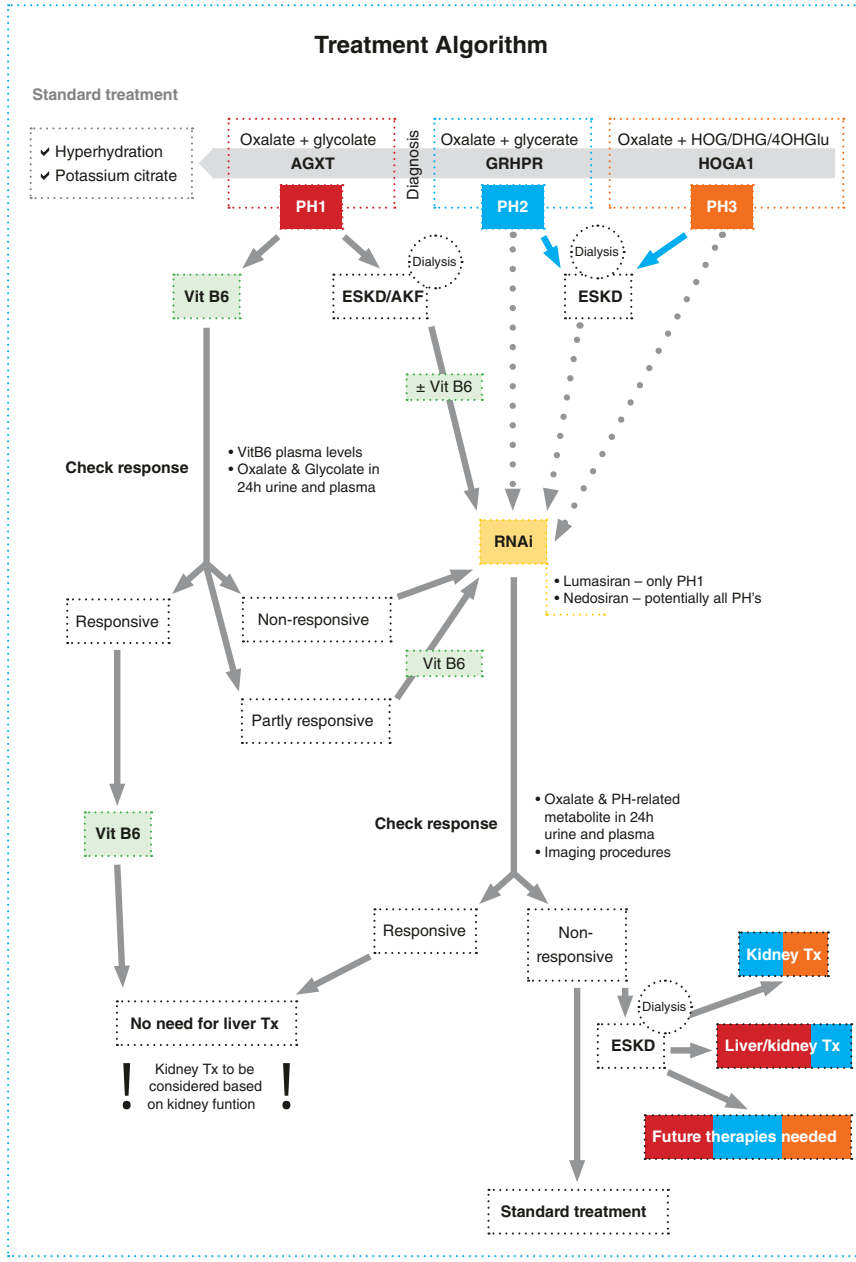


Fig. 29.4 Treatment algorithm in patients with the primary hyperoxalurias implementing the new RNAi interference (RNAi) treatment option. All patients should receive hyperhydration and alkaline citrate medication at start of treatment. In patients with primary hyperoxaluria type 1 (PH1), vitamin B6 (Vit B6) response has to be checked primarily before considering RNAi medication. In those patients who only show partial or no responsiveness to Vit B6, treatment decision is challenging and must be personalized by clinical follow up, quality of life and socio-economic evaluation. No liver Tx is needed in patients with adequate Vit B6 or RNAi response as indicated by normalized or nearly normalized urinary oxalate excretion. In patients with PH2&3 there currently is no RNAi treatment available. *PH1–3* primary hyperoxaluria types 1–3, *ESKD* end stage kidney disease, *AKF* acute kidney failure, *Tx* transplantation, *sqm* square meter, *Vit B6* vitamin B6

depletes oxalate precursors/substrates) to be independent of the effect of vitamin B6 treatment (which partially restores proper targeting of AGT to the peroxisome), so synergistic benefits could be expected from combining RNAi and vitamin B6 in patients with susceptible missense genotypes, e.g. partly B6 responsive patients (Fig. 29.4).

Side effects are rarely seen and even at higher dosages sensory neurotoxicity is uncommon according to investigator brochures and retrospective case series [95] (Table 29.2).

RNA Interference Therapeutics

RNA interference (RNAi) therapeutics are a revolutionary new approach to the treatment of PH. RNAi compounds work at the level of messenger RNA (mRNA) translation. These synthetic small double-stranded RNA molecules (small interfering RNA, siRNA) bind to a cytoplasmic protein complex (RNA-induced silencing complex, RISC), which specifically degrades the targeted mRNA and thus prevents translation into the corresponding protein [119–121]. This renders placing false information at the site that normally produces an enzymatic protein involved in oxalate metabolism (in the liver). If this is not produced, oxalate production in the liver can be significantly reduced or even completely blocked.

A first RNAi drug, Oxlumo® (Lumasiran, Alnylam Pharmaceuticals, USA), has recently been approved and can be prescribed since January 2021 [122]. Oxlumo® targets the mRNA of glycolate oxidase (GO) and, thus, prevents its translation in patients with PH1. This reduces the production of glyoxylate (i.e. the precursor to oxalate) and hence also oxalate production. Subcutaneous administration in animals showed reduction of urinary oxalate excretion by 98% [119, 120]. In healthy volunteers, Oxlumo® blocked about 80% of the corresponding mRNA without relevant side effects. In PH1 patients, urinary oxalate excretion is reduced by an average of 65% [122]. The downregulation of glycolate oxidase leads to an increased production and urinary excretion of the supposedly non-toxic compound glycolate. In patients with >20 kg body weight Oxlumo® is injected subcutaneously at a dosage of 3 mg/kg at monthly intervals for the first four injections, followed by quarterly injections. Smaller children are started on 6 mg/kg per month for the first 4 dosages, followed by 3 mg/kg monthly in children <10 kg and 6 mg/kg quarterly in patients with 10–20 kg body weight.

Another RNAi medication, Nedosiran® (Dicerna Pharmaceuticals/NovoNordisk, USA/Denmark) interferes with the final step towards oxalate production, the translation of liver-specific lactate dehydrogenase A (LDHA), preventing the conversion of glyoxylate to oxalate in all three types of PH [123]. Its efficacy in reducing urinary oxalate excretion has been demonstrated in animal models [124]. In a pilot study with long-term follow-up a significant decrease in urinary oxalate excretion, up to normalization, was achieved [119]. Nedosiran® is also injected subcutaneously, at a fixed dose of 170 mg per month in adults >50 kg and 136 mg in those <50 kg, and corrected to body weight in children (3.5 mg/kg). In a recent pivotal study Nedosiran® achieved equivalent reduction of urinary oxalate excretion in patients with PH1 as Oxlumo® [119, 123]. However, the expected positive results were not reached in patients with PH2.

The recent advent of RNAi therapeutics for clinical use mark a new era in the treatment of PH. Although long-term outcome data are not yet available, these drugs promise to allow better preservation of renal function and even prevention of progression to ESKD and systemic oxalosis at excellent tolerability. The major drawback of RNAi based drugs is their very high cost.

Oxalate Degrading Bacteria

The human intestine is physiologically colonized by oxalate degrading bacteria. Among these is *Oxalobacter formigenes*, an obligate anaerobic microbe residing primarily in the intestines of vertebrate animals [125]. This bacterium is unique among oxalate-degrading organisms as it has become totally dependent on oxalate for energy metabolism [125]. During the past three

decades compelling evidence has been gathered that the intestine plays an important role in oxalate homeostasis in states of health and disease and that *Oxalobacter formigenes* exerts a symbiotic relationship regarding the regulation of oxalic acid absorption in the gut [92, 93]. In various animal models of primary and secondary hyperoxaluria it was demonstrated that endogenous oxalate can be eliminated by *Oxalobacter* colonization of the intestinal tract [116, 126, 127]. Such treatment also showed efficacy in two pilot clinical trials with orally administered *Oxalobacter formigenes*, as paste or capsule, in PH patients with normal renal function or in ESKD [128, 129]. UOx or POx levels decreased significantly during 4 weeks of *Oxalobacter formigenes* administration, confirming the findings in the animal models [116, 126]. Significant reduction of POx was also observed in a phase 2 study with PH1 patients on chronic renal replacement therapy [128]. However, these results were not reproduced in two subsequent phase III trials [130]. A double blind multicenter trial was recently finalized but did not reach the primary endpoint, so the manufacturer stopped the developing process. A potential reason for the disappointing results in humans may be an inefficient delivery of the drug to the colon. Alternatively, the human gut flora may be deranged and microbial interplay be severely hampered by administration of *Oxalobacter* at excessive doses [130]. It is also possible that difficulties of the production process, with a less active *Oxalobacter* preparation used in the latter studies, might explain the findings [127].

Other Medication

Like in all other kidney stone formers, an increased excretion of urinary stone inhibitory parameters is regarded as an important measure. Both, alkaline citrate and orthophosphate treatment increases urinary pH (pH values above 7.5 should be avoided to prevent precipitation of calcium-phosphate crystals) and urinary citrate excretion. With the increase in citrate excretion, urinary calcium to oxalate binding is reduced, but also the urinary calcium excretion *per se* can be reduced by approximately 30% [131, 132].

Dialysis

No blood purification modality sufficiently removes the endogenously overproduced oxalate [58, 133–135]. Not even daily hemodialysis, nor its combination with peritoneal dialysis achieves a negative oxalate balance [135]. Body oxalate accumulation increases rapidly in dialyzed children and cannot prevent systemic oxalosis from taking its disastrous course. Hence, the period of dialysis prior to transplantation should be kept as short as possible. Since oxalate, a small hydrophilic molecule, is rapidly cleared by hemodialysis, frequent relatively short hemodialysis sessions, e.g. 5–6 times a week for 3 h, are more efficient than extended less frequent dialysis protocols [133, 134]. High-flux filters have a slight advantage in oxalate elimination [133]. Post dialysis POx should be well below <30 μmol/L, the cutoff value for plasma CaOx supersaturation [23]. A rapid POx rebound occurs after hemodialysis, making nocturnal peritoneal dialysis a valuable option for further oxalate elimination [135]. However, intense dialysis protocols are also fraught with higher technical complication rates [133]. Also, dialysis prescription should keep the families' quality of life in mind.

Transplantation Strategies

The transplant procedure is different for either form of PH, with no specific procedures for transplantation reported so far in PH type 3 patients. Liver transplantation cures the enzyme defect in PH1 and hence, pre-emptive liver transplantation and sequential or combined liver/kidney transplantation are possible procedures. However, due to the extreme clinical heterogeneity of PH1 even within families and in patients with the same genotype, individualized transplantation strategies are necessary [57, 61].

Combined liver/kidney transplantation is the method of choice in patients with ESKD and in vitamin B6 unresponsive patients without severe systemic oxalosis. Patient and liver allograft survival following combined liver and kidney transplantation were 80% and 72% at 5 years, respectively in European registry data [21, 57]. Pre-emptive liver transplantation might be an option in patients with a more rapid decline in kidney function [136], but timing of that procedure is difficult due to the variability of the disease course. Due to mobilization and renal elimination of systemically deposited oxalate, kidney function may still deteriorate after preemptive liver transplantation and kidney transplantation may still become necessary [57, 61]. In patients with infantile oxalosis, sequential liver and kidney transplantation is sometimes reasonable for technical/anatomical reasons [57]. In patients with severe systemic oxalosis sequential transplantation should also be considered to avoid prompt recurrence of oxalosis within the kidney graft.

Isolated kidney transplantation was generally not recommended in PH1. There is substantial evidence that PH1 patients with isolated kidney transplantation develop renal and systemic oxalate deposition [76, 77]. Allograft oxalosis clearly limits the duration of graft function: Following isolated kidney transplantation, graft survival rates were only 46%, 28%, and 14% at 1, 3, and 5 years in PH patients, as compared to 95%, 90%, and 85% in non-PH patients [21]. By contrast, urinary and plasma oxalate normalize following combined liver and kidney transplantation. Isolated kidney transplantation might be considered in elderly patients with late onset of ESKD and a pyridoxin-sensitive genotype [137, 138]. Also, a recent report from the OxalEurope registry suggested that isolated kidney transplantation in patients with pyridoxine sensitive genotypes treated with vitamin B6 may be equivalent in long term outcome to combined transplantation procedures [139].

The advent of the RNAi therapies opens the option to reduce oxalate overproduction pharmacologically, potentially removing the need for curative liver transplantation in patients with PH1. However, it is currently still too early to say how many patients can eventually be spared liver transplantation in the long run by RNAi therapy, comparable to those patients being sensitive to vitamin B6 medication and reduce their urinary oxalate excretion to merely normal. Long-term follow-up data will be required to address whether liver transplantation will really become obsolete and ESKD can be prevented by this therapy. Our current approach is shown in Fig. 29.4.

In the rare patients with PH2 who reached ESKD, isolated kidney transplantation has mostly been performed since the clinical picture clearly is less severe than in PH1 and liver transplantation does not truly eliminate oxalate overproduction. Although the currently followed very small group of PH2 patients with a kidney transplant show good overall graft survival, patients with oxalate related graft dysfunction, which appeared earlier than chronic rejection induced graft failure, have been described [27]. In a few PH2 cases even combined liver kidney transplantation was found to be necessary [140].

Conclusions and Outlook

The primary hyperoxalurias are challenging diseases. Diagnosis is still frequently delayed despite the availability of genetic screening as a rapid diagnostic tool. Any urolithiasis in a child should prompt an investigation of the underlying cause to treat adeuqately and not only with symptomatic empiric therapy and/or repeated stone removal procedures. This also applies to adult patients with multiple or recurrent kidney stones. The relevance of an early diagnosis for improved long-term outcomes has markedly increased with the advent of RNAi therapeutics as another effective therapy of PH1 aside vitamin B6. It may be now possible to largely prevent the hitherto dismal course of the disease, preserve kidney function and avoid liver transplantation as a curative therapy.

Further curative treatment approaches are under development. Gene therapy might be considered in patients with PH1, as new, safe and highly liver-selective vectors are becoming available which would deliver the targeted gene into the liver cells. In *AGXT* deficient mice it has already been demonstrated that PH1 can be cured with such a therapy [141]. Currently, new vector technologies are being developed since a singular gene therapy might not be sufficient in PH patients as more or less all hepatocytes must be transfected. Multiple administrations will induce immune reactions of the host, which may limit the tolerability of the procedure [142]. Induced pluripotent stem cell-derived hepatocytes, which have first been used experimentally to model human metabolic liver diseases [143], can now be generated from blood leukocytes and dermal fibroblasts of PH1 patients (hiPSC) [144, 145]. In the future, such cells could be gene-edited and used for autologous hepatocyte-like cell transplantation [146] if a proliferative advantage of the hISPC over native diseased hepatocytes can be achieved. Gene editing of the *HAO1* gene by Crispr-Cas9 has already been applied in the animal model [147].

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Cystinosis

30

Elena Levtchenko, Leo Monnens, and Aude Servais

Introduction

Cystinosis is an autosomal recessive disorder characterized by an accumulation of the amino acid cystine in lysosomes throughout the body. The responsible gene *CTNS* is located on the short arm of the chromosome 17 (p13) and encodes the lysosomal cystine carrier cystinosin [1, 2]. Lysosomal cystine accumulation is the hallmark of the disease.

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Depending on the age of presentation and the degree of disease severity, three clinical forms of cystinosis are distinguished:

- Nephropathic infantile form (MIM #219800), which is the most frequent and the most severe form of the disease
- Nephropathic juvenile form (MIM #219900); synonyms: intermediate cystinosis, late-onset form, adolescent form
- Non-nephropathic adult form (MIM #219750); synonyms: benign non-nephropathic cystinosis, ocular non-nephropathic cystinosis

All three forms of the disease are caused by mutations in the *CTNS* gene and have phenotypic overlap. Unless specified otherwise, this chapter focuses on infantile nephropathic cystinosis which affects ~95% of the patients.

Historical Aspects

Cystinosis was first described as a clinical entity by the chemist Abderhalden in 1903 [3] and recognized as a main cause of generalized proximal tubulopathy, called de Toni-Debré-Fanconi syndrome in the late 1940s/early 1950s [4–6]. Real progress in the investigation of cystinosis started when amino acid chromatographic analysis allowed measuring elevated cystine concentrations in tissues of cystinotic patients [7, 8]. In the

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1980s it was demonstrated that cystine was stored within the lysosomes due to the impairment of cystine transport across the lysosomal membrane [9, 10]. The availability of renal transplantation and treatment with the amino thiol cysteamine dramatically improved the prognosis of cystinosis patients allowing them to survive into adulthood [11, 12]. Cloning the *CTNS* gene in 1998 was pivotal for understanding the genetic basis of the disease and performing genetic counseling of the families [1]. Moreover, the *CTNS* gene discovery opened a new chapter in studying the disease pathogenesis and searching for novel molecular therapies [13].

Epidemiology

The estimated incidence of cystinosis is 1 in 100,000–200,000 live births [2] with clustering reported in some populations [14–16]. Cystinosis affects all races, with specific hotspot mutations reported in different nations [16, 17].

Males and females are equally affected. Overall cystinosis patients account for approximately 1–2% of the pediatric end-stage renal failure population or 0.85/million of age related population [18, 19].

Genetics

Mutation Spectrum

Cystinosis is caused by mutations in the *CTNS* gene, which has been identified by positional cloning strategy [1]. *CTNS* maps to 17p13.2 (Fig. 30.1a) [1] and encodes a 367 amino acid protein, cystinosin, with a 7-transmembrane domain structure which is highly glycosylated at the N-terminus [21] (Fig. 30.1b). Cystinosin contains 2 lysosomal-targeting sequences, one situ-

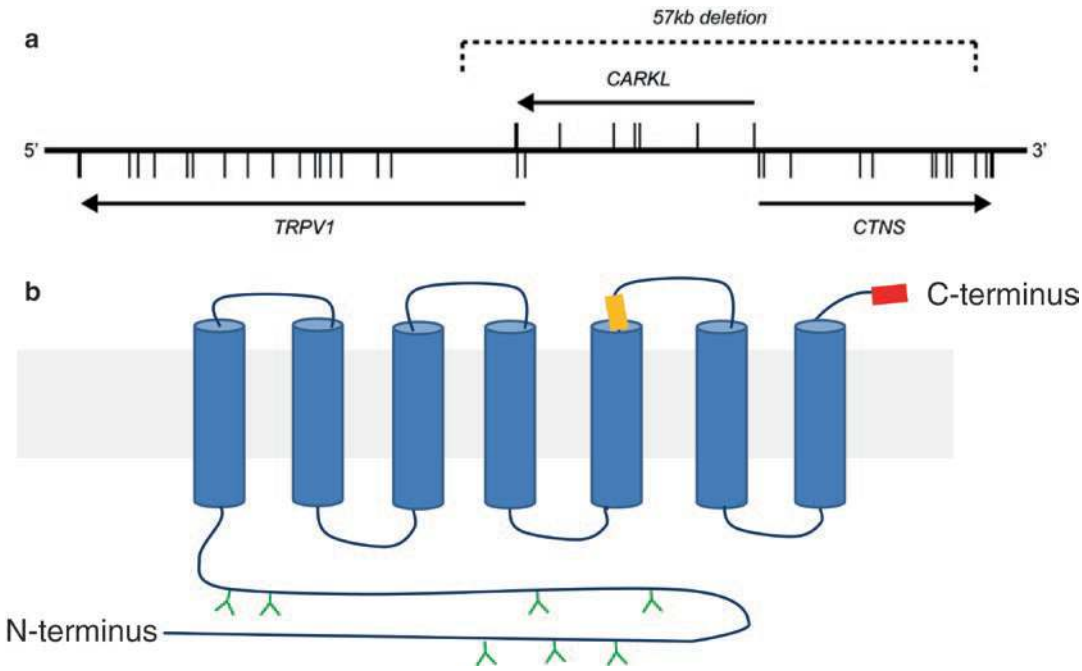

Fig. 30.1 Molecular basis of cystinosis. (**a**) The *CTNS* gene is mapped at chromosome 17p13.2 and is composed of 12 exons, with the open reading frame starting in exon 3. The *CTNS* shares promoter sequence with the *CARKL* gene. Common 57 kb deletion is the most prevalent mutation in the Northern-European population which affects *CTNS*, *CARKL* and the first two non-coding exons of the adjacent *TRPV1* gene (Used with permission of The American Physiological Society from Wilmer et al. [20]); (**b**) *CTNS* encodes lysosomal membrane protein cystinosin with seven transmembrane domain structure. Cystinosin contains two lysosomal-targeting sequences: GYDQL at C-terminus (red box), and YFPQA—in the fifth inter-transmembrane domain loop (yellow box). Y—predicted glycosylation sites

ated in its C-terminus, a tyrosine-based motif (GYDQL) and the second (YFPQA) in the fifth inter-transmembrane domain (TM) loop [21].

Mutations in *CTNS* have been detected in all three clinical forms of cystinosis, demonstrating that these forms are allelic [1, 22, 23]. The most common anomaly is a large 57 kb deletion spanning the first nine exons and a part of exon 10 of the *CTNS* gene, the upstream 5-prime region that encodes the *CARKL* (carbohydrate kinase-like) gene, and the first two noncoding exons of the *TRPV1* gene [1, 24–26]. *CARKL* encodes the enzyme sedoheptulokinase [26] and *TRPV1* encodes the protein transient receptor potential one [27]. Patients with the homozygous 57 kb deletion have increased sedoheptulose levels in tissues, serum and urine [26, 28], but no clinical disorders related to CARKL deficiency has been reported so far. Decreased sensitivity to capsaicin and altered sensation of heat due to strongly reduced activity of TRPV1 channel may account for the reported sensory alterations and thermoregulatory deficits (such as impaired sweating) in patients carrying homozygous 57 kb deletion [27]. The common 57 kb deletion can be detected by FISH analysis, by a rapid PCR assay with the 57 kb deletion breakpoint primer sets, or by MLPA [29–31]. While being frequent in the Northern-European population, the 57 kb deletion is rare in Southern Europe and in patients originating from other ethnical groups [16].

Over 140 other mutations in *CTNS* have been reported, which include small deletions, insertions, nonsense, missense, splicing mutations, mutations in the promoter region or small genomic rearrangements (uniparental disomy on chromosome 17) [1, 16, 22, 25, 30–34].

In a recently published European cohort, 33% of patients were homozygous and 23% were heterozygous for the 57 kb deletion, and 45% had other pathogenic variants of the *CTNS* gene [35]. Missense variants, splicing variants and out-of-frame deletions were the other most frequent types of mutations. If standard analysis using PCR technique and Sanger sequencing fails to demonstrate *CTNS* mutations in patients with the clinical diagnosis of cystinosis, the *CTNS* gene transcripts analysis for detection of exon-skipping mutations should be performed [34]. Maternal heterodisomy should be suspected when no mutation is found in the father and can be detected using analysis of microsatellite markers on chromosome 17 [33].

Genotype: Phenotype Correlations

In infantile forms, individuals have severe mutations in both alleles, leading to the complete loss of cystinosin function. No differences in kidney survival and severity of the extra-renal disease between patients that inherited the 57 kb deletion in homozygous or in heterozygous state and patients with other severe pathogenic *CTNS* variants were found [35–37]. In contrast, point mutations in the *CTNS* gene that do not disrupt the open reading frame of cystinosin are more commonly associated with the late-onset phenotype and generally affect the inter-transmembrane loops or the N-terminal region [22, 23, 38–42]. In the late-onset form, patients are usually homozygous for mild mutations or compound heterozygous for a mild mutation and a severe mutation [22]. Mild mutations impair, but do not completely abolish cystine transport [43]. Moreover, the level of transport inhibition often correlates with the severity of symptoms. Some tissues might be spared in benign cystinosis due to tissue-specific splicing factors mitigating the effect of splice-site mutations in renal tissue by favoring the expression of residual normal message [23, 42, 43]. Mutations in the promoter region have also been described in patients with ocular cystinosis [25]. In addition, contrasting to almost 100% detection rate in infantile cystinosis, *CTNS* mutations are not found in all patients in late-onset forms [39, 41]. Mutations in the non-coding regions of the *CTNS*, such as the regulatory regions, may also be involved in these patients.

Pathogenesis

Following the seminal studies by Schneider et al. [7, 8], electron microscopy of lymph nodes of patients with cystinosis first suggested that cystine accumulation occurs in lysosomes [44]. The lysosomal localization of cystine was later confirmed in cystinotic leucocytes [45]. Subsequently, kinetic studies of cystine clearance from lysosomes of cystinotic leucocytes and fibroblasts provided evidence that impaired lysosomal cystine efflux represents the primary defect causing cystine accumulation in cystinosis [9, 10, 46].

The discovery of the gene defective in cystinosis allowed the functional characterization of the gene product cystinosin as a proton-driven lysosomal cystine carrier [47]. A transcript variant of the *CTNS* originating from alternative splicing of exon 12, which replaces the lysosomal targeting motif GYDQL at the C-terminus by a longer amino acid sequence (termed CTNS-LKG based on the sequence of the three last amino acids leucine (L), lysine (K), glycine (G)) shows expression in the plasma membrane, in lysosomes, in the endoplasmatic reticulum, in the Golgi apparatus, and in small intracellular vesicles [48]. In most tissues, CTNS-LKG represents 5–20% of *CTNS* transcripts with the highest expression level found in the testes [49].

The source of lysosomal cystine *in vivo* is uncertain. Because kidney proximal tubules are the first affected cells of the body, it has been suggested that the endocytosis of disulfide-reach plasma proteins with subsequent hydrolysis in lysosomes can lead to extensive cystine accumulation and early damage of proximal tubular cells. Blocking megalin-driven endocytosis in a mouse model of cystinosis substantially decreased crystal formation and prevented proximal tubular atrophy, providing a proof-of-concept that the megalin pathway can be a potential therapeutic target [50]. On the other hand, inhibiting the lysosomal membrane cysteine transporter MFSD12 responsible for the import of cytosolic cysteine to the lysosomal lumen substantially decreases lysosomal cystine accumulation in fibroblasts from patients in cystinosis, pointing that cytosolic cysteine is an important source of lysosomal cystine [51].

ATP Depletion

Prior to the development of the knockout mouse model, cystine-loaded proximal tubules were used to study renal pathogenesis in cystinosis [52, 53]. Whether cystinosis causes a direct alteration of mitochondrial ATP generation remains controversial [54–56]. While studies in skin fibroblasts and immortalized proximal tubular cells derived from cystinosis patients showed overall normal ATP generating capacity and unaltered activity of Na-K ATPase [57–60], decreased mitochondrial levels of cyclic-AMP, reduced complex I and V activity and altered mitochondrial morphology and dynamics were demonstrated and point to altered mitochondrial function [61, 62] (Fig. 30.2).

Increased Apoptosis

Another pathologic mechanism involved in cystinosis is an altered regulation of cell survival and death due to enhanced apoptosis.The rate of apoptosis is increased in fibroblasts and proximal tubular cells of patients with nephropathic cystinosis compared to normal cells [63, 64].

Enhanced apoptosis has been postulated to cause the specific atrophy of renal proximal convoluted tubules adjacent to glomeruli (“swan-neck” deformity), that has been demonstrated in cystinotic kidney biopsies starting from sixth month of age [65, 66]. Transcripts of pro-apoptotic caspase 1, caspase 4 and caspase 12 are increased in cystinotic tissues, with significant increase in caspase 3 activity being demonstrated in proximal tubular cells of cystinosis mouse model [67].

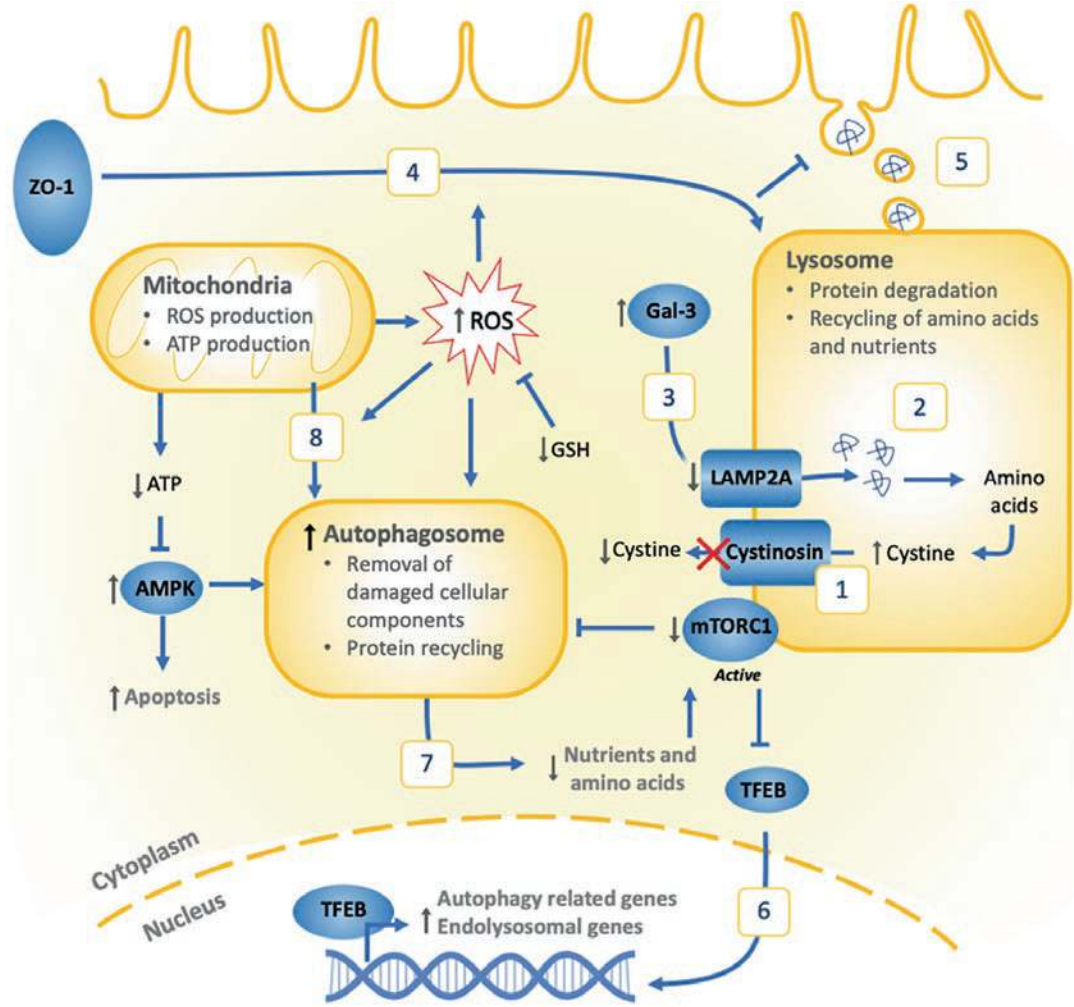

Fig. 30.2 Simplified scheme of the current knowledge on pathogenesis of cystinosis. (1) Dysfunctional cystinosin leads to the accumulation of cystine within the lumen of the lysosome. (2) Reduced protein degradation due to defective lysosomal enzyme activation. (3) Reduced chaperone-mediated autophagy leads to galectin-3 overexpression and has been associated with chronic kidney disease progression. (4) Phosphorylation of tight junction adapter protein ZO-1 results in its misrouting to endolysosomal compartments and disruption of tight junction integrity. (5) Disruption of tight junctions leads to epithelial dysfunction and dedifferentiation, repressing apical endocytic receptors and megalin/cubilin-mediated endocytosis. (6) Increased TFEB nuclear translocation by inhibiting mTOR activates transcription of autophagy related genes and can reduce cystine accumulation via increased exocytosis. (7) Reduced autophagic flux and degradation. (8) Abnormal mitophagy can lead to increased oxidative stress, further promoting epithelial dysfunction, dedifferentiation, and apoptosis. *AMPK* 5′ adenosine monophosphate-activated protein kinase, *ATP* adenosine triphosphate, *GSH* glutathione, *Gal-3* galectin-3, *LAMP2A* lysosome-associated membrane protein 2A, *mTORC1* mammalian target of rapamycin complex 1, *ROS* reactive oxygen species, *TFEB* transcription factor EB, *ZO-1* zonula occludens 1. (Reproduced with permission from Jamalpoort et al. Trends Mol Med 2021)

Altered Redox Homeostasis and Increased Oxidative Stress

Multiple studies have demonstrated that cystinosis cells are prone to oxidative stress and that the mitigation of oxidative stress is an important therapeutic target in cystinosis [68]. Cystine is composed of two molecules of cysteine and the cysteine/cystine couple represents one of the major cell thiol/disulfide systems involved in the regulation of the cell redox state. In the cytosole cystine is reduced to cysteine through electron transfer from the other major cell thiol/disulfide systems, mainly free and oxidized glutathione (GSH/GSSG) [69]. Altered GSH metabolism has been demonstrated in cystinosis cells with decreased GSH levels [58, 70], decreased GSH/GSSG ratio or inability to upregulate GSH synthesis upon oxidative stress [71]. These alterations might be a direct consequence of cystinosin dysfunction as cystinosin expression is regulated by the intracellular Cys/CySS redox state [72]. On top, mitochondrial dysfunction and altered autophagy of damaged mitochondria have been shown to increase the generation of reactive oxidative species (ROS) which can further exhaust the cellular anti-oxidative capacity and lead to cell damage [73]. Reducing lysosomal cystine accumulation with cysteamine interferes with this cascade, increases cellular glutathione levels, attenuates oxidative stress and decreases the rate of apoptosis promoting cell survival in cystinosis [60, 63, 64].

Altered Vesicle Trafficking and Cell Signaling

The fact that renal Fanconi syndrome is not fully responsive to cysteamine treatment suggested that not all cellular mechanisms of cystinosis are due to cystine accumulation and pointed to potential other functions of cystinosin [20, 68]. This hypothesis was substantiated by recent studies demonstrating that the absence of cystinosin led to alterations of cellular trafficking mechanisms, impaired cell signaling and autophagy (Fig. 30.2).

Altered vesicle movements with a predominance of slow moving lysosomes was found in murine and human cystinotic proximal tubular cells, accompanied by decreased expression of the small GTPase Rab27a [74]. In addition, altered autophagic flux was demonstrated in human cystinotic fibroblasts, proximal tubular cells and in patient kidney biopsies [75, 76].

Proximal tubular cells of the *ctns* $^{-/-}$ mouse showed signs of dedifferentiation with decreased expression of the endocytotic receptors megalin and cubilin and elevated expression of zonula occludens-1 (ZO-1)-associated nucleic acid-binding transcription factor (ZONAB) and proliferation markers PCNA and cyclin D1 characteristic of immature proximal tubular cells [77]. In line with the development of the "swan-neck" lesion demonstrated in human cystinotic kidneys [65], the first segment of proximal convoluted tubules in the *ctns* $^{-/-}$ mice was first affected with lesions gradually progressing towards more distal parts of the proximal tubule [78]. Increased oxidative stress might be a link between cystinosin dysfunction and altered endocytosis as it stimulates Gα12/Src-mediated phosphorylation of tight junction protein ZO-1 and triggers a signaling cascade involving ZO-1-associated Y-box factor ZONAB, which leads to dedifferentiation of proximal tubular cells, cell proliferation and transport defects [73]. It is still a working model without explanation for decreased transport for glucose, phosphate and other small solutes.

Altered mammalian target of rapamycin (mTOR) signaling might be another potential therapeutic target in cystinosis, unrelated to cystine accumulation. While an interaction between cystinosin and Vacuolar H+—ATPase-Ragulator-Rag complex controlling mTOR has been demonstrated [79], studies of mTOR activity in cystinosis cells provided inconsistent results [80, 81]. Nevertheless, inhibiting mTOR activity by everolimus reduced the number of large lysosomes, decreases apoptosis, and activates autoph-

agy in kidney organoids derived from *CTNS*-deficient human induced pluripotent stem cells [82]. The expression of transcription factor EB (TFEB), which inhibits mTOR activity, is reduced in cystinosis proximal tubular cells, and overexpressing TFEB improves the altered cellular phenotype [83]. Inhibiting mTOR stimulates nuclear transplocation of TFEB and can reduced cellular cystine accumulation by increasing exocytosis of cystine loaded lysosomes [68] (Fig. 30.2).

A drug-repositioning strategy combined with high-throughput screening showed that the flavonoid luteolin improves the lysosome-mediated degradation of the autophagy cargoes, restores lysosomal distribution, and stimulates endocytosis in cystinotic proximal tubular cells, opening a new therapeutic prospective in cystinosis [84].

Mechanism of Chronic Interstitial Damage

The progression of cystinotic nephropathy is characterized by the development of diffuse tubulo-interstitial lesions and fibrosis. Cystinosis is also associated with early podocyte dysfunction characterized by excessive losses of podocytes into urine and the development of glomerular proteinuria [81].

Reabsorption of excessively filtered proteins may contribute to the renal interstitial injury in analogy to other proteinuric conditions by stimulating local inflammatory response [85, 86]. Furthermore, higher circulating levels of pro-inflammatory interleukin-1β and interleukin-18 were attributed to inflammasome activation by cystine crystals [87]. Macrophage activation in the kidney is likely to contribute to the development of interstitial renal damage caused by cystine accumulation. Furthermore, studies have demonstrated an additional new role for cystinosin in inflammation through its interaction with the lectin and β-galactoside-binding protein family 21 galectin-3 (Gal-3), enhancing macrophage infiltration and CKD progression [88] (Fig. 30.2).

Clinical Presentation

Kidney Disease

Infantile nephropathic cystinosis is the most frequent cause of inherited renal Fanconi syndrome in childhood. The dysfunction of different proximal tubular transporters develops gradually after birth with aminoaciduria being present already during the first month of life [89]. Full-blown renal Fanconi syndrome characterized by excessive urinary excretion of amino acids, phosphate, bicarbonate, glucose, sodium, potassium, low molecular weight proteins and other solutes, handled in renal proximal tubules is usually present by the age of 6 months. Clinically, patients are asymptomatic at birth and develop normally until 3–6 months, when they manifest with failure to thrive, vomiting, constipation, polyuria and excessive thirst, periods of dehydration and sometimes rickets [90, 91]. Growth retardation in case of late diagnosis may reach −4 SD [90]. Renal loss of sodium and potassium can result in hyponatremia and hypokalemia, which may be life threatening. Hypouricemia, decreased plasma carnitine and medullary nephrocalcinosis related to increased calcium excretion are also observed [92, 93]. Total protein excretion can reach several grams per day and contains both low molecular weight proteins, albumin and high molecular weight proteins [85].

In untreated patients glomerular filtration declines gradually and progresses towards kidney failure before the age of 10 years [94, 95]. At start of kidney replacement therapy patients with cystinosis have better blood pressure control and lower serum phosphate levels compared to patients with end stage kidney disease (ESKD) due to other causes because of still ongoing renal Fanconi syndrome with polyuria and urinary sodium and phosphate losses [96].

Renal Pathology

The age at which the first morphological changes appear in the kidneys of patients with cystinosis

is unknown. No significant renal changes were observed in the fetus [97]. Serial renal biopsies in two cystinotic patients demonstrated that the typical "swan-neck" deformity of proximal convoluted tubules appeared only after 6 months of life [65]. In a large series of kidney specimens, the most striking feature were the marked irregularities of renal tubular cells with the presence of flat cells with focal disappearance of the brush border and very large cells with a prominent and hyperchromatic cytoplasm (Fig. 30.3a) [97, 98]. Glomeruli can appear normal but most contain peculiar giant multinucleated podocytes (Fig. 30.3a) [85]. Podocyte foot process effacement seen in other proteinuric disorders is also present in cystinosis (Fig. 30.3b) [85]. Cystine crystals located in the lysosomes or in cytoplasm are seen mostly within interstitial cells and rarely within podocytes [97]. Patients with late-onset or juvenile cystinosis can demonstrate focal and segmental glomerular sclerosis (FSGS) undistinguishable from idiopathic FSGS [39].

The deterioration of kidney function is accompanied by progressive tubulo-interstitial lesions, including interstitial fibrosis, tubular atrophy and marked arteriolar thickening. The progressive glomerular damage, leading to increasing albuminuria and hematuria, consists of segmental or global collapse of the capillary tuft, accumulation of mesangial matrix material, and, observed by electron microscopy, irregular thickening of glomerular basement membrane [97].

In transplanted kidneys cystine crystals, sometimes seen at graft biopsy, have no clinical relevance as they are present in the host mononuclear cells [99].

Kidney Transplantation

Kidney transplantation is the best therapeutic option for ESKD in young patients [100]. In pediatric cohorts, the outcome of kidney transplantation in patients with cystinosis is generally better than that of other patients undergoing transplantation [96, 101–103]. In adults, a similar rate of graft survival at 5 and 10 years among patients with cystinosis and control patients has been reported [104]. Furthermore, long term graft survival is higher in patients with cystinosis compared to controls by multivariate analysis. Proximal tubular disease does not occur in the transplanted kidney because the metabolic disorder is not present in the engrafted kidney. However, retention of a native kidney can result in the persistence of renal tubular Fanconi syndrome. Renal transplantation does not correct the systemic meta-

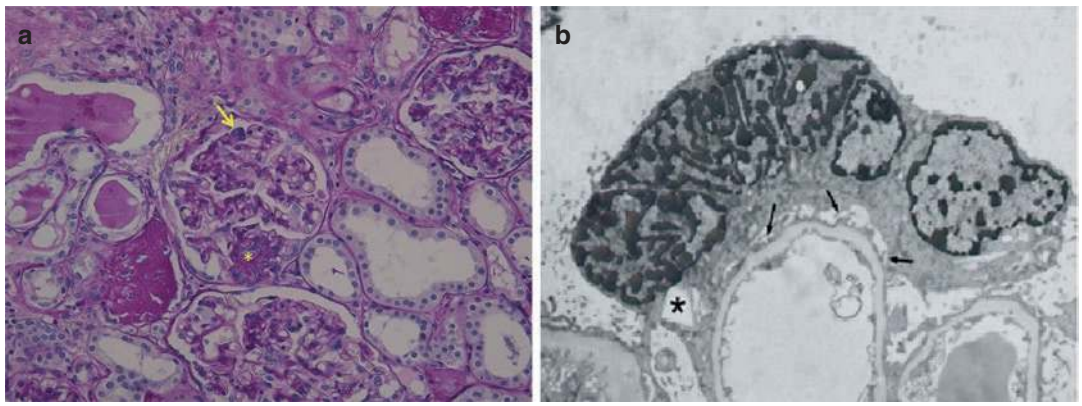

Fig. 30.3 Renal pathology in cystinosis. (**a**) Renal tissue of a 9-year-old patient with cystinosis. Some glomeruli contain giant multinucleated podocytes (yellow arrow) or show FSGS lesions (yellow asterisk). The tubules are often delineated by cuboidal cells. PAS, original magnification ×200 (Courtesy of Prof. Dr. E. Lerut, Department of Pathology, University Hospitals Leuven); (**b**) Electron microscopy of cystinotic podocyte showing multinucleation which is pathognomonic for cystinosis. Black arrows indicate podocyte foot process effacement. Black asterisk indicate cystine crystal in the cytoplasm (Used with permission of Elsevier from Wilmer et al. [85])

bolic defect of cystinosis and cystine continues to accumulate in most organs and may lead to late systemic complications.

Patients with cystinosis are at risk of developing post-transplant diabetes. However, the risk of diabetes is not increased compared with control patients [104]. Immunosuppressive maintenance treatment regimens should be equivalent between patients with or without cystinosis.

Extra-renal Involvement

Ocular Impairment

Accumulation of cystine crystals occurs in all ocular structures, including the cornea, the conjunctiva, the iris and the retina causing symptoms of photophobia, blepharospasm and other complications. The corneal crystals, pathognomonic of the disorder, are absent at birth, but generally may be visible on ophthalmological investigation from around 16 months of age in most patients with cystinosis (Fig. 30.4a). If untreated, patients develop increasingly severe photophobia, refractory blepharospasm, peripheral corneal neovascularisation and band keratopathy, eventually progressing to involvement of the posterior segment of the eye with hypopigmentary mottling of the retinal pigment epithelium, glaucoma and visual impairment [105–107].

Novel techniques such as *in vivo* confocal microscopy and anterior segment optical coherence tomography of the cornea allow not only to visualize corneal cystine crystals with high sensitivity, but also to quantify the crystals and the

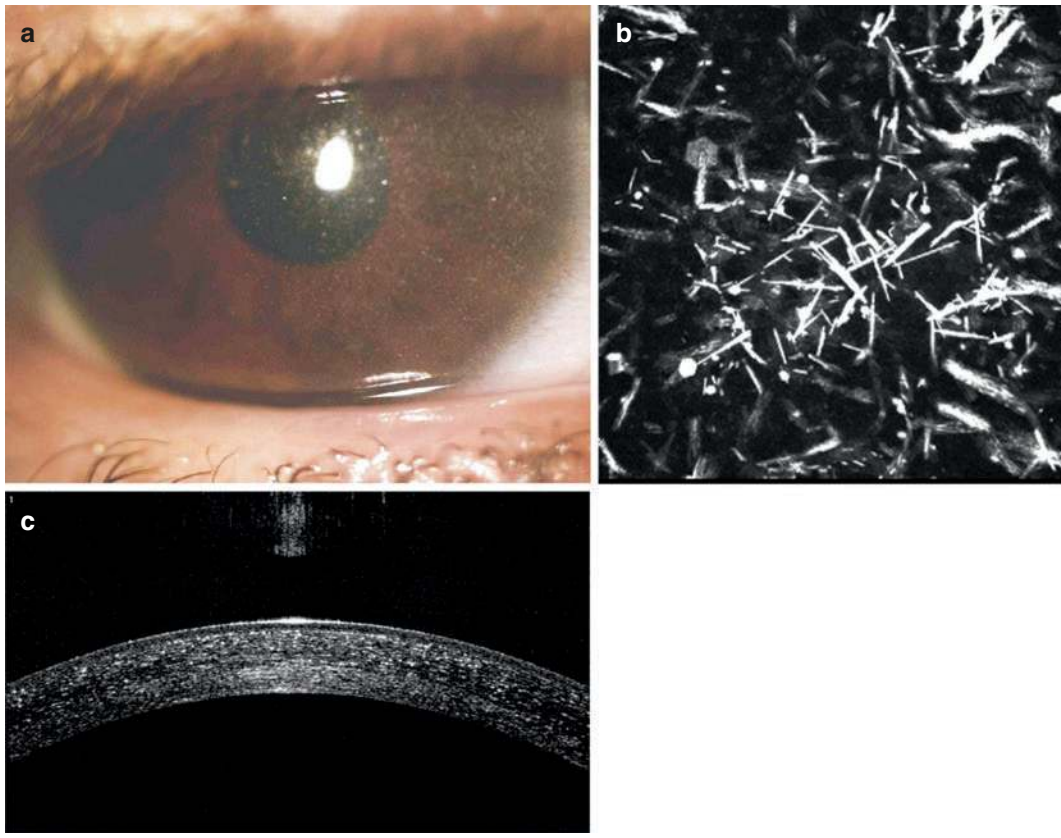

Fig. 30.4 Eye examination of cystinosis. (**a**) Slit-lamp photography of corneal cystine crystals. Corneal crystals typically appear as needle-shaped and highly reflective. (**b**) *In vivo* confocal microscopy images of crystals detected in the cornea stroma. (**c**) Anterior-Segment Optical Coherence Tomography (OCT) Z-axis: infiltration of crystals in depth of the cornea. (Courtesy of Dr Liang, Hôpital des Quinze Vingt, Paris, France)

thickness of the cornea, improving the longitudinal monitoring of the patients (Fig. 30.4b). The prevalence of anterior segment complications increases with age [108].

Growth Impairment

Growth retardation is severe in untreated cystinosis compared to other patients with renal failure [96, 109]. In historical cohorts of patients not treated with cysteamine, only very few patients attained an adult height >150 cm [110]. The causes of growth retardation are multi-factorial and encompass poor nutrition, metabolic disturbances due to renal Fanconi syndrome, rickets, renal function impairment and possibly a direct effect of cystinosis on the bone formation.

Significantly improved linear growth is associated with early use of cysteamine [12, 35, 111]. However, cysteamine does not induce catch-up growth in children who are already growth retarded [111, 112]. Growth hormone treatment is indicated in patients in whom adequate feeding, good metabolic control and cysteamine treatment fail to normalize growth even if renal function is still not severely compromised [108]. Most of the catch-up growth occurs during the first 3 years of treatment [108]. Long-term GH treatment improves final height to a range of −2.6 to −2 SD in the majority of patients [112, 113].

Endocrine Impairment

The continuing accumulation of cystine crystals leads to the impairment of endocrine organs. Cystine accumulation in thyroid follicular cells causes fibrosis, atrophy and dysfunction of the thyroid gland.

Thyroid impairment starts with a compensated hypothyroidism defined by a high TSH but a free T4 still in the normal range, followed by a confirmed hypothyroidism with clinical symptoms [114]. Hypothyroidism has been found in more than 70% of adult patients In historical cystinosis cohorts [36, 37]. Treatment requires thyroxine supplementation.

Cystine also accumulates in the bêta cells of the islets of Langerhans with massive crystal deposits in the pancreas and complete architectural disorganisation. Glucose intolerance is characterized by a slow, progressive loss of insulin secretion and C-peptide production [115]. Hypothyroidism and diabetes are less frequent and delayed when cysteamine has been administered early [36, 37, 107].

While puberty generally proceeds normally in females, it is delayed in males, who may show primary hypogonadism and do not always complete pubertal development [116]. Testosterone treatment allows puberty but does not prevent infertility. Azoospermia is present in almost all males with infantile cytinosis, even in those treated with cysteamine started from early age and maintaining normal hormonal tests, and is caused by obstruction at the level of epididymis [117, 118]. Testicular fibrosis without germinal dysplasia and with sufficient spermatogenesis is observed [117]. One successful conception induced by a cystinosis male has been reported through assisted reproductive technology [13]. Several females with cystinosis have given birth [119, 120].

Central Nervous System and Muscle Impairment

Although rare, central nervous system complications have been described in adults with cystinosis [107, 121–124]. In these patients, the occurrence of central neurological complications did not correlate with other extra-renal complications of cystinosis, but their frequency correlated with patient age in untreated patients. Cystine is increased in all parts of the brain, especially in basal ganglia and in the arterial walls.

Two forms were observed: a cystinosis encephalopathy [121, 124, 125] and stroke-like episodes. Clinical encephalopathy often started with cerebellar signs and/or motor difficulties, mainly of the lower limbs. Decrease of oral expression was also characteristic [121, 124, 125]. Motor coordination difficulties and muscular hypotonia were initially recognized and then

corroborated with more recent studies of motor performance [126–128]. The other form of central nervous system complications resembled stroke-like episodes with coma and hemiplegia or milder symptoms. These symptoms may still be observed in adult patients, in particular with poor adherence to treatment [129]. Idiopathic intracranial hypertension has also been described, associated with headaches, or without clinical manifestation, and might be revealed during a routine ophthalmic examination [121, 124, 130].

By computed tomography (CT) scan or magnetic resonance imaging (MRI), cortical atrophy is observed in all patients with central nervous system symptoms [121, 124]. Cerebral atrophy is also reported in patients without gross central nervous system clinical abnormalities [129, 131] and in patients with minor alterations in cognitive performance, particularly those with impairment of visual memory [132]. Gahl et al. observed a 22% incidence of cerebral calcifications in a large retrospective cohort of cystinosis patients, but this was not confirmed in a more recent cohort [129, 133].

Neuro-imaging studies by MRI showed selective changes in cerebral white matter early during the development [134]. Children with cystinosis evidenced diminutions in mean fractional anisotropy and corresponding elevation in mean diffusivity in component areas of the dorsal visual pathway. In adults, a neuro-imaging study showed a significant grey matter decrease in the middle frontal gyrus compared with healthy controls and a significant negative correlation between the cystine blood level and rest cerebral blood flow was observed in the right superior frontal gyrus, a region associated with executive function [129].

Cystine also accumulates in muscular tissue and distal myopathy is found in 50–70% of patients in historical cohorts. Hand muscles are mostly initially affected (Fig. 30.5) [36, 37, 133]. In addition Vester et al. demonstrated electrophysiological signs of myopathy in patients with cystinosis without obvious weakness [135]. This distal myopathy also results in an extra-parenchymal pattern of restrictive lung disease with inspiratory and expiratory dysfunction in adults who have not received long-term cystine depletion [136]. Gahl et al. have shown that pulmonary dysfunction decreased in frequency and severity with time on cysteamine therapy, but very few patients had been treated for more than 20 years [133]. Dysphagia and swallowing difficulties for solid food appear to develop with age in nearly all patients with cystinosis [137, 138]. In a historical cohort severity increased with the number of years without cysteamine therapy. Swallowing dysfunction in cystinosis correlates with the presence of muscle atrophy and presents a risk of fatal aspiration or aspiration pneumonia.

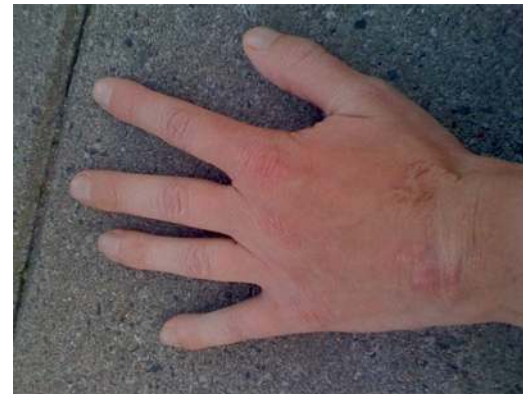

Fig. 30.5 Hand muscle atrophy in a 18-year-old cystinosis patient

Intelligence and School Performance

Clinical studies have shown that patients have generally normal intelligence but may have mild neurocognitive abnormalities [127, 128, 139], with specific impairments in the processing of visual information. Relative weakness was found in visual motor, visual spatial and visual memory skills, and may be associated with academic difficulties, primarily in arithmetic. A fine-motor coordination deficit in children and adolescents with cystinosis has also been documented [126].

Gastro-intestinal Disease

Cystine excessively accumulates in the stomach and in the intestine of cystinosis patients [140].

However, nausea and vomiting are mostly caused by cysteamine treatment due to increased gastric acid secretion [141].

Hepatosplenic complications have become rare with the routine use of cysteamine [36, 37]. Liver enlargement with normal synthetic function was a frequent finding in historical cohorts, while hepatic fibrosis with portal hypertension and hypersplenism, eventually causing bleeding problems, and cholestatic liver disease due to nodular hyperplasia can still be observed. Electron microscopy showed extensive cystine crystal accumulation in Kŭppfer cells [110, 142, 143].

Bone Disease

Bone impairment is also described in patients with cystinosis. International recommendations concerning the management of cystinosis associated bone disease have been published in 2019 [144]. Even though the exact pathophysiology of bone impairment remains unclear, at least five distinct but complementary entities can explain it: consequences of renal Fanconi syndrome, malnutrition and copper deficiency, hormonal disturbances, myopathy, and intrinsic bone defects [144, 145]. Bone complications have a significant impact on patients' quality of life due to an increased frequency of bone pain, deformations and fractures occurring in late teenage and early adulthood [146–148].

Other Clinical Features

Diminished skin, hair and iris pigmentation is frequently observed in cystinosis patients and is explained by reduced melanin synthesis [149]. Impaired sweating has been reported in some patients. Premature skin aging is a typical feature in adult patients [150, 151].

Cystine crystals are found in the bone marrow of the patients, but has usually they have few consequences for the hematopoiesis, although some patients with bi-or pancytopenia have been reported [152, 153].

Cystinosis in Adults

Renal transplantation has transformed cystinosis from a fatal pediatric disease into a multisystem adult disease. The series of patients described in the 1990s revealed a high rate of mortality and morbidity. The main causes of death occurring before 35 years of age were aspiration, pseudobulbar palsy and uremia. The most incapacitating morbidities included blindness or severely impaired vision, and swallowing difficulties due to myopathy [122]. The long-term prognosis of patients has been substantially improved during the past two decades due to the administration of cysteamine with reduced morbidity and mortality rates [107, 133, 154], especially in patients in whom cysteamine was started before the age of 5 years [36, 37]. Transition from pediatric to adult care providers remains an area of concern due to the generally lacking expertise of internists-nephrologists with this ultra-rare disorder with historically rare survival into adulthood. Good communication between pediatric and internists-nephrologists is required to guarantee the continuation of adequate medical care [155, 156]. Management of systemic disease involvement is mandatory and requires a multi-disciplinary team.

Late Onset and Ocular Forms of Cystinosis

Less severe clinical forms of cystinosis account for less than 5% of all patients. Patients with the nephropathic late-onset form (MIM #219900) manifest with a spectrum of symptoms, varying from a milder proximal tubulopathy to apparent nephrotic syndrome, focal segmental histological lesions and generally have a slower rate of renal disease progression [2, 39, 41, 157, 158]. Cystine crystals accumulate also in the cornea and are diagnostic. In terms of the age at presentation there is probably a continuum between the infantile and the late-onset forms; however, most of the described patients were older than 10 years. Extra-renal organs may also be affected in these patients and progression towards end stage kidney disease may also occur.

Ocular non-nephropathic cystinosis (MIM #219750) affects the cornea with cystine crystal deposits causing photophobia. The kidneys, retina and other organs are clinically spared in these patients, but they do have elevated cystine leukocyte content and cystine crystals in bone marrow [23, 159]. The co-existence of the ocular form with late-onset nephropathy in the same family has been reported, warranting monitoring of kidney function in patients with ocular cystinosis [39].

Diagnosis

The diagnosis of cystinosis has to be suspected in all infants and children presenting with renal Fanconi syndrome. Other inherited and acquired forms of Fanconi syndrome should be excluded. Urine dipstick usually shows low specific gravity, overt glucosuria and mild albuminuria. Serum creatinine is generally normal in young children, unless patients are dehydrated [2].

The diagnosis is based on the measurement of elevated white blood cell (WBC) cystine levels. The isolation of polymorphonuclear (PMN) leukocytes is recommended for WBC cystine determination because cystine preferentially accumulates in this type of blood cells and not in the lymphocytes [160, 161]. It is important at a younger age when lymphocytes are the predominant cells type (up to about 60% of WBC) until the age of 5–7 years when neutrophils predominate. As leukocyte isolation and cystine measurement (by either high performance liquid chromatography or tandem mass spectrometry) require specific expertise, this analysis should only be performed in specifically certified laboratories. Cystine values in healthy individuals, heterozygous subjects, patients at diagnosis and under cysteamine therapy are provided in Table 30.1. The detection of corneal cystine crystals is pathognomonic for the diagnosis in patients above the age of 1.5 years. Molecular analysis of the *CTNS* gene should be performed for confirming the diagnosis and genetic counselling of the families [162]. Including cystinosis in the list of diseases to be detected via neonatal screening programs using next generation sequencing (NGS) in on the research agenda in many countries. The feasibility of this approach and the benefit for the patients' prognosis has been demonstrated [163, 164] (Fig. 30.6).

Table 30.1 Reference values for intracellular cystine

Healthy subjects	
PMN leukocytes	0.04–0.16 nmol cystine/mg protein [a]
Mixed Leukocytes	0.05–0.17 nmol cystine/mg protein [a]
Fibroblasts	0.0–0.23 nmol cystine/mg protein [a]
Patients at diagnosis	
PMN leukocytes	>2 nmol cystine/mg protein
Heterozygotes	
PMN leukocytes	0.14–0.57 nmol cystine/mg protein
Patients under cysteamine therapy	
PMN leukocytes	<1 nmol cystine/mg protein

[a]Values are provided by Laboratory of Genetic and Metabolic diseases, Radboud University Nijmegen Medical Centre, The Netherlands and presented as Percentile 5 and 95

Cystine is frequently expressed as nmol ½ cystine per mg protein. Conversion factor ½ cystine/mg protein = 2× cystine/mg protein

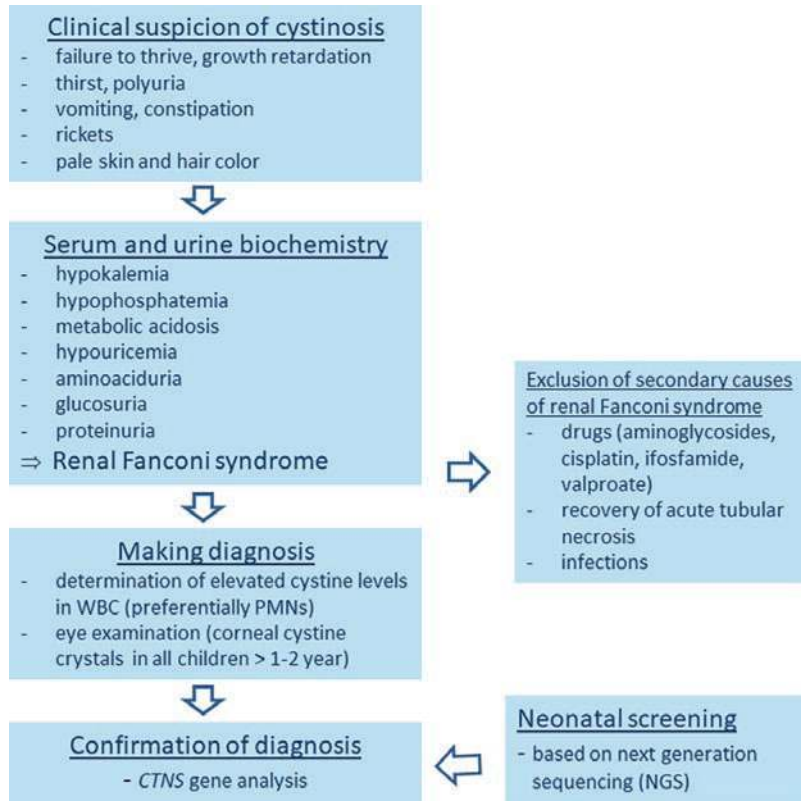

Fig. 30.6 Proposed diagnosis algorithm for cystinosis. Other genetic, metabolic and secondary causes of renal Fanconi syndrome should be excluded (see Chap. 32 for differential diagnosis of renal Fanconi syndrome)

Treatment

Treatment of cystinosis includes adequate feeding, the symptomatic replacement of substances lost into urine due to renal Fanconi syndrome, hormone replacement therapy and specific cystine lowering therapy with cysteamine. Kidney replacement therapy in cystinosis patients reaching ESKD is not different compared with other underlying diseases and is beyond the scope of this chapter. Both hemodialysis and peritoneal dialysis can be successfully applied.

Feeding Recommendations

Early gastric tube placement should be considered in all children who have poor appetite or frequent vomiting [165]. Gastric tubes also facilitate administration of oral medications and fluid. Patients with frequent vomiting and gastroesophageal reflux may respond to proton pump inhibitor therapy [166]. Overall, children should receive at least 100% of the recommended dietary allowance (RDA) for their age; however, no current evidence indicates that any higher caloric intake is useful. The composition of the diet should be balanced and should include salt and fluid supplementation.

Symptomatic Therapy of Renal Fanconi Syndrome

Patients with cystinosis should have free access to water and toilet privilege, because of pronounced polyuria and polydipsia. Prolonged exposure to heat and sun should be avoided because to photophobia, and the risk of dehydration and heat stroke due to impaired sweating.

Symptomatic therapy aims to maintain fluid and electrolyte balance, to prevent rickets and to

Table 30.2 Symptomatic therapy of renal Fanconi syndrome in cystinosis

Drugs [a]	Doses
Sodium potassium citrate	
Suspension (Na 50 g = 1.2 mmol/mL, K 55 g = 1.1 mmol/mL, Citrate 33.5 g = 2.3 mmol/mL, sir Simplex ad 500 mL)	2–10 mmol K/kg/day, divided in 4 doses
Potassium citrate	
Powder (100 mg: K 37 mg = 1 mmol, Citric Acid 62 mg = 0.3 mmol)	2–10 mmol K/kg/day, divided in 4 doses
Sodium bicarbonate	
Suspension 8.4% (Na 1 mmol/mL, bicarbonate 1 mmol/mL)	2–15 mmol/kg/day, divided in 4 doses
Powder (100 mg: Na 27 mg = 1.2 mmol, bicarbonate 73 mg = 1.2 mmol)	
Potasium phosphate	
Suspension (K dihydrophosphate/K_2 hydrophosphate) 7.5 g, aqua ad 100 mL (K 39 mg = 1 mmol/ml, phosphate 18 mg = 0.6 mmol/mL	0.6–2 mmol phosphate/kg/day, divided in 4 doses
Powder (100 mg: K 52 mg = 1.3 mmol, phosphate 23 mg = 0.6 mmol)	
L-carnitine	
Suspension 100 mg/mL	20–30 mg/kg/day, divided in 3 doses
Tablets 330 mg	
Indomethacin	
Suspension 5 mg/mL	0.5–3 mg/kg/day, divided in 2–3 doses
Vitamin D	
1 alpha-hydroxycholecalciferol	0.5–2 μg/day in one dose

improve growth. An overview of frequently prescribed drugs is shown in Table 30.2. Excessive administration of phosphate, 1,25-dihydroxycholecalciferol and bicarbonate is not indicated as it may aggravate nephrocalcinosis and stimulate renal stone formation [93]. A decrease of the renal function requires adaptation of the doses.

The aim of symptomatic therapy is to maintain serum potassium levels >3 mmol/L, serum bicarbonate level 22–25 mmol/L, serum phosphate level 0.8–1.6 mmol/L with normal for age levels of alkaline phosphatases. The administration of high doses of phosphate and vitamin D can increase nephrocalcinosis. Blood for electrolyte measurements should preferentially be drawn before the next dose of electrolyte supplements as their levels fluctuate substantially during 24 h. Carnitine replacement normalizes plasma and muscular carnitine levels; however, it is not established whether carnitine administration results in improved muscular performance [167, 168]. The recommended dose of 50 mg/kg causes changes in plasma acetylcarnitine and several short and medium-chain acylcarnitines indicating oversupplementation [169]. In patients with muscular complaints lower doses of carnitine (20–30 mg/kg) might be considered, but no clinical evidence supports carnitine supplementation in all patients.

Maintaining fluid and electrolyte balance in young patients can be challenging and can be improved by the administration of indomethacin [170]. Indomethacin is a non-selective inhibitor of cyclooxygenase (Cox). The Cox-1 and Cox-2 metabolites have a direct effect on salt and water transport along the nephron. Indomethacin increases NaCl reabsorption in the medullary ascending limb and in the collecting duct and augments the antidiuretic effect of the antidiuretic hormone. These effects compensate for the proximal tubular losses [170].

The inhibition of renin-angiotensin-aldosterone system (RAAS) decreases albuminuria [171] and might improve renal function survival [112]; however, this class of drugs should be used with caution, because of the risk of hypotension and renal function decline in patients having extracellular volume and salt depletion. Concomitant administration of RAAS inhibitors and indomethacin is contra-indicated to avoid acute kidney injury. Of note, in a large European cohort of cystinosis patients no beneficial effect of RAAS inhibitors on kidney function survival could be demonstrated [35]. Long-term administration of indomethacin didn't have a detrimental effect on the kidney function [35].

Hormone Replacement Therapy

Treatment with recombinant GH improves growth in children with cystinosis, allowing them

to catch-up and to maintain normal growth velocity [13]. Hence, GH therapy should be considered in children with cystinosis and subnormal growth rates even in the presence of a still normal glomerular filtration rate [109]. Levothyroxine is indicated in patients with hypothyroidism, as is insulin in case of diabetes. Testosterone supplementation should be considered in male patients with hypogonadism.

Specific Treatment with Cysteamine

Cysteamine, first introduced for the treatment of cystinosis in 1976 [172] and approved for clinical use in the 1990s, is still the only available treatment that efficiently decreases lysosomal cystine accumulation [173]. Inside lysosomes the drug reacts with cystine and breaks it into cysteine and cysteamine-cysteine mixed disulfide that can exit the lysosome via transporters other than the defective cystinosin (Fig. 30.7). Cysteamine therapy has fundamentally transformed the prognosis of the disease. In patients born between 1970 and 2000, early initiation of cysteamine delayed end stage renal disease by ~6 to 10 years [35, 36, 96, 112]. In well-treated patients in whom cysteamine was administered before the age of 2 years, renal Fanconi syndrome could be attenuated and renal function remained preserved until young adult age [36, 112, 174]. Cysteamine treatment has beneficial effects also on extra-renal symp-

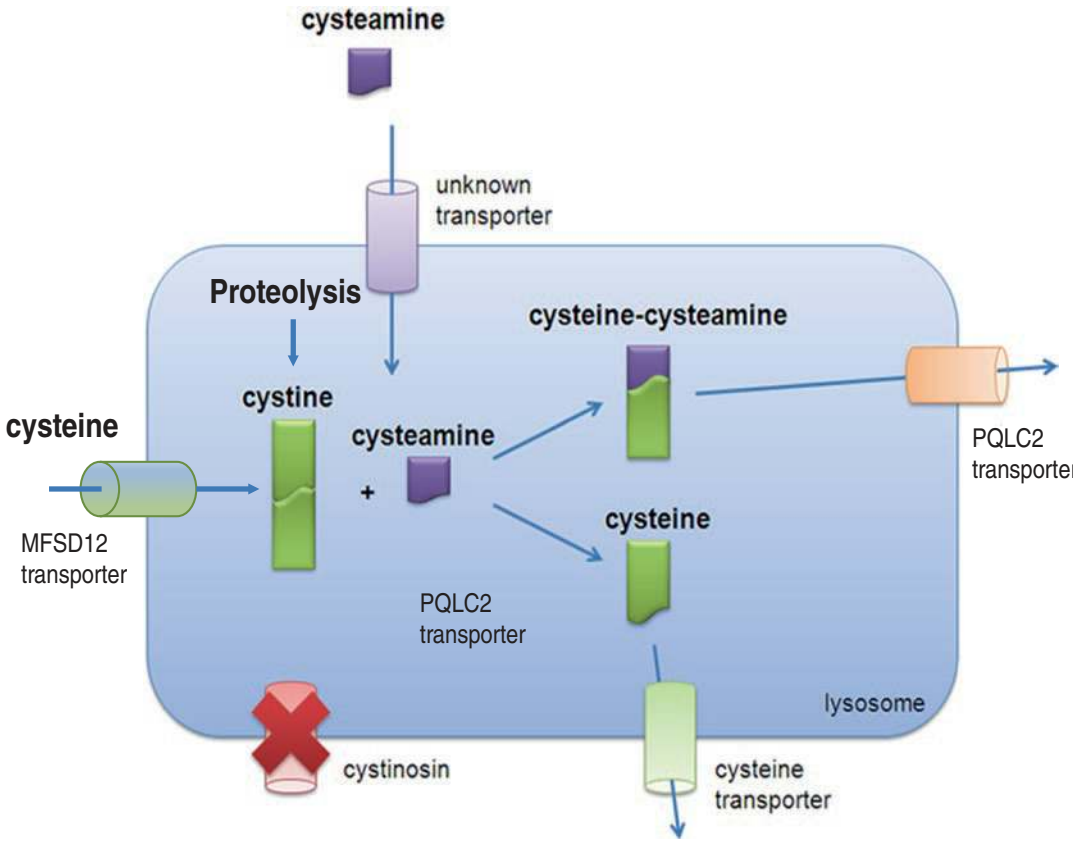

Fig. 30.7 Schematic drawing of cysteamine action in cystinotic cells. Cysteamine can enter the lysosome trough an unknown transporter. Once inside the lysosome it breaks the disulfide bond in cystine, leading to the formation of cysteine and a cysteamine-cysteine disulfide which can leave the lysosome trough the cysteine and PQLC2 transporters respectively. Lysosomal cystine is formed by the proteolytic degradation of proteins and by oxidation of cytosolic cysteine which enters lysosomes via MFSD12 transporter [51] (Used with permission of Elsevier from Besouw et al. [173])

toms of cystinosis with decreased frequencies of hypothyroidism, diabetes, myopathy, pulmonary dysfunction, swallowing difficulties, and death [36, 133, 154, 155, 175, 176]. The drug also prevents the development of visual loss due to retinopathy [105, 177].

The most widely used cysteamine salt, cysteamine bitartrate, is available as an immediate release (Cystagon®) and as a delayed release (Procysbi®) preparations. Cystagon® has to be administered every 6 h including the night period [178]. Procysbi® is a micro-spheronized enteric-coated cysteamine bitartrate with an improved pharmacokinetic profile, allowing twice daily dosing. Non-inferiority of the effect of the delayed release formulation compared to immediate-release cysteamine on WBC cystine levels has been demonstrated [179, 180]. The recommended daily dose of cysteamine is 1.3 g/m^2 of cysteamine base (or 2 g/day in patients older than 12 years). The maximum cysteamine dose should not exceed 1.95 g/m^2/day. To avoid the development of side-effects cysteamine has to be started at ~1/6 of the target dose and gradually increased over 6–8 weeks. Patients using delayed release formulation show better adherence to treatment compared with immediate release preparation ([181], see below)

WBC cystine levels are used as a biomarker to monitor the effectiveness of cystine depletion; the upper limit of asymptomatic heterozygous carries (<1 nmol ½ cystine/mg protein) is usually considered as an indication for adequate cysteamine dosing (Table 30.1). WBC cystine levels have to be measured 6 h after last dose of immediate-release cysteamine or ideally 30 min after last dose for delayed-release cysteamine, however, a pre-dose sampling is frequently performed in routine practice.

The most commonly reported side effects comprise gastro-intestinal complaints, for which the concomitant use of proton pump inhibitors is advised [141, 166] although reducing the acidity of the stomach will influence the pharmacokinetics of Procysbi®. Gastrointestinal tolerability may be improved with the slow-release cysteamine formulation [179].

Cysteamine causes disagreeable breath and sweat odor due to the conversion of cysteamine to methanethiol and dimethylsulphide [182]. This side effect may lead to significant psychosocial issues, which sometimes limit the long-term compliance with the medication especially during adolescence [181, 183].

Three cystinosis patients with lupus nephritis while receiving cysteamine were reported [173]. Eight cystinosis patients, mostly treated with high cysteamine doses, developed skin adverse events, consisting of vascular proliferative lesions on the stretchable skin surfaces, striae and severe bone and muscular pains (Fig. 30.8a, b) [184]. An abnormal morphology of dermal collagen fibers was found on EM in these patients and suggested to be caused by copper deficiency due to urinary losses that could inhibit collagen cross-linking (Fig. 30.8b) [185].

Animal studies showed an increased risk for intrauterine death, intrauterine growth retardation and fetal malformations (especially cleft palate and kyphosis) in pregnant rats given high cysteamine doses of 100 and 150 mg/kg [186]. While human teratogenicity information is lacking, FDA classified cysteamine as a category C drug based on the animal data. It is advised in female cystinosis patients to stop cysteamine administration at the diagnosis of pregnancy until the baby is born, although one normal pregnancy in a cystinosis female receiving 900 mg cysteamine per day has been reported [187].

Cysteamine Eye Drops

Corneal cystine accumulation is resistant to oral cysteamine and must be treated by topical administration of cysteamine eye drops, which dissolve corneal cystine crystals and alleviate symptoms at all ages [105, 188]. Cysteamine collyrium containing 0.55% cysteamine base needs to be administered six to ten times daily [188]. A commercial 0.44% cysteamine ophthalmic solution (Cystaran®, Sigma-Tau Pharmaceuticals, Gaithersburg, MD, USA) has been approved for clinical use in the US and should be administered 6–12 times per day. In this formulation, cyste-

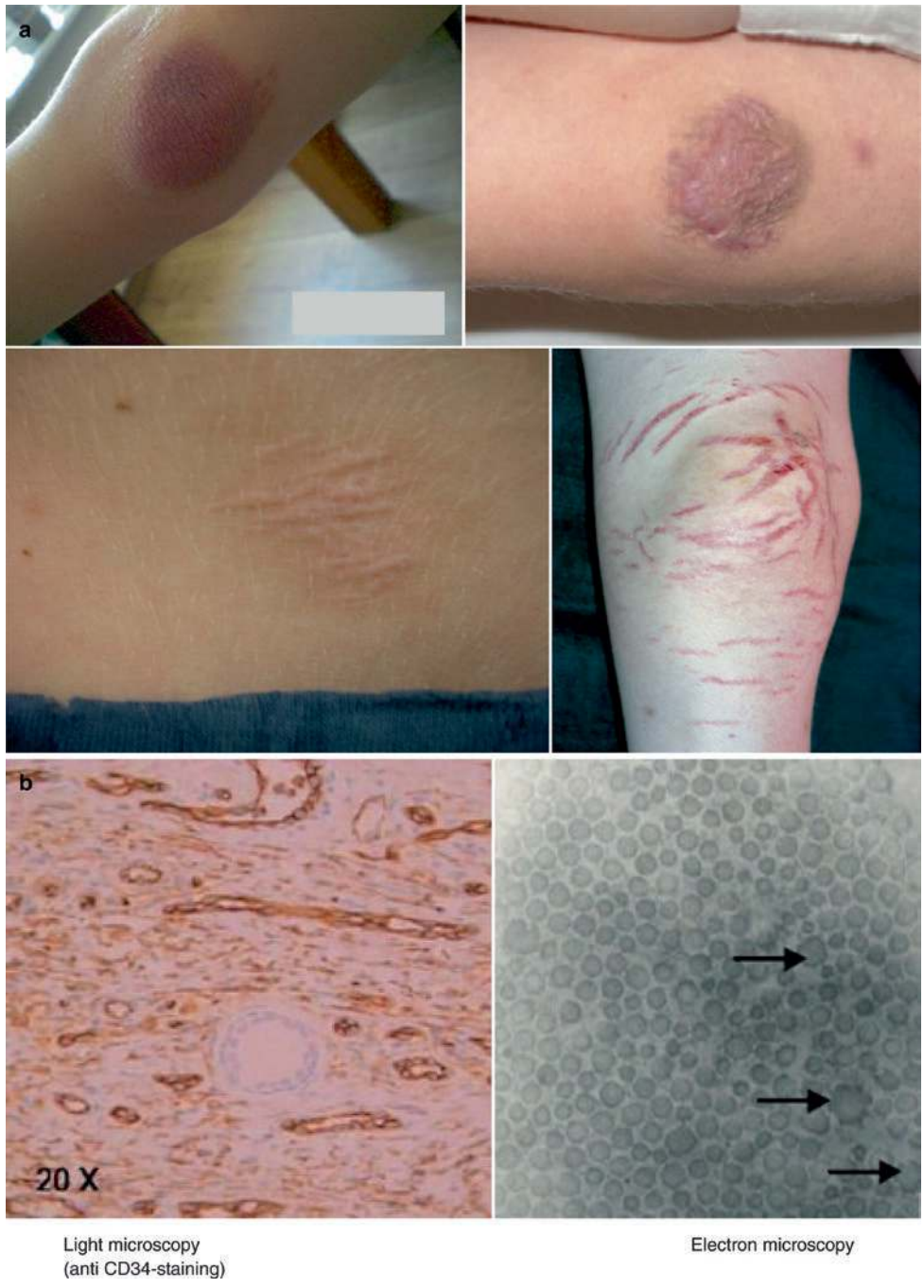

Fig. 30.8 Cutaneous signs of cysteamine toxicity. (**a**) Upper row: Bruise-like lesion at the elbow at early stage (left) and in regression (after the decrease of cysteamine dose) (right). Lower row: the lesion above lumbar vertebrae in regression, the skin is becoming loose and wrinkly (left); skin striae at the stretchable surface of the knee (right). (**b**) Skin biopsy from the elbow lesion. Left: light microscopy, anti-CD34 immuno-staining, original magnification ×20. Plump CD34-positive endothelial cells line numerous vascular structures, some of them completely developed and others with immature features. The CD34− negative sweat gland epithelium serves as negative control. Right: electron microscopy. Variability of collegan fibre caliber: focal diameter increase (arrows) (Used with permission of Elsevier from Besouw et al. [184])

amine oxidises at room temperature, necessitating cold storage. Another topical 0.55% cysteamine gel formulation (Cystadrops® Recordati Rare Diseases, Puteaux, France) for less frequent (three to four times) daily administration has been proven to be safe, efficacious and tolerable [189, 190]. The chemical stability of the formulation was improved, allowing the 0.55% cysteamine drops to be kept at room temperature for up to 7 days after opening, although refrigeration is still required for long-term storage. Eye drop administration can cause eye burning due to the low pH of the solution, especially in patients with corneal erosions. Using hydrating eye drops prior to cysteamine might improve discomfort related to cysteamine eye drop administration.

Prognosis and Future Treatments

The overall prognosis in patients with cystinosis has dramatically improved during the past decades due to the availability of renal replacement therapy and wide use of cysteamine therapy starting from the 1990s. The oldest patients with infantile cystinosis are reaching now their 50th and sometimes 60th birthday.

Although cysteamine treatment has substantially improved the prognosis of cystinosis patients, the drug offers no cure of the disease. Therefore continued efforts are undertaken to develop more definitive therapies. In a mouse model of cystinosis, allogeneic hematopoietic stem cell (HSC) transplantation significantly reduced cystine tissue content in all tested organs (by 70% in the kidney) and resulted in long-term preservation of kidney function [191, 192]. HSCs differentiate into tissue-resident macrophages forming tunneling nanotubes transferring cystinosin-bearing lysosomes into CTNS-deficient cells, even crossing tubular basement membrane. More recently, autologous HSC transplantation after *ex vivo* lentiviral *CTNS* gene transduction and subsequent injection has proven to be efficient in cystinosis mice [193]. Allogenic full HLA-matched HSC transplantation in one cystinosis patient resulted a temporary stabilization of the kidney function and demonstrated *de novo* expression of wild type cystinosin in different tissues of the recipient. The procedure, however, was complicated by therapy-resistant graft-versus-host disease with fatal outcome [194]. A clinical trial evaluating the safety and efficiency of autologous HSC transplantation after *ex vivo* gene therapy in humans is ongoing (https://clinicaltrials.gov/ct2/show/NCT03897361).

Novel pharmacological therapies targeting cystine accumulation and/or other altered pathways in cystinosis cells are currently at different stages of development and have been reviewed elsewehere [68]. It can be expected that during the next decade early diagnosis of cystinosis in pre-symptomatic patients via neonatal screening based on NGS and administration of novel thera-

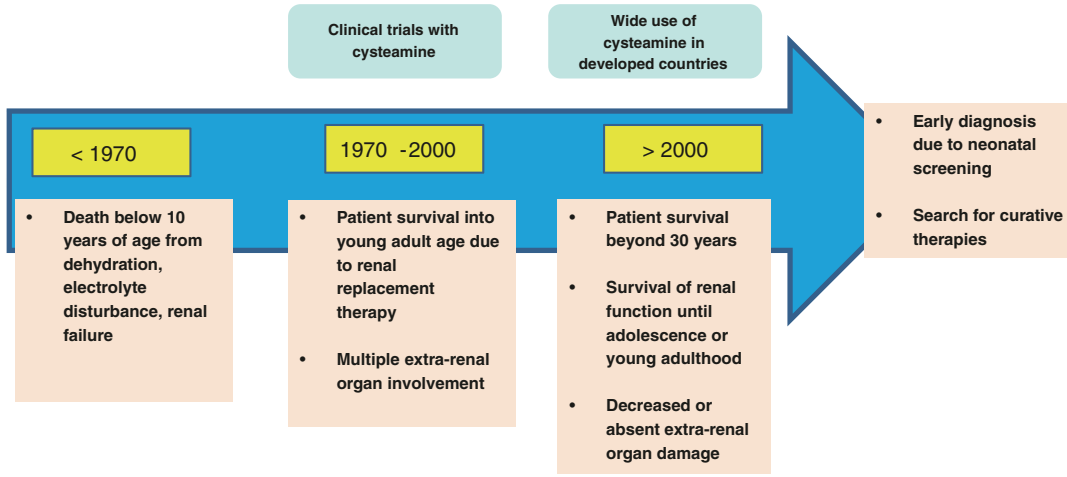

Fig. 30.9 Improved prognosis of nephropathic cystinosis. The overall prognosis of patients with cystinosis has substantially improved by the availability of renal replacement therapy allowing patients to survive into young adult age, and by wide use of cysteamine treatment prolonging renal function survival and protecting extra-renal organs. Early diagnosis in pre-symptomatic patients and the administration of novel therapies will further improve patients' survival and quality of life

pies will further improve patients survival and quality of life (Fig. 30.9).

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The Kidney in Sickle Cell Disease

31

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Sickle cell disease (SCD) is the most common inherited red blood cell disorder in the United States, impacting approximately 100,000 Americans and 1 in 365 African American births [1]. SCD is most prevalent in sub-Saharan Africa due to its protective inheritance against malaria. About 1000 children are born in Africa each day with SCD [2]. Some Hispanic and Indian populations have also been identified with up to 40% of residents having at least one sickle gene mutation.

The sickle cell mutation causes a hydrophobic valine to replace a hydrophilic glutamic acid in the sixth amino acid position of the β-globin protein. This mutation allows polymerization of hemoglobin S in the deoxyhemoglobin state. Two inherited β-globin sickle cell mutations result in the diagnosis of hemoglobin SS (HbSS). Different mutations in one of the β-globin subunits cause other forms of SCD. These include mutations that lead to no beta globin synthesis (β0 thalassemia) or minimal globin synthesis (β+ thalassemia) as well as other mutations, including hemoglobin C (HbC). The most prevalent, severe forms of SCD are HbSS and hemoglobin Sβ0 (HbSβ0) thalassemia; these genotypes are referred to as sickle cell anemia (SCA) and occur in about 70% of SCD patients. Other genotypes usually have less severe disease, and include hemoglobin Sβ+ (HbSβ+) thalassemia and hemoglobin SC (HbSC).

Sickle cell trait, which occurs in 1 in 13 African Americans, has been studied for its association with progressive kidney disease in adults, hematuria, renal papillary necrosis, and pyelonephritis during pregnancy. Sickle cell trait is associated with the rare cancer, renal medullary carcinoma. Patients with sickle cell trait do not have a significant pediatric clinical disease course due to the protective effect of one normal β-globin gene. Therefore, patients with sickle cell trait do not have SCD and, aside from counseling, do not receive follow-up care by a pediatric hematologist.

Hyposthenuria, Renal Papillary Necrosis, and Nocturnal Enuresis

Hyposthenuria

Pathophysiology: The development of hyposthenuria and renal papillary necrosis begin with sickling of the red blood cells in the vasa recta, leading to marked vascular changes. Necropsy studies have demonstrated almost complete destruction of the vasa recta and medullary

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capillaries in patients with SCA as compared to a reduced number of vasa recta in patients with sickle cell trait and HbSC disease [3]. These findings may be related to the impact of the hyperosmolar environment of the kidney on red cell rheology. In vitro models have demonstrated that sickle red blood cells exposed to even mildly hypertonic environments (sodium levels of 141 mEq/L) experience a delay in transit time, an increase in red cell rigidity and an increase in red cell adherence to vascular walls [4]. The renal medulla is an extremely hypertonic, hyperosmotic environment (800–1200 mOsm/kg) relative to the milder in vitro environments studied, which likely contributes to the more pronounced pathophysiologic changes seen in vivo. In addition, the renal medulla is a relatively hypoxic environment. Sickle red blood cells are stable in normoxia, but hemoglobin S begins to polymerize during the hypoxic state. Finally, acidic environments promote sickling of RBCs. Therefore, the combination of hypertonicity, hypoxia, and the acidotic environment in the renal medulla causes sickling of red blood cells within the vasa recta, with subsequent ischemic and reperfusion injury.

Epidemiology: A diminished urine concentrating ability is a well-established complication in patients with SCD [5–8]. The prevalence of hyposthenuria increases with disease severity (SCA > HbSC disease > sickle cell trait) [9, 10]. Urine concentration defects are first noted in infants and toddlers with SCA. In the baseline analysis of the BABY HUG study of children with SCA, only 30% of infants concentrated their urine >500 mOsm/kg, and only 13% of infants concentrated their urine >2 times their serum osmolality [11]. Of note, infants whose urine osmolality was >500 mOsm/kg after fluid deprivation had higher mean fetal hemoglobin concentrations, and the urine osmolality correlated with glomerular filtration rate (GFR).

Treatment: The BABY HUG study treated infants with 24 months of hydroxyurea, which increases fetal hemoglobin, versus placebo. While treatment with hydroxyurea for 24 months did not affect GFR, it did result in a significantly higher mean urine osmolality (495 mOsm/kg hydroxyurea vs. 452 mOsm/kg placebo) and a higher percentage of infants with a urine osmolality >500 mOsm/kg after fluid deprivation [12]. Murine models also suggest that a higher percentage of fetal hemoglobin and higher hemoglobin levels are associated with less severe concentrating defects [13]. Thus, hydroxyurea may decrease the urine concentrating defect early in life in SCA patients. Chronic transfusion therapy may also improve hyposthenuria [5, 6, 14]. However, preventing hyposthenuria is not an indication for initiating chronic transfusion; it is restricted to patients with increased risk of a poor central nervous system outcome or a very severe clinical course.

Interventions to prevent hyposthenuria should be initiated early in life. Red blood cell transfusions before the age of 10 years are effective in reversing hyposthenuria, but this reversibility was lost when initiated after the first decade [3, 5]. In a study of HbSC patients with a mean age of 11 years, hydroxyurea treatment for 12 months did not improve urinary concentrating ability [15]. This contrasts with the BABY HUG study and suggests loss of reversibility in older children.

Renal Papillary Necrosis

Another complication resulting from destruction of the renal vasculature is renal papillary necrosis. The pathophysiology is likely due to the renal medullary environment that promotes a higher level of sickling of red blood cells. It is believed that occlusion of the blood supply causes ischemia-induced necrosis of the renal medulla and papillae. This necrosis initiates subclinical and clinical hematuria. Pathology studies in SCD patients identified papillary necrosis in about one-third, most often located in the tips of the papillae [16]. Hematuria is secondary to changes in the permeability of the vasculature that allows red cell leakage into the collecting system.

Epidemiology: In radiologic studies, 30–75% of SCD patients have evidence of papillary necrosis [17–19]. Studies in Africa suggest 2% of

patients will develop symptomatic renal papillary necrosis [20]. Risk factors for renal papillary necrosis are female sex, older age, more severe anemia and hypertension. Renal papillary necrosis is more common in the left kidney.

Diagnosis: While renal papillary necrosis is a common etiology of hematuria in pediatric patients with SCD, a standard diagnostic workup for hematuria should be performed on initial presentation. Since hemoglobinuria may cause a positive urine dipstick result, microscopic evaluation of the urine is necessary to confirm hematuria. SCD patients should also have a complete blood count (CBC) and creatinine to identify acute changes in hemoglobin and kidney function. Patients should be queried regarding other acute symptoms of SCD, prior history of hematuria, and medication use.

Several diagnostic imaging techniques can be considered in the initial evaluation of hematuria in a SCD patient. Ultrasound, which does not require contrast and is readily available, will identify hydronephrosis, and may provide evidence for a kidney stone as a cause of hematuria. A renal mass suggesting renal medullary carcinoma is more often identified in patients with sickle cell trait than SCA [21]. Findings on ultrasound suggestive of renal papillary necrosis include filling defects and necrosed papillae in cavities [22]. However, ultrasound is not the optimal imaging for identifying renal papillary necrosis. Most patients require a contrast evaluation, including intravenous urography, retrograde pyelography, or CT with contrast [23]. Intravenous urography and retrograde pyelography have less radiation exposure than CT [23]. Findings on a contrast study will often demonstrate filling defects of the renal calyx, including deformities of the renal papillae (hooks, spurs) and a blunted calyx [22].

Treatment: Some cases of hematuria due to papillary necrosis will be mild, painless, and self-resolve; other cases may require therapy. There are no guidelines addressing the efficacy of supportive care or when patients should be admitted to the hospital for care. Treatment may include intravenous (IV) fluids, analgesia, bedrest, alkalization, and low-dose aminocaproic acid [24, 25]. Patients who develop severe anemia require transfusion. Surgical interventions such as papillary tamponade, shunt placement, or nephrectomy are rarely indicated unless a pediatric patient is experiencing severe, persistent, life-threatening hemorrhage [16, 26].

The goal of fluid therapy is to ensure adequate hydration and to maintain high urine output. For mild cases, aggressive oral hydration as an outpatient is sufficient. For more severe cases, IV fluids can be prescribed in either outpatient day hospital settings or inpatient units. Patients can be administered fluids at maintenance to 1.5 times maintenance with or without a loop diuretic to further ensure adequate urinary output [27]. Since acidosis promotes sickling, adding base to the IV fluids to create a more alkaline environment to reduce sickling is theoretically appealing; however, there are no trials demonstrating benefit in SCD patients with papillary necrosis [28]. Bedrest may reduce the risk for dislodging of clots. Clinicians should closely monitor for the development of respiratory symptoms or fluid overload in patients receiving higher rates of IVF and/or suggested bedrest. Analgesia is used in patients with painful hematuria. If patients presenting with painless hematuria progress to painful hematuria, additional imaging may be required to evaluate new obstructive disease due to blood clots. Low dose aminocaproic acid at 20–50 mg/kg IV or po every 8 or 12 h has been used in severe cases as well as lower maintenance oral dosing [24, 25, 29]. SCD is a hypercoagulable state so close monitoring for new clot formation or ureteral obstruction should occur when using aminocaproic acid. Transfusion therapy can improve the anemia and reduce the concentration of sickle red blood cells, which should reduce sickling. In very severe cases, exchange transfusion may be used to significantly reduce the concentration of sickle cells, often to a sickle cell concentration of less than 30%. Reducing the sickle cell concentration may not treat the acute complication, but may allow more rapid recovery and prevent early recurrence of another renal injury.

Nocturnal Enuresis

Epidemiology: Primary nocturnal enuresis (PNE) is bedwetting that occurs in an individual who has never been dry at night, and is usually not associated with daytime wetting symptoms. PNE affects approximately 15% of children aged 5 years in the general population, and spontaneously remits at a rate of 15% per year [30]. The prevalence of PNE in SCD is much higher; children with SCD aged 14–17 years old were five times more likely to have PNE than controls [31–35]. Similar to the general population, PNE is more common in males, younger children and children with a positive family history of PNE [31–35]. Children with SCA and PNE are more likely to have sleep-disordered breathing [36]. In a study of 8-year-old Jamaican children, the prevalence of enuresis was 52% for boys and 38% for girls with HbSS disease, and 10% for boys and 20% for girls with HbSC disease. The prevalence of PNE in HbSS disease was significantly more common than HbSC disease or controls, but there was no significant difference between HbSC disease and controls. There was no significant difference by sex [37].

Risk Factors: PNE in HbSS disease has been attributed to hyposthenuria and polyuria. However, in a study comparing HbSS patients with and without enuresis, there was no difference in urinary concentrating ability or overnight urine volume [38]. However, the bladder capacity corrected to body surface area was lower, and the ratio of overnight urine volume divided by bladder capacity was higher in enuretic compared to non-enuretic children. A high prevalence of daytime symptoms of overactive bladder was also present in those with nocturnal enuresis and SCA [34]. These results suggest that hyposthenuria-induced polyuria may not be the primary cause of PNE in HbSS disease. In addition to urinary tract pathology, an association has been identified between sleep disordered breathing and PNE [36].

Therapy: Several pharmacological and behavioral therapies have been identified to treat patients with PNE in the general populations [39]. However, there are no controlled trials demonstrating benefit in children with SCD. One prospective study of 10 patients with SCD treated with desmopressin reported improvement in six patients [32]. Hence, the evidence for pharmacologic treatment is inadequate, and thus the focus of therapy is watchful waiting and bed-wetting alarms in some patients [40].

Albuminuria

Epidemiology: Fifty to seventy percent of adults with SCA have albuminuria in large cross-sectional studies [41–43]. Children with SCD develop albuminuria around 5–10 years of age and the prevalence increases throughout adolescence. The reported prevalence of albuminuria in cross-sectional pediatric SCA studies is 20–40% [44–46]. There is a need for longitudinal data on the natural history of the progression of kidney disease and development of end-stage kidney disease (ESKD) among pediatric patients with albuminuria as they transition into adulthood [47].

Pathology: Patients with SCA develop albuminuria due to either direct glomerular injury or impaired tubular reabsorption of albumin. In adults with SCD, glomerular complications include glomerular hypertrophy, focal segmental glomerulosclerosis, and membranoproliferative glomerulonephritis [48]. Kidney pathologic data was reported from 36 pediatric patients that underwent renal biopsy for proteinuria or low estimated GFR (eGFR) [49]. The majority had glomerular hypertrophy. In addition, the majority of the biopsies had mesangial hypercellularity and/or increased mesangial matrix. All patients in this cohort with mesangial hypercellularity, had proteinuria, including 88% with nephrotic range proteinuria. Eleven of the 36 patients had focal segmental glomerulosclerosis, six of the 11 also had global sclerosis. Five patients had membranoproliferative glomerulonephritis. On electron microscopy, more than 50% of biopsies had podocyte effacement, and a quarter had mesangial deposits. This study supports the importance of nephrology evaluation of children with SCD and significant proteinuria or decreased GFR.

Impaired tubular reabsorption of albumin may also contribute to albuminuria independent of glomerular disease in patients with SCA. Some albumin is normally filtered at the glomerulus and then reabsorbed by receptor-mediated endocytosis by megalin and cubilin in the proximal tubule [50]. However, internalization of albumin is subject to competition from many other proteins. Importantly in SCD, hemoglobin dimers can also be reabsorbed in the proximal tubule. In preclinical models, the addition of oxyhemoglobin to proximal tubule cells significantly reduced albumin uptake [51]. As SCD patients have daily variations in the amount of hemolysis consequent hemoglobinuria, it is plausible that albuminuria may vary depending on the amount of hemolysis. Studies have demonstrated that a single urine sample with albuminuria may not represent persistent albuminuria [47, 52]. While free hemoglobin or heme reuptake may lead to non-glomerular albuminuria, the uptake of free heme by the proximal tubule may induce pathologic changes and kidney disease progression, as described in the section on acute kidney injury.

Risk factors: Older age is a risk factor for albuminuria in SCD [43, 47]. Inheritance of two apolipoprotein L1 (APOL1) risk alleles, which are common in people of African descent, is associated with increased risk of albuminuria and CKD in SCD [53, 54]. Albuminuria in SCD patients with two APOL1 risk alleles begins in the first decade of life [44]. Serum hemoglobin is inversely related to risk of albuminuria [45, 55–57]. Patients with severe anemia in their second year of life are more likely to develop albuminuria earlier in life [45]. There is inconsistent data on the association of albuminuria with leukocytosis, hemolytic markers (lactate dehydrogenase), and blood pressure [46, 58–61].

Similar to diabetes, hyperfiltration is a risk factor for albuminuria in SCD. In cross-sectional studies, there are conflicting results on the association of eGFR with albuminuria [55, 62, 63]. A large, prospective, pediatric cohort evaluated the impact of hyperfiltration on progression of albuminuria; patients with hyperfiltration in the first decade of life were more likely to develop albuminuria at an earlier age [64]. Patients that developed albuminuria had a significant increase in eGFR prior to developing albuminuria while patients without albuminuria did not experience a significant rise in eGFR during the first decade of life. Adult data also suggests that hyperfiltration is associated with albuminuria [65].

Diagnosis: The National Heart, Lung, and Blood Institute (NHLBI) guidelines recommend screening for albuminuria by age 10 years, with annual screening thereafter [66]. Patients with a positive screening result should have a first morning test for albuminuria (albumin/creatinine ratio [ACR] >30 mg/g), with referral to a kidney specialist if positive. If a first morning void is not available or feasible, scheduling patients for an early morning appointment to obtain a second morning void may be of benefit. This second urine measurement is important as patients with SCD experience intermittent albuminuria, but may not have persistent albuminuria. About 25–50% of SCA patients with ACR <100 mg/g do not have persistent albuminuria; patients with ACR >100 mg/g are more likely to have persistent albuminuria [47, 67].

Albuminuria can begin prior to 10 years of age; therefore, some centers may begin screening for albuminuria earlier than 10 years of age [44, 64]. This is especially relevant in patients with two APOL1 risk alleles since almost 25% of these patients with SCD develop albuminuria prior to age 10 years.

The presence of severe albuminuria (ACR >300 mg/g) in SCD is less likely in pediatric patients than adults. As severe albuminuria is a rare complication in children with SCA, patients presenting with severe albuminuria require a complete diagnostic workup for proteinuria.

Treatment: The therapeutic approach in patients with SCD and albuminuria focuses on traditional modifiers of SCD and interventions to reduce hyperfiltration.

SCD Modifying Therapies: All patients with SCA should be offered hydroxyurea, a daily oral therapy, starting at 9 months of age regardless of clinical complications [66]. Therefore, patients that have progressed to albuminuria should be encouraged to begin hydroxyurea or improve adherence if nonadherence is present.

Hydroxyurea induces fetal hemoglobin, raises hemoglobin, and reduces hemolysis and inflammation. The dose of hydroxyurea may be increased to 35 mg/kg in children to allow for maximal effect without reducing the absolute neutrophil count below 1000–2000/μL [68]. Patients on hydroxyurea have reduced hospitalizations, pain events, and episodes of acute chest syndrome; this may provide downstream renoprotective effect [69]. Pediatric patients on hydroxyurea with higher fetal hemoglobin levels have a lower prevalence of albuminuria [70]. As hydroxyurea improves anemia and reduces hemolysis, biologic plausibility suggests that early use of hydroxyurea should decrease glomerular hyperperfusion and reduce free hemoglobin exposure to proximal tubule cells; however, randomized controlled data demonstrating this benefit is lacking. Longitudinal adult studies have demonstrated some benefit of hydroxyurea when started prior to the development of severe albuminuria [71].

Chronic transfusion therapy is a proven therapy to reduce progressive SCD and treat acute SCD complications. By performing chronic monthly transfusion therapy, patients are maintained with a very low sickle cell concentration, often to a lower percentage of sickle cells than patients with sickle cell trait. Chronic transfusion protocols have demonstrated a clear benefit to prevent a first or second stroke in patients at risk. The use of transfusion therapy would likely benefit pediatric patients with albuminuria or other renal complications, but using chronic transfusion for this indication has not been systematically studied for benefit or cost-effectiveness in large studies [72]. One complication of chronic transfusion is the development of iron overload. After 10–12 transfusions, many patients will be started on iron chelation, which can have nephrotoxic effects [73].

A few SCD modifying therapies have been recently approved by the Food and Drug Administration (FDA), but these approvals were not based on a renal indication. Crizanlizumab is a P-selectin inhibitor administered intravenously monthly that can prolong the time to the next pain event in patients with 2–10 pain events annually [74]. It is currently being studied for renoprotective effects (NCT04053764). Voxelotor is a daily oral medication that increases the oxygen affinity of sickle red blood cells. This increased oxygen affinity reduces sickling of red blood cells and was FDA approved for its ability to raise hemoglobin [75]. As a medication that reduces hemoglobinuria and likely reduces heme exposure in the kidneys, this therapy may have renoprotective effects and is being studied in adults with high risk for CKD (NCT04335721). Finally, L-glutamine received FDA approval for the reduction in painful events among pediatric and adult patients with two or more painful events in the last year [76]. L-glutamine may protect SCD patients by reducing oxidative stress through an increase in the availability of glutathione. Future studies will need to be performed to determine the effect of glutamine on renal endpoints.

Angiotensin Blockade: Based on the benefit of angiotensin converting enzyme inhibitors (ACEI) and angiotensin receptor blockers (ARBs) in patients with diabetes to reduce proteinuria and lower intraglomerular pressure, SCD patients with albuminuria may be started on these medications. The American Society of Hematology (ASH) Guidelines for the management of SCD includes an evidence-based review of ACEI/ARBs in patients with albuminuria [77]. Based on a low certainty in the evidence, the guideline panel decided to "suggest" the use of ACEI/ARBs for albuminuria rather than "recommend" it for albuminuria. One randomized controlled trial and several observational studies that suggested benefit were evaluated [78]. The methodologic experts and guideline panel members determined from these studies that 63% of patients who received ACEI showed improvement in albuminuria; the panel also identified that 100% of 30 patients who received ARBs had improved albuminuria at some point during the treatment [77–82]. Patients started on ACEI/ARBs should be reassessed in one week and then serially for changes in GFR and serum potassium. In addition, these medications should be held during acute illness, planned IV contrast administration, or prior to surgery or other procedures.

Endothelin Receptor Antagonist: Endothelin receptor A (ETA) is responsible for vasoconstriction, inflammation, and adhesion. Endothelin B receptor mediates nitric oxide (NO) production and vasodilation. It is likely that specific blockade of the ETA receptor could improve renal and SCD outcomes as compared to dual blockade. Murine sickle cell data showed a benefit after 10 weeks of ETA receptor blockade, as compared to a combined ETA/B antagonist, in reducing plasma ET-1 levels, urinary protein and albumin excretion and maintaining a stable GFR [83]. In adults with SCA, a phase I double blind study of ETA antagonist ambrisentan has been completed [84]. The data suggests a potential benefit for albuminuria and improved microvascular function.

Glomerular Filtration Rate

Epidemiology: Patients with SCD experience hyperfiltration early in life. Infants enrolled in a randomized trial underwent measured GFR (mGFR) at around one year of age and again at study exit 2 years later. At one year of age, participants had a normal mGFR; 2 years later, the mGFR had increased by a mean of 20 mL/min/1.73 m^2 to 146 mL/min/1.73 m^2 [69]. This increase in GFR occurs throughout early life and cross-sectional studies have identified that around 40–80% of SCD patients will develop hyperfiltration [12, 46, 58, 85]. After this early rise in GFR, pediatric patients likely experience a plateau in GFR around 6–12 years of age before they begin a decline in GFR [58, 64]. Some pediatric patients will progress to a GFR <90 mL/min/1.73 m^2; these patients should be evaluated to investigate non-SCD related causes of CKD [62, 86].

Risk Factors: Many cross-sectional studies have identified potential associations with GFR. Data is conflicting for the role of anemia, leukocytosis, blood pressure, male sex, and LDH [12, 58, 85, 87]. A few studies have explored the association of uric acid and GFR in SCD; these studies suggest that uric acid may be an early marker of risk for GFR decline [88–90]. As discussed in the albuminuria section, several studies have identified a link between hyperfiltration (elevated GFR) and albuminuria.

Treatment: Treatment approaches to prevent hyperfiltration or glomerular injury have not been fully explored. Treatment of infants with hydroxyurea for 2 years did not prevent the increase in eGFR or reduce the number of participants who developed hyperfiltration [12]. In contrast, some older participants started on hydroxyurea experience a decrease in eGFR that was associated with change in fetal hemoglobin and LDH [91]. The studies of angiotensin targeted therapies often focused on albuminuria as the primary outcome; however, some of these studies have also reported the impact of treatment on change in GFR. In a well-designed study of enalapril in adults, treatment for 2 weeks did not change the mean GFR, effective renal plasma flow, or filtration fraction [80]. Two studies using losartan therapy for 6 months or 1 year also did not identify a change in GFR while on treatment [92, 93].

One concern in clinical care and conducting research to evaluate eGFR or change in eGFR in patients with SCA is that the equations used to estimate GFR in children and adults have low precision and accuracy [12, 94–96]. In addition to the concern for the bias in a single eGFR measurement, individual patients do not demonstrate concordance between eGFR and mGFR in longitudinal studies [96]. Therefore, clinicians and researchers need to evaluate GFR changes at several time points to reduce these inaccuracies. Formulas using either cystatin C alone or cystatin C and creatinine may have the best precision and accuracy among the current formulas. A current study is attempting to develop novel eGFR equations for children and adult participants with SCA (NCT 04380610). A second concern is that after a period of hyperfiltration in early childhood, a decline in eGFR occurs. Several studies have evaluated the change in eGFR after an intervention or in the evaluation of risk factors. A rapid decline in eGFR in adults is associated with higher morbidity and mortality [97–99]. However, shorter-term longitudinal pediatric studies of patients with baseline hyperfiltration

struggle to determine whether a decline in eGFR during the study is related to an improvement in renal function back to baseline, the beginning of a progressive decline in renal function to CKD, or regression to the mean among participants with higher eGFR at one time point. Long-term pediatric research is vital to determine whether a change in eGFR represents a benefit or risk.

Progressive CKD, End-Stage Kidney Disease, and Mortality

It is important to monitor pediatric patients with SCA for progression from glomerular hypertrophy with elevated GFR to CKD with decreased GFR due to sclerosis and fibrosis [80]. CKD in SCA is an independent risk factor for early death [100]. The management of CKD in SCD is similar to other patients with CKD, including control of hypertension and proteinuria with an ACEI or ARB. Currently, there are no FDA-approved medications for the specific treatment of sickle cell nephropathy.

Renal Replacement Therapy: The average age of initiation of renal replacement therapy (RRT) in SCD patients is 40–45 years [101–103]. Patients with SCD are currently a small minority of U.S. dialysis patients, but this population is expected to grow as overall life expectancy improves [104]. A longitudinal cohort study of SCA patients published in 1991 reported that the median survival of patients requiring dialysis was a mere 4 years [105]. In a more contemporary study (2005–2009), the hazard ratio for mortality among SCD patients with ESKD was 2.8 (95% CI 2.31–3.38) compared to those without SCD as the primary cause of renal failure, and 26.3% of incident SCD ESKD patients died within the first year of dialysis [101].

SCD patients are less likely than other ESKD patients to have a functioning arteriovenous fistula at the time of hemodialysis initiation, an important quality metric tied to improved RRT survival [103]. SCD patients on RRT experience greater rates of bacteremia and sepsis, atrial flutter and fibrillation, congestive heart failure exacerbations, and major hemorrhage than other ESKD patients [103]. SCD patients on RRT have more RBC transfusions than other RRT patients. SCD patients not receiving erythropoietin stimulating agents (ESAs) have the highest transfusion burden, while those treated with ESAs and hydroxyurea have the lowest transfusion burden [103]. The 2019 ASH guidelines recommend that hydroxyurea and ESAs be used in combination to promote fetal hemoglobin production. Clinicians should use a lower hemoglobin threshold (<10 g/dL) when prescribing this combination therapy as higher hemoglobin levels, especially with higher HbS percentage, may be associated with increased SCD complications [77].

Renal Transplantation: For those with advanced CKD, the ASH evidenced-based guidelines suggests referral for renal transplant. As SCD is associated with chronic inflammation, the guidelines suggest judicious use of corticosteroids in post-transplant protocols due to the risk for vaso-occlusive pain with the increase in WBCs that accompanies steroid use [77]. Referral rates for transplantation for SCA patients are lower than other ESKD patients, even after adjusting for covariates [102]. One potential explanation for this disparity may be related to historical data reporting high rates of complication and poor graft survival in SCD patients. An analysis of the U.S. Renal Data System data from 1984–1996 showed that SCA patients and other renal transplant recipients have similar 1-year cadaveric graft survival [106]. Recipients had higher rates of 3-year graft loss (RR 1.60) and a significantly higher adjusted mortality rate at 1 year (RR 2.95) and 3 years (RR 2.82) compared to non-SCA transplant recipients [106]. However, more recent data (2000–2011 compared to 1988–1999) showed that 6-year survival among SCA recipients improved in the more recent era compared to the early era (78% versus 55.7%, $p < 0.001$) [107]. While the 6-year patient survival was still significantly lower than non-SCA recipients (HR for mortality 2.03, 95% CI 1.31–3.16), it was equal to that of black diabetic transplant recipients [107].

An additional concern that may hinder referral for renal transplantation is the perceived risk of alloimmunization if blood transfusion is required

in anemic SCD transplant recipients. It is not surprising, given the lifetime exposure to RBC transfusions, that a greater proportion of SCA renal transplant recipients had allosensitization, with panel reactive antibodies >20% [107]. One retrospective multicenter study compared the proportion of *de novo* donor specific antibodies (DSAs) and graft survival among SCD renal transplant recipients who received regular automated exchange blood transfusions (EBT) pre or post renal transplant versus those who did not receive regular EBT [108]. Goals for EBT were to maintain hemoglobin >9 g/dL, to reduce HbS to <30%, and to reduce sickling-related complications. The median number of red blood cell units transfused per year was 37 and 8 in the EBT and non-EBT group, respectively. Overall, patient survival, graft survival, and graft function were superior in those who were on EBT, and the proportion of patients who developed *de novo* DSAs was not different (20% and 21%) between the groups. In addition, the incidence of rejection was lower in those on EBT (28% vs 54%). These data, while limited by sample size, indicate that blood transfusions peri-transplant are effective, safe and lead to improved outcomes in the SCA renal transplant population.

SCA patients with advanced CKD should therefore be counseled on the shortened graft survival and increased complication rates expected after transplantation, but should not be restricted from renal transplant access. Moreover, they should receive blood transfusions as needed pre or post transplantation.

Acute Kidney Injury

Patients with SCD may be at increased risk for acute kidney injury (AKI) due to high use of nephrotoxic medications and hemolysis causing proximal tubule injury from free heme and hemoglobin exposure [51, 109]. Acute pain events are a leading cause of hospitalization in children with SCD and repeated acute pain events can progress to chronic pain. Aggressive and early pain management is important, and many centers utilize individualized pain plans for home and during hospitalizations [110]. These pain plans often include non-steroidal anti-inflammatory medications (NSAIDs) during home pain events and inpatient IV ketorolac as adjunctive therapy to opioids [111–113]. The 2014 NIH guidelines provide a moderate recommendation for the use of NSAIDs for mild to moderate pain in the absence of contraindications [110]. The 2019 ASH guidelines suggest a short course of NSAIDs in addition to opioids for acute pain management based on a low certainty of evidence [113]. The ASH guidelines remark that patients with known risk for renal toxicity should be identified as the mild potential benefit to NSAIDs for pain may not outweigh the risks associated with NSAID use. One concern with these guidelines is that pediatric patients may utilize a significant amount NSAIDs without appropriate monitoring of kidney function or fluid intake. Rarely, a single dose of ketorolac in the absence of volume depletion may precipitate irreversible renal failure [114].

In addition to acute pain events, infections may lead to use of nephrotoxic medications in SCD patients, who are increased risk for infection with pneumococcus and other encapsulated bacteria [115–117]. Patients may receive vancomycin or other nephrotoxic antibiotics for acute chest syndrome, fever, sepsis, or skin infections. It is important to consider the pros and cons of nephrotoxic medications given the potential long-term exposure and underlying risk of developing sickle cell nephropathy; monitoring of kidney function is needed when patients with SCD are at risk of AKI from nephrotoxins, volume depletion or infection.

When pediatric SCD patients are admitted for pain events or acute chest syndrome, AKI, when defined as an increase in creatinine occurs in 10–20% [118–120]. Risk factors for AKI in these studies include ketorolac exposure and an acute drop in hemoglobin, which probably reflects increased hemolysis and tubular exposure to free heme and hemoglobin. In another study using coding data, 1.4% of hospitalized SCD patients develop AKI, with risk factors including HbSS genotype, older age and greater number of total hospitalizations [121].

Adult studies of SCD patients demonstrate a higher incidence of AKI during hospitalization than described in children [122]. In a prospective study of adult SCD patients observed for a median of 5.5 years, 46% developed AKI [122]. Patients with AKI were older, had lower hemoglobin levels, higher white blood cell counts, and higher use of vancomycin. Moreover, genetic variants of heme catabolism (HMOX1) were independently associated with the development of AKI. Finally, adults with AKI were likely to develop CKD sooner, with the highest risk associated with more severe AKI.

Tubular Abnormalities and Acidification Defects

Along with defects in urinary concentrating ability (see above), SCD patients have well-described defects in other tubular functions, including urinary acidification and potassium excretion. SCA patients have an incomplete distal renal tubular acidosis as evidenced by decreased urine acidification in response to a systemic acid load [10, 123]. In one study, 42% of adult SCA patients had a metabolic acidosis [124]. Defects in acid excretion are associated with poor outcomes. In one adult study, the lowest tertile of urinary ammonia excretion increased the risk of ESKD [125]. Acidification of the urine was impaired in 52% of adults SCD patients, and was associated with older age, higher serum uric acid, increased hemolysis, lower eGFR, and lower serum bicarbonate [121]. Poor urinary ammonium excretion, as a measure of acid excretion, is associated with poor urinary concentrating ability [121].

SCA patients also have impaired potassium excretion [126]. In this study, patients had a normal renin-aldosterone axis and normal GFR, but had impaired potassium excretion, urine acidification and urinary concentrating ability, indicating that a severe distal tubular dysfunction is present in SCA patients despite preservation of glomerular function.

In one study of 24 children without decreased GFR, 75% had hyperphosphatemia, but serum calcium was normal [127]. Seventy-nine percent of participants had elevated FGF-23 levels, which is the expected physiologic response to hyperphosphatemia and would be expected to increase renal phosphate excretion. However, patients had evidence of impaired phosphate excretion, suggesting t that SCA patients have a proximal tubular resistance to FGF-23 before evidence of GFR decline.

Hypertension

A meta-analysis conducted for the 2014 NHLBI guidelines demonstrated that patients with HbSS genotype have lower diastolic and systolic blood pressures than healthy children. A large cohort study developed 90th percentile curves for children with SCD [128]. Hence, it may be appropriate to use SCD-specific blood pressure (BP) tables when evaluating BP in children with SCD.

There is an association in SCD patients between elevated BP and higher hemoglobin values [128, 129]. Hypertension in SCD patients has been associated with increased risk of acute stroke, although causality has not been established [128, 130]. Finally, data suggests that there is a direct correlation between higher blood pressure and increased mortality [128].

SCD patients have a high prevalence of masked hypertension and "white coat" hypertension in studies using 24-h ambulatory blood pressure monitoring (ABPM). Patients with SCA have only moderate concordance between in-clinic blood pressure and ABPM, with 25–33% having masked hypertension and 60% having white coat hypertension [61, 90, 131–133]. This may explain why some studies of in-clinic BP did not find a correlation between albuminuria and hypertension while there was an association when using ABPM [90, 132, 133]. Hence, since ABPM is the gold standard for measurement of BP, it is especially important to follow the American Academy of Pediatrics Guidelines and perform ABPM prior to initiation of antihypertensive treatment in SCD patients. Screening for masked hypertension may be appropriate in children with SCD and evidence of kidney disease. Patients identified with nocturnal

hypertension or abnormal nocturnal dipping may have an increased risk for more rapid annual decline in GFR, albuminuria, and silent cerebral infarcts [61, 90, 131–133].

The ASH evidence-based guidelines for the management of SCD patients recommends a lower BP goal (<130/80) than the usual goal in adults without additional co-morbidities (<140/90) [77]. This strong recommendation was based on a moderate certainty in the evidence. The guidelines did not address BP management in children, and thus there are no recommendations to have a lower BP goal in SCD patients than in healthy children. Hence, BP management in children with SCD should follow the guidelines for healthy children.

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Diabetic Kidney Disease

32

Allison B. Dart

Introduction to Diabetic Kidney Disease

While diabetic nephropathy is the most common cause of end-stage kidney disease (ESKD) in adults in many countries [1, 2], it has become increasingly relevant to children and adolescents. Traditionally, the chronic kidney disease (CKD) associated with diabetes in adults has been termed diabetic nephropathy in both type 1 and type 2 subtypes. As the clinical phenotype of kidney complications associated with diabetes has broadened, the term diabetic kidney disease (DKD) is increasingly utilized. It has been defined as CKD, with diabetes being partially involved in the pathogenesis of the kidney disease. DKD in adults can include diabetic nephropathy, ischemic nephropathy, hypertensive nephrosclerosis, and non-diabetic kidney disease, with multiple entities possible in the same patient [3]. In general, the natural history of DKD is similar in adult type 1 and type 2 diabetes populations. In children, the natural history and pathology of kidney complications in the diabetes subtypes is quite different. Currently, DKD is used to describe the kidney complications in all youth with diabetes. Important differences in DKD associated with youth onset type 1 and type 2 diabetes will be outlined in each section. This chapter will outline the clinical risk factors, natural history, pathology, and treatment of DKD.

Epidemiology of Diabetes in Children

Youth onset diabetes, defined as disease onset prior to 18 years of age, is an important clinical problem in children and adolescents, with an increasing incidence of both type 1 and type 2 diabetes in the last 20 years [4, 5]. While type 1 diabetes is the most common endocrine condition in children, the proportion of children with type 2 is increasing, especially in disadvantaged populations and minority ethnic groups, including children of African, Arab, Asian, Hispanic, and Indigenous descent [6]. The increasing prevalence of obesity in children is an important cause; however, most important are the social determinants of health such as poverty, colonization and systemic racism that are driving the increased risk of chronic disease in disadvantaged populations [7, 8].

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The unadjusted estimated incidence of type 1 diabetes and type 2 diabetes in the United States (US) between 2002–2003 and 2011–2012 increased annually by 1.4% and 7.1%, respectively [9]. Overall, 87% of youth with diabetes in North America have type 1 diabetes [10], but type 2 diabetes accounts for up to 50% of cases in some clinical settings [11]. The incidence of type 1 diabetes is now 1–3 per 100,000 per year in South America, China and other Asian countries; 10–20 per 100,000 in South European countries and the US; and 30–60 per 100,000 in Scandinavia [12, 13]. In North America, the prevalence of type 2 diabetes is 12.5 cases per 100,000 and the prevalence of diabetes in youth overall is now up to 0.8% [14, 15].

Clinical Risk Factors for Diabetic Kidney Disease

The most well-characterized and clinically important risk factors for DKD are glycemic control and hypertension. Additional risk factors include hyperlipidemia, smoking, duration of diabetes, and post-pubertal status. More common in youth with type 2 diabetes are risk factors for non-diabetic kidney disease, such as in-utero exposure to diabetes and obesity, which affect nephron endowment, and an increased risk of immune-mediated kidney disease, such as IgA nephropathy, in Indigenous and Asian populations [16, 17].

Hyperglycemia is a consistent and important determinant of renal injury. The Diabetes Control and Complications Trial (DCCT) [18] and the UK Prospective Diabetes Study (UKPDS) [19] evaluated intensive glycemic control vs. conventional regimens and provided clear evidence that optimized glycemic control was associated with improved long-term kidney health. In the DCCT study, the 9-year risk of microalbuminuria was reduced by 34% and macroalbuminuria by 56% in the intensive therapy group when compared with standard therapy. The intensive therapy group also had improved long-term outcomes with respect to estimated glomerular filtration rate (eGFR) [20].

However, target glycemic control is often very difficult to achieve in adolescents. Many observational studies have reported hemoglobin A1c (HbA1c) levels well above the recommended <7% [21, 22]. There are important psychological factors related to adolescence that make adherence to the complex care regimens of diabetes management unattainable. There is also increasing recognition of mental health co-morbidities that both affect adolescents ability to self-manage their chronic disease [23].

Hypertension has also been consistently identified as an important modifiable risk factor for DKD in type 1 diabetes [24]. Youth onset type 2 diabetes studies that included 24 h ABPM data also support the importance of this clinical risk factor [25, 26]. Additional risk factors include duration of diabetes, and post-pubertal status [27]. Hyperlipidemia and smoking have also been associated with an increased risk of albuminuria [28, 29]. Obesity is common in youth with type 2 diabetes; however, it has not independently been shown to increase the risk of DKD early in the disease course [30].

Natural History

The natural history of DKD has 5 stages: glomerular hyperfiltration with a period of subclinical morphological changes; onset of albuminuria; progressive increases in albumin excretion; declining GFR; and ultimately ESKD (Table 32.1). The period of glomerular hyperfiltration is fairly consistent, but its pathogenicity is controversial [31]. Following this period, albuminuria starts to manifest, which once consistent, is considered the first clinical marker of DKD [3]. Historically, thresholds for microalbuminuria, and macroalbuminuria were described, but these thresholds are no longer recommended; albuminuria should be evaluated as a continuous outcome.

Traditionally, DKD developed in 25–35% of patients with type 1 diabetes, but typically manifested 10–20 years after diagnosis [32]. In modern adolescent cohorts, persistent albuminuria occurs in 0.7–9.2% of youth with type 1 diabetes

Table 32.1 Typical stages of diabetic kidney disease

	Clinical feature	Typical timeline after diagnosis
Hyperfiltration	eGFR >140 mL/min/1.73 m^2	0–<5 years
Albuminuria (Incipient Nephropathy)	UAE 30–300 mg/day ACR > 30 mg/g	5–15 years
Progressive albuminuria (Overt Nephropathy)	Increasing UAE >300 mg/day Declining eGFR	10–20 years
Progressive nephropathy (Chronic Kidney Disease)	Declining eGFR	15–25 years
End Stage Kidney Disease	eGFR <15 mL/min/1.73 m^2	>20 years

UAE Urinary Albumin Excretion, *ACR* Albumin:Creatinine Ratio, *eGFR* estimated Glomerular Filtration Rate

[29, 33] and 5.1–30.5% of youth with type 2 diabetes [25, 34]. Indigenous youth are at particularly high risk of early onset albuminuria and progression of CKD [30, 34–36]. This may reflect the important impact of developmental risk factors in this population. Autopsy studies have identified larger and fewer glomeruli in Indigenous adults, especially in individuals exposed to diabetes in pregnancy [37] and living in remote locations [38].

DKD is generally slowly progressive, typically taking 5 years to progress through each stage, with a decrease in GFR manifesting after many years of progressive albuminuria. GFR generally starts to decrease after more severe albuminuria has developed. Early in the course, the GFR decreases by 1–2 mL/min/1.73 m^2/year, but this can accelerate to 5–10 mL/min/1.73 m^2/year subsequently [3]. Once more severe albuminuria has developed, rates of progression are very high (cumulative risk of ESKD are 24.4%, 43% and 52% at 5, 10 and 15 years) [39].

With modern therapy, however, rates of ESKD have improved. Long-term, 30-year follow-up from the DCCT trial has shown nephropathy in only 9% of the trial cohort 30 years after diagnosis, and only 2% require kidney replacement therapy [40]. Similarly, a Swedish study reported a rate of persistent albuminuria of only 8.9% at 25 years, presumed to be secondary to improvement in glycemic control [41]. Studies in which glycemic control remains suboptimal continue to show high rates of kidney complications [42].

Rates of progression in youth with type 2 diabetes are much higher. Long-term follow-up of Indigenous youth from Canada and Australia with type 2 diabetes have shown rates of ESKD up to 50% [30, 43]. The modern type 2 diabetes cohorts are only now reaching young adulthood; therefore, more knowledge regarding eGFR trajectories in this population will be available [44, 45].

Ultimately, individuals with type 1 [46] and type 2 [47] diabetes that develop kidney disease are at increased risk of mortality; hence, prevention and delay of progression of kidney disease are very important.

Pathophysiology

The mechanisms proposed to contribute to hyperfiltration in diabetes include up-regulation of sodium-glucose cotransporter-2 (SGLT-2) in the proximal tubule due to the higher load of glucose. Up-regulation of SGLT-2, which co-transports sodium and glucose back into the circulation, decreases distal sodium delivery to the macula densa, thereby promoting renin release, and thus over-activation of the renin-angiotensin-aldosterone system (RAAS) [48]. Glomerular hypertrophy also leads to an increased filtration surface area. Additionally, abnormal vascular control decreases afferent glomerular arteriolar resistance and increases efferent glomerular resistance, ultimately causing an increase in renal blood flow [49].

Metabolic factors are also important, including advanced glycation end products (AGEs), which are thought to increase production of reactive oxygen species; stimulate intracellular molecules such as protein kinase C and NF-kB; and activate growth factors, including TGF-B and vascular endothelial growth factor. These factors, along with hemodynamic changes, contribute to podocyte injury, oxidative stress, inflammation and, ultimately, fibrosis.

Exacerbating factors in youth with type 2 diabetes may include a decreased functional nephron mass, as well as a higher prevalence of immune-mediated glomerular diseases [16]. Adults with diabetes may have hypertensive nephrosclerosis or ischemic nephropathy from atherosclerotic changes, either overlapping with traditional DKD or on their own.

Biomarkers

Albuminuria is the most established biomarker of early kidney disease, but there is a subset of patients that develop progressive CKD in the absence of proteinuria [50, 51]. This suggests that albuminuria may not identify all patients with DKD. There are several potential additional biomarkers that are predictive of progressive kidney disease. These include uric acid, tumor necrosis factor receptors, markers of oxidative stress and profibrotic cytokines [52], but their role in clinical practice is not defined.

Pathology

The typical pathological changes in DKD include mesangial matrix expansion, glomerular basement membrane thickening, diffuse or nodular glomerulosclerosis, and ultimately tubulointerstitial inflammation and fibrosis (Fig. 32.1). Biopsy studies in youth with type 1 diabetes between 1.5 and 5 years after diabetes onset show early pathological changes [53–55].

Pathologic classification of diabetic nephropathy has been divided into four hierarchical glomerular lesions [56]. Class I is defined by isolated glomerular basement membrane thickening and only mild changes on light microscopy. Class II includes mild (IIa) and severe (IIb) mesangial expansion. Class III includes nodular sclerosis (Kimmelstiel-Wilson lesions) in at least one glomerulus. Class IV, advanced diabetic glomerulosclerosis, includes glomerulosclerosis in more that 50% of glomeruli, along with lesions from Classes I-III. Interstitial and vascular lesions including interstitial fibrosis and tubular atrophy (IFTA) and arteriolar hyalinosis and arteriosclerosis develop concomitantly.

Biopsy findings in youth with type 2 diabetes often occur early in the disease course, but have frequently shown features other than typical diabetic nephropathy [57]. In Canadian First Nation children with type 2 diabetes, histologic changes included enlarged glomeruli; focal, mild hyaline arteriolosclerosis; and focal and mild glomerular basement membrane thickening (Fig. 32.2). Additional findings included immune complex deposition, and focal segmental or global glomerulosclerosis [57]. Most adults with type 2 diabetes exhibit typical DKD [58]; however, the prevalence of non-diabetic kidney disease varies (3–82.9%), with IgA nephropathy being a common finding, occurring in 3–59% of biopsies in a meta-analysis [17].

Kidney biopsy may therefore be indicated if a patient's course is not following the expected natural history of DKD in order to identify alternative causes of albuminuria or decreased eGFR. Non-diabetic kidney is more likely in patients with diabetes if there is nephrotic range proteinuria with a disease duration of <5 years; persistent hematuria suggestive of an immune-mediated process; rapidly progressive proteinuria or renal insufficiency; or a family history of non-diabetic kidney disease, [3] and may be an indication for kidney biopsy. The absence of retinopathy has been utilized as a criterion for biopsy is adult populations, but the association between retinopathy and DKD in youth with type 2 diabetes is unknown.

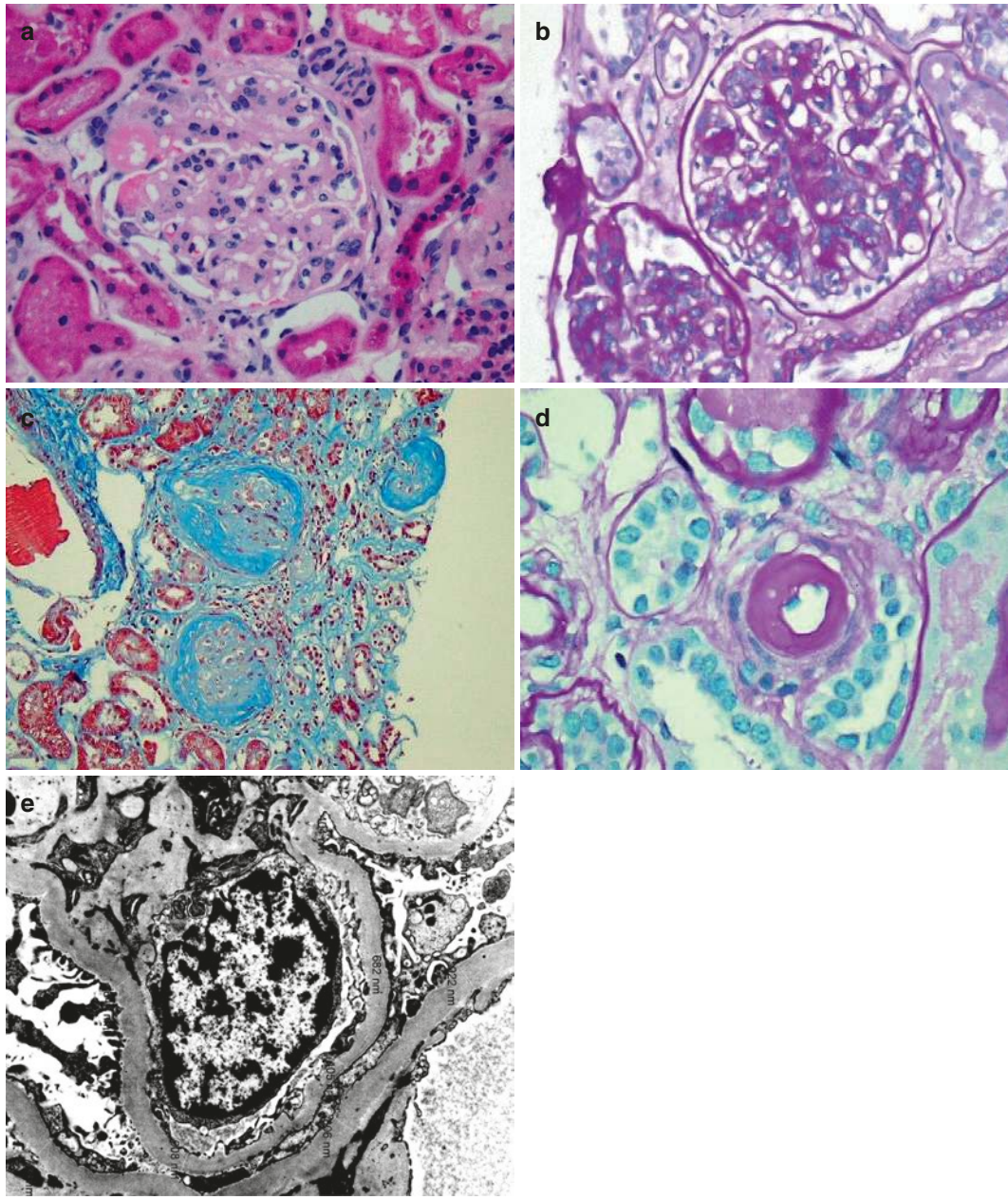

Fig. 32.1 Kidney pathology in an adolescent with type 1 diabetes for 10 years with poor glycemic control showing characteristic features of diabetic kidney disease. (**a**) Hematoxylin + Eosin [H+E] stain showing diffuse diabetic glomerulosclerosis with mesangial matrix expansion and secondary focal segmental glomerulosclerosis and hyalinosis. (**b**) Periodic acid-Schiff [PAS] stain showing moderate mesangial matrix expansion. (**c**) Trichrome stain showing three globally sclerosed glomeruli and associated severe tubular atrophy and interstitial fibrosis. (**d**) PAS stain showing nodular hyaline arteriolosclerosis. (**e**) Electron micrograph showing diffuse glomerular basement membrane thickening (>600 nm) and patchy epithelial cell foot process effacement. (*Images courtesy of Dr. Ian Gibson, University of Manitoba*)

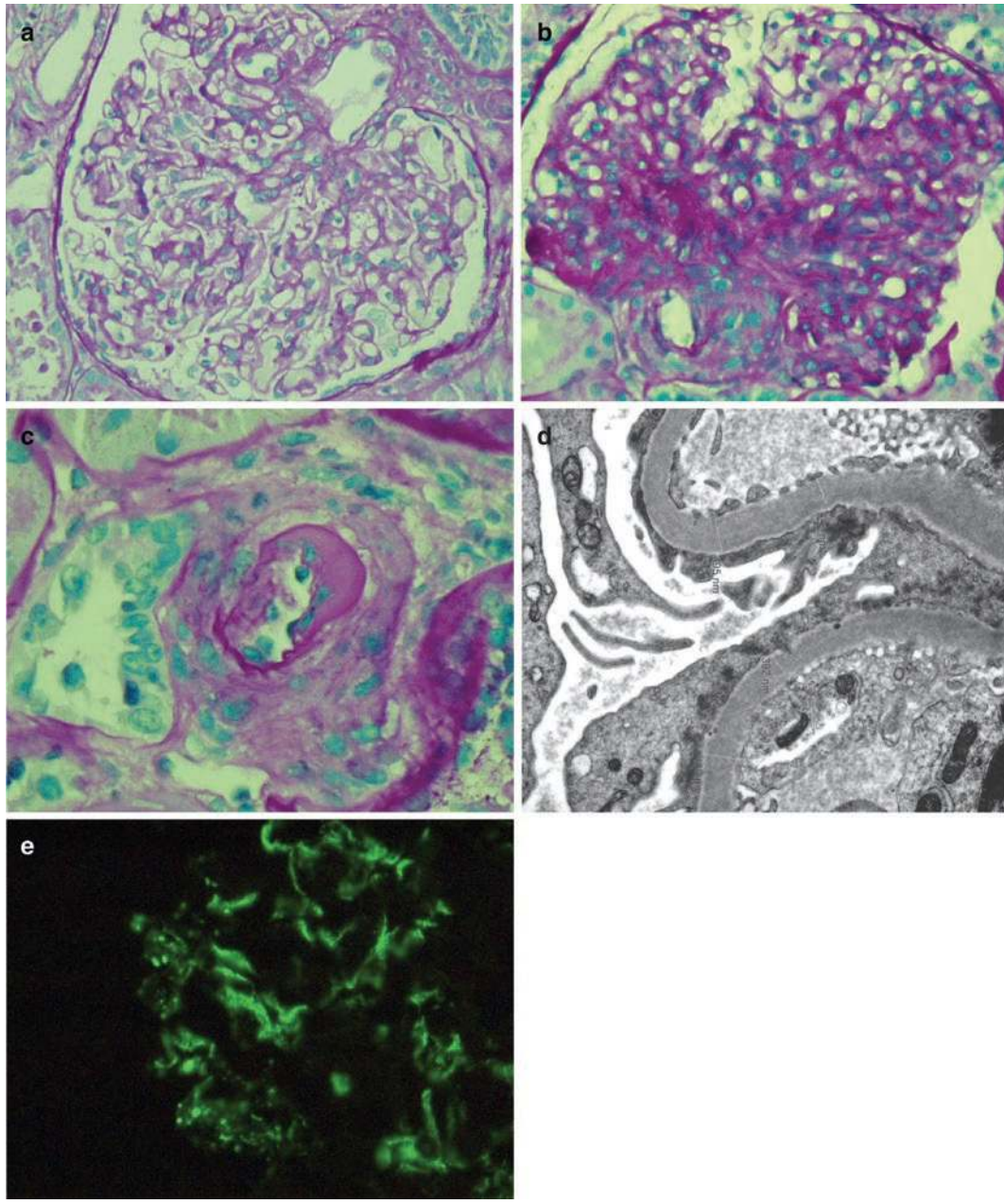

Fig. 32.2 Kidney pathology in a youth with type 2 diabetes and albuminuria. (**a**–**c**) Light microscopy (Periodic acid-Schiff stain), (**d**) Electron microscopy. (**e**) Immunofluorescence. Findings include (**a**) Glomerular hypertrophy; (**b**) Hilar focal segmental glomerulosclerosis; (**c**) Nodular arteriolar hyalinosis; (**d**) Early diffuse diabetic glomerulosclerosis with glomerular basement membrane thickening up to 500 nm; (**e**) Mesangial staining for IgA indicating co-existent non-proliferative IgA nephropathy. (*Images courtesy of Dr. Ian Gibson, University of Manitoba*)

Screening and Monitoring

Albuminuria

Yearly screening for albuminuria is recommended when patients with type 1 diabetes are at least 11 years of age and have had 5 years of diabetes [59] and at diagnosis for youth with type 2 diabetes [60]. Random urines are acceptable as initial screening tests. However, a first morning urine or overnight urine collection is required to confirm the diagnosis of non-orthostatic albuminuria if the random sample is abnormal [2]. The confirmation of persistent albuminuria also requires albuminuria in 2/3 samples at least 1 month apart over a 6-month time period [61]. Thresholds for albuminuria differ slightly across consensus guidelines. The Diabetes Canada urine albumin:creatinine ratio (ACR) threshold for albuminuria is >2 mg/mmol in adults with DKD (equivalent to a 24 h albumin excretion >30 mg/day) [3]; the American Diabetes Association utilizes an ACR threshold of 30 mg/g (3.39 mg/mmol); and the International Society for Pediatric and Adolescent Diabetes ACR threshold is >3.5 mg/mmol for males and >2.5 mg/mmol for females. KDIGO utilizes an ACR threshold of >3 mg/mmol [61]. Limitations of this test are false positives due to the high prevalence of orthostatic proteinuria in adolescents and intercurrent illness causing transient albuminuria. In addition, reduction in kidney function in the absence of albuminuria occurs in a subset of patients with DKD [62].

Glomerular Filtration Rate

Annual measurement of creatinine to determine the eGFR is also recommended, although the best equation for estimating eGFR in this population is unknown. As many youth have hyperfiltration, elevated eGFRs are quite common. For youth with type 1 diabetes, options for eGFR include the CKiD equation [63], with or without the addition cystatin C, or other cystatin C based equations if cystatin C is available [64]. Equations utilized in the youth onset type 2 diabetes population include the Zappitelli equation [65, 66] and the iCARE study equation, which was developed and validated in an Indigenous population in Canada [67]. All existing formulas have limitations, and therefore should be interpreted with caution.

Blood Pressure

Blood pressure should be measured at each healthcare encounter. Based on the American Academy of Pediatrics clinical practice guidelines, blood pressure is considered elevated at a threshold of >90th percentile for age, sex and height or >120/80 in adolescents >13 years of age. Hypertension is defined as >95th percentile for age, sex and height or>130/80 in adolescents >13 years of age. In addition, routine performance of 24-h ambulatory blood pressure is strongly recommended in children with diabetes to assess hypertension severity and to evaluate circadian blood pressure patterns [68].

Lipids

Screening for dyslipidemia is recommended after stabilization of hyperglycemia in children with type 1 diabetes after age 11. As fasting lipid profiles are not always feasible, non-fasting screening is acceptable. If abnormal, a fasting sample should be obtained [69].

Treatment

Preventive strategies should be considered early in the disease course of diabetes. Intervention during childhood and adolescence prevents or delays complications [70]. Specific treatment targets for DKD include glycemic and blood pres-

sure control. Optimization of glycemic control is critical for preventing or delaying albuminuria and progression in youth with both types of diabetes. The target HbA1c and blood pressure is the same for both types of diabetes: HbA1c <7% and the blood pressure <90th percentile for age, sex and height or <130/80 in adolescents >13 years.

Blood Sugar Control

Treatment with insulin is required in youth with type 1 diabetes. Pharmacologic therapy approved for youth with type 2 diabetes includes insulin or metformin [60]. Efforts to address lifestyle related risk factors for diabetes, especially in patients with type 2 diabetes, include healthy eating, with a diet high in vegetables, fruits, whole grains, fiber, legumes, plant-based proteins, unsaturated fats, and nuts, with few processed meats, refined carbohydrates and sweetened beverages; light and vigorous exercise; and adequate sleep quality and quantity [60]. Specific recommendations include 60 min of daily moderate-to-vigorous physical activity, screen time limited to no more than 2 h per day, limiting sitting, and time spent indoors [71]. Team-based, multidisciplinary care, including nutrition specialists, to support patients with these recommendations is critical. In addition, the screening and treatment of mental health co-morbidities is essential [25, 72]. It is important, however, to provide counselling to patients that is non-judgmental, and considers the social context of the individual, the impact of food insecurity [73] and access to safe places to be active [74]. Many youth with type 2 diabetes confront stigma, which can significantly add to the challenges of managing a chronic disease [75]. Healthcare providers have a responsibility to educate themselves about systemic barriers that families confront, and to focus less on individual behavior strategies and blame.

Smoking Cessation

Implementation of strategies to promote smoking prevention or cessation is also important [76].

Lipid Management

The intervention for hyperlipidemia depends on the severity of the problem. An LDL cholesterol >2.6 mmol/L warrants a low fat diet and increased exercise. An LDL >3.4 mmol/L is the threshold for initiation of a statin in children over age 10, with a target of <2.6 mmol/L [69].

Hypertension Management

The first line medications for the treatment of hypertension and albuminuria are RAAS inhibitors, including angiotensin-converting enzyme inhibitors (ACEi) and angiotensin receptor blockers (ARBs). Many intervention studies in adults have documented decreased progression of albuminuria and attenuated decline of GFR with these therapies [77, 78]. These drugs are safe and effective in children with CKD [79]. The AdDIT trial, which randomized youth with type 1 diabetes in the upper tertile of ACR to an ACEi, a statin or both, did not identify a significant effect on albuminuria progression over 2–4 years [33]. Hence, initiation of these medications prior to the onset of hypertension or albuminuria is not recommended.

Combinations of ACEs and ARBs have been associated with safety concerns, including increased risk of acute kidney injury and hyperkalemia [80], and are therefore not recommended. Side effects of ACEi include hyperkalemia, cough, and a decrease in eGFR. They should be avoided if eGFR <30 mL/min/1.73 m^2. Monitoring of serum creatinine and potassium is indicated within 2 weeks of starting the medication. In addition, due to the potential teratogenicity in pregnancy, pre-conception counselling in postmenarchal girls is essential [81].

Novel Therapies

SGLT2 inhibitor trials, including The Canagliflozin and Renal Events in Diabetes with Established Nephropathy Clinical Evaluation (CREDENCE) trial, have demonstrated improved

glycemic control, weight loss, and renal protection in adults with type 2 diabetes [82]. Randomized trials which include adolescents with type 2 diabetes are ongoing. These drugs are associated with an increased risk of euglycemic diabetic ketoacidosis; therefore, they likely warrant significant caution in adolescents. Guidelines now consider SGLT2 inhibitors standard of care for adults with type 2 diabetes [83]. They have the potential to significantly delay DKD progression in adolescents if safety is demonstrated.

Glucagon-like peptide-1 agonists have been studied and approved by the United States Food and Drug Administration in children and adolescents with type 2 diabetes [84]. The potential kidney benefits, beyond glycemic control, are not fully understood. Newer studies are also focusing on AGE inhibitors, metabolic memory resulting from epigenetic changes from hyperglycemia, and incretin-related drugs for their effects on renal outcomes [85] (Table 32.2).

Table 32.2 Screening, risk factors and treatment of diabetic kidney disease in youth with type 1 and type 2 diabetes

	Type 1 diabetes	Type 2 diabetes
Timing of screening	11 years with 2–5 years diabetes duration	At diagnosis
Screening method	Random urine for albumin:creatinine ratio annually Serum creatinine and/or Cystatin C for calculation of eGFR annually	
Follow-up screening	If random urine ACR 30mg/g to align or 3mg/mmol then collect 2–3 first am urine samples over the next 6 months, at least 1 month apart	
Risk factors	Hyperglycemia Hypertension Lipid abnormalities Smoking Duration of diabetes Post-pubertal status	Hyperglycemia Hypertension Minority ethnicity Socioeconomic factors
Treatment	Diet and exercise, smoking cessation Angiotensin converting enzyme inhibitors or angiotensin receptor blockers for persistent urine ACR 30mg/g or 3mg/mmol and/or blood pressure >130/80	
Targets	HbA1c <7% Blood pressure <130/80 >13 years or <90th percentile for age, sex, height <13 years	
Treatment considerations	Multidisciplinary teams Mental health assessment and treatment	

eGFR estimated glomerular filtration rate, *ACR* albumin:creatinine ratio, *HbA1c* glycosylated hemoglobin A1c

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Disordered Hemostasis and Renal Disorders

33

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Hemostasis

Hemostasis constitutes a physiologic response of the human body to injury regulated by the dynamic equilibrium between pro-coagulant and natural anticoagulant mechanisms. An inherited and/or acquired defect in any of those pro- or anticoagulant forces may "tip the clotting balance", resulting in either bleeding or excessive hypercoagulability.

Endothelial cells are one of the major components of the hemostatic system, covering the vascular structures and having a primordial anticoagulant role when the body is in a steady state. However, after an endothelial injury occurs, a process entitled primary hemostasis ensues, where platelets play a major role towards thrombus formation particularly under high shear stress forces (e.g., arterial circulatory component). Secondly, in conjunction with the now activated platelets and their newly exposed negatively charged phospholipid (PL) membranes, the coagulation factors that are circulating in a non-active state are activated in sequence to promote the formation of an insoluble thrombus, which will ultimately anchor the platelet plug to the newly formed wound site, preventing excessive bleeding. The steps summarized above are part of the accepted model that integrates the hemostatic response, entitled the "cellular model" of the coagulation cascade [1]. The mechanisms of the second wave, called secondary hemostasis, are counterbalanced by the progressively increased activation of natural anticoagulant pathways, which will ultimately tailor down the formation of insoluble thrombus in an attempt to restrain its growth to the wound site. Additionally, the fibrinolytic system will also help contain the newly formed thrombus to the wound site by promoting a local thrombolytic effect to digest thrombus formed in excess (Fig. 33.1). Moreover, non-coagulation components in blood, such as red blood cells (RBC) and white blood cells (WBC), also have a contributory role in the hemostatic system. Understanding normal hemostasis is helpful and necessary to explain the pathophysiology of hemorrhage and thrombosis in patients with renal diseases.

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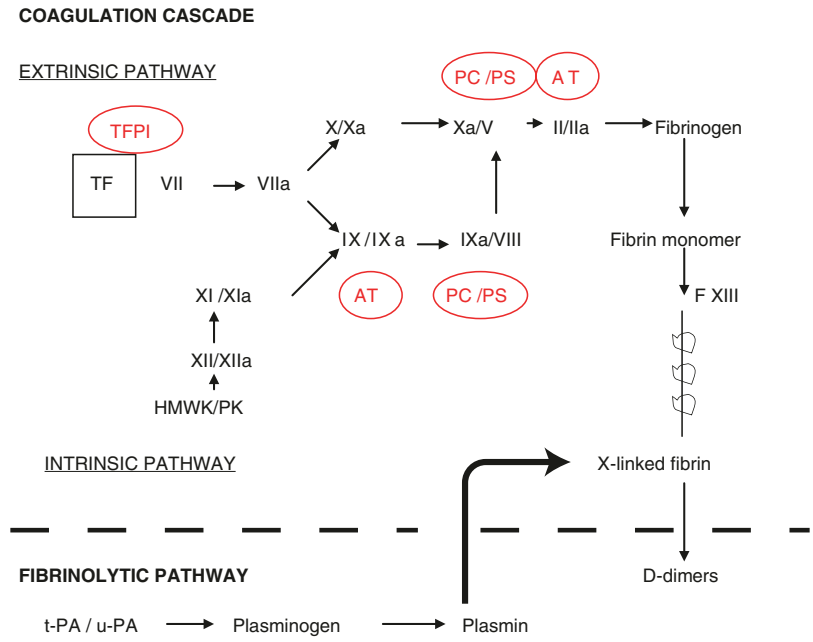

Fig. 33.1 The coagulation cascade and the fibrinolytic pathway. *Red*: natural coagulation inhibitors; *black*: pro-coagulant system: *bold*: fibrinolytic pathway. *PC* protein C, *PS* protein S, *AT* antithrombin, *TFPI* tissue factor pathway inhibitor, *TF* tissue factor, *VII* factor VII, *VIIa* activated factor VII, *X* factor X, *Xa* activated factor X, *V* factor V, *II* prothrombin, *IIa* thrombin, *IX* factor IX, *IXa* activated factor IX, *FXIII* factor XIII, *XII* factor XII, *XIIa* activated factor XII, *HMWK* high molecular weight kininogen, *PK* pre-kallikrein, *X-linked* cross linked, *t-PA* tissue plasminogen activator, *u-PA* urokinase

Primary Hemostasis

Vessel Wall

When a vessel wall is damaged, many different pro-coagulant components of the hemostatic system are activated to generate a clot (i.e., thrombus). The release of cytokines leading to vasoconstriction and activation of the local endothelial cells occurs immediately after injury. This process is a result of the neurogenic reflex when endothelin, a vasoconstrictive agent, is released from endothelial cells in a process that sustains hemostasis for an initial short period of time [2]. Additionally, serotonin, a substance which is released from dense granules of activated platelets, and thromboxane A2 (TXA2), a derivative of platelet membrane PL metabolism, stimulate smooth muscles of the vessel wall and augment the process of vasoconstriction [3].

Endothelium

Endothelial cells have a great influence on hemostasis because of their interaction with all parts of the hemostatic system. Endothelial cells produce several important substances directly or indirectly related to the hemostatic processes: [2, 3]

- Vasoactive substances
  - Nitric oxide (NO) and prostacyclin (PGI2), which are both potent vasodilators.
  - Heparan sulfates, a group of "heparin-like" compounds that prevent the initiation phase of the coagulation cascade.
- Pro-coagulant activation
  - Von Willebrand factor (VWF).
  - Coagulation cascade: tissue factor (TF) and factor VIII (FVIII).
- Anticoagulation mechanisms

(a) Natural anticoagulant pathways
  - Protein C (PC)
  - Protein S (PS)
  - Thrombomodulin (TM)
  - Endothelial PC receptor (EPCR).
  - Tissue factor pathway inhibitor (TFPI).

(b) Fibrinolytic system
  - Tissue-type plasminogen activator (t-PA)
  - Urinary type plasminogen activator (u-PA).
  - Plasminogen activator inhibitor type 1 (PAI-1).

In addition, the disruption of endothelial cells at the site of vascular injury exposes subendothelial matrix, which is an activator of platelets and of the coagulation system [3–5].

Platelets

Platelets are disc-like, anucleated cells generated by megakaryocytes in the bone marrow that have a major role in primary hemostasis. After the exposure of subendothelial matrix, circulating platelets adhere to the injured site in a process called "platelet adhesion." This first step of platelet activation results from the interaction between several glycoprotein (GP) complexes expressed by the platelet surface (e.g., GP Ib/IX/V, GP Ia/IIa and GP VI), with the subendothelial matrix. For instance, the complex named GP Ib/IX/V interacts with VWF under high shear conditions as the VWF molecule unfolds, exposing the domains which will bind to the GP complex expressed by the platelet membrane, promoting the platelet to vessel wall interaction that leads to adherence of platelets to subendothelial matrix. In addition, GP Ia/IIa and GP VI adhere directly to collagen fibers in subendothelial matrix [5, 6].

After platelet adhesion, platelet activation occurs rapidly. During this process, the many pro-coagulant substances contained within platelet granules are released, including calcium (Ca^{++}) and ADP from dense granules, FV, FXI, FXIII, VWF, platelet factor-4 (PF4) and other substances from alpha granules, all ultimately leading to further activation of other platelets. During this second step of platelet activation, platelets also change their shapes by remodeling their platelet cytoskeleton, to fully spread with pseudopodia and expose important platelet membrane PL, phosphatidylserine and phosphatidylethanolamine [4]. This process further increases the amount of activated platelet surface area, which is necessary for the next step of secondary hemostasis [4, 7].

The major platelet GP activated during this step is GP IIb/IIIa, which works with fibrinogen under low shear flow and VWF under high shear flow to promote platelet-platelet interactions, also known as platelet aggregation. Several agonists, such as ADP, collagen, arachidonic acid and thrombin at the damaged vessel wall, also play a role on specific receptors in this process [4, 5].

Secondary Hemostasis

The secondary hemostatic wave combines serial proteolytic reactions to activate coagulation proteins (i.e., coagulation factors), culminating in the formation of an insoluble fibrin clot (Fig. 33.1). Postulated in 1964, the original waterfall cascade model of coagulation is still helpful to explain the in vitro phenomenon evaluated by screening coagulation tests, namely the prothrombin time (PT) for the extrinsic pathway and the activated partial thromboplastin time (APTT) for the intrinsic pathway of the coagulation cascade [7, 8]. Coagulation factors are most often synthesized in the liver, and can be classified by their functions into a few distinct groups as follows: contact factors (include prekallikrein, high-molecular-weight kininogen, FXII and FXI), extrinsic tenase complex (comprises TF and activated FVIIa), intrinsic tenase complex (consists of both FVIIIa and FIXa), prothrombinase complex (includes FXa and FVa), thrombin (FIIa) (activates fibrinogen and FXIII, which stabilizes fibrin), and fibrinogen and factor XIII (FXIII), which work together to form a fibrin clot.

Anticoagulation System

There are three major systems of proteins that counteract the coagulation system by inactivating coagulation proteins.

(a) *Antithrombin* (AT; formerly known as ATIII) inhibits mainly FIIa and FXa. In addition, it also inactivates FIXa, FXIa and FXIIa. The liver synthesizes AT, which potentiates unfractionated heparin anticoagulant power by 1000-fold.
(b) *TFPI* inactivates the extrinsic tenase complex. TFPI is released from endothelial cells and platelets.
(c) *PC*, *PS and TM* work together to inhibit FVa and FVIIIa. After being released from endothelium and activated by thrombin, TM combines with TM receptor and EPCR to form a complex with PC. Then activated PC (APC) is released from the complex and works with PS to inhibit target factors. Both PC and PS are vitamin K-dependent enzymes and synthesized by the liver [2–7].

Fibrinolytic System

The fibrinolytic system is a complex system that lyses or "digests" fibrinogen and non-cross linked and cross-linked fibrin. There are many proteins involved in the fibrinolytic system [9] and these include:

(a) *Plasminogen*, *t- PA and u- PA* work together to lyse a clot.
(b) *α 2 - antiplasmin* (*α 2–AP*) is synthesized in the liver and inactivates plasmin by forming a 1:1 ratio complex.
(c) *α 2 - macroglobulin* (*α 2–M*) is a four-polypeptide protease that can inhibit plasmin and tPA.
(d) *Thrombin-activatable fibrinolytic inhibitor* (*TAFI*) is synthesized by the liver and activated by thrombin and plasmin. It inhibits fibrinolysis by cleaving some parts of fibrin, which prevent fibrin degradation by plasmin.

The final products of fibrinolysis are called fibrin degradation products (FDP), which consist of different-sized lysed fibrin-derived fragments. One of the FDP- laboratory tests, which is commonly used in clinical practice, is called D-dimer.

Other Components

(a) *Red Blood Cells* (*RBCs*): in normal blood flow conditions, RBCs circulate in the central part of the flow within vessels while platelets flow along the vessel wall [4]. This blood rheology facilitates platelets reaching injured sites quicker. Patients with decreased red cell mass lose this mechanism, potentially having impaired platelet function. Nonetheless, hemolytic anemias may cause a hypercoagulable state leading to an increased risk for thromboembolic events (TEs) by other mechanisms [10].
(b) *White Blood Cells* (*WBCs*): an extremely high number of WBC causes hyperviscosity of the blood and can result in thromboembolism in leukemic patients. In addition, monocytes can be activated and express TF in some specific conditions, contributing to thrombus formation [11].
(c) *Neutrophil Extracellular Traps (NETs):* an original defense mechanism by neutrophils and leukocytes to extrude their DNA and protein components (e.g., histones) to trap and kill pathogenic microorganisms, which can lead to pathogenic thrombosis [12].

Age-Appropriate Development of Hemostasis (Developmental Hemostasis)

Hemostasis evolves from the fetal period, starting at 10 weeks of gestational age.

Primary Hemostasis

(a) Vessel wall and endothelial cells: increased levels of glycosaminoglycans in the vessel

walls of neonates promote antithrombotic property by working with AT, while increased levels of VWF and large VWF multimers from endothelial cells counteract this effect [13].

(b) Platelets: there is no difference in platelet numbers between healthy neonates and children. Conversely, the platelet count in preterm neonates can be lower than term neonates due to several factors. Platelets in full-term neonates express a lower quantity of specific receptors on the platelet surface. The response to several agonists may also be less pronounced in comparison to the response found in adult platelets. However, these observations suggestive of a likely platelet function defect in neonates is counterbalanced by a higher red cell mass, mean corpuscular volume (MCV), higher circulating VWF level and a higher proportion of large VWF multimers [14].

Secondary Hemostasis

At birth, the levels of vitamin K-dependent factors (prothrombin, FVII, FIX and FX), contact factors and FV are lower than in adults. In contrast, FVIII, VWF and TF levels are higher than in adults during the first 6 months of life, subsequently decreasing [15–18]. The levels of fibrinogen and FXIII are similar from birth to adulthood [15, 17, 18]. However, a study of endogenous thrombin potential (ETP) showed almost two-times higher levels in adults compared to children less than 5 years of age [18].

Anticoagulation System

At birth, the levels of AT and TFPI are lower than circulating adult levels. The same finding applies for PC and PS [15, 17, 18]. PS gradually increases to adult levels at 6 months of age; whereas, for PC circulating levels reach adult values only after 11 years of age [16].

Thrombolytic System

The levels of plasmin, PAI-1 and α_2-AP are lower at birth than in adults. In contrast, t-PA and α_2-M are much higher at birth than in adults. Most of the fibrinolytic proteins reach adult levels within 5 days of life, except plasminogen, which increases to adult levels at 6 months of age, and α_2-M, which is still elevated until the second to third decade of life. The level of u-PA measured in neonates remains controversial [8, 15].

Ultimately, healthy neonates and infants do not bleed spontaneously when challenged during birth despite their distinct platelet-related laboratory testing results. Likewise, despite having lower circulating levels of many of the natural anticoagulant pathways, neonates, infants, and children have an incidence of thrombosis that is much lower than the one reported in adults [19]. Moreover, venous thrombotic events in neonates and infants are almost invariably provoked. This contrast between laboratory and clinical findings highlights some of the limitations of current laboratory testing, as well as the yet unraveled aspects of developmental hemostasis.

Bleeding in Renal Disease

Uremic Coagulopathy

The association between uremia and bleeding was first described in 1764 by GB Morgagni. In 1836, Richard Bright published on 100 cases of patients with albuminous urine and also noted the connection between purpura and uremia. The observation that bleeding in uremic patients occurs despite having normal clotting factors led to the supposition that the primary abnormality must be within the platelet system [20]. Despite many theories and suppositions, the exact etiology of uremic coagulopathy remains poorly understood.

Clinical Manifestations

The occurrence of bleeding in uremia is twofold higher in those with chronic kidney disease (CKD) and has been widely reported [20–22]. This includes potential bleeding in many locations, including the skin and mucosa, the gastrointestinal tract, the retroperitoneum, ocular tissues, genitourinary system, and intracranial. There are potential risks of bleeding during surgery or post-operatively and from venipuncture and renal biopsy sites. Pleural and pericardial hemorrhagic effusions have also been described. Most of these reports have been in the adult population with only a few scattered reports of increased bleeding risk in uremic children [20]. Whether the adult risks of bleeding can be extrapolated to children is a question that is still unanswered, given the developmental hemostatic differences.

Pathogenesis

The levels and function of coagulation factors are normal in patients with CKD [23]. From these data, it is assumed that platelet dysfunction is primarily responsible for the increased bleeding risk due to uremia. When placed into normal plasma, uremic platelets demonstrate normal function, implying that causative factors are present in the surrounding uremic plasma. However, research has found that there are both intrinsic and extrinsic platelet abnormalities that result in the uremic coagulopathy.

Intrinsic Platelet Abnormalities

In CKD and uremia, the content of ADP and serotonin is reduced in the platelet granules. This is felt to be either an acquired storage pool defect or a defect in secretory mechanisms [23]. Cyclic adenosine monophosphate (cAMP) has been reported to be increased in CKD, which can affect the mobilization of calcium in response to stimulus and, ultimately, platelet activation. It may be through an imbalance among ADP, serotonin and cAMP that results in platelet activation defects. Other defects include low levels of GP Ib/V/IX in association with elevated levels of glycocalicin, a proteolytic byproduct released by GP Ib/V/IX when damaged on the platelet surface [24]. Thromboxane A2 levels, generated from free arachidonic acid, are also low in uremia and result in poor platelet adhesion and aggregation [25].

Platelet contractility defects may be another factor contributing to platelet dysfunction by reducing its mobility and secretory capacity [26]. In uremic states, platelets have deficient cytoskeletal proteins, such as α-actin and tropomyosin, with the abnormalities becoming more pronounced after activation by thrombin.

Platelet: Vessel Wall Abnormalities

Levels of VWF and fibrinogen are normal in uremic states [22, 23]. There is normal surface expression of the platelet receptor GP Ib/V/IX, although the total levels of GP Ib/V/IX have been found to be suboptimal [24]. It has also been shown that there is impaired binding of VWF to GP 1b/V/IX and that this results in lower levels of TXA2 and ADP, both necessary to stabilize hemostatic plugs [27]. Another noted abnormality is reduced binding capacity of VWF and fibrinogen to GP IIb/IIIa, resulting in reduced platelet adhesion to injured endothelium. This may be secondary to receptor blockade by fibrinogen, or through substances that are dialyzable, as dialysis improves this anomaly.

Other extrinsic factors that might come into play include platelets and prostaglandins [28–30]. Anemia in CKD may influence platelet function through changes in laminar blood flow, as previously noted. A reduction in hematocrit can change platelet travel from where it is normally at the periphery of a blood vessel to the central part where erythrocytes traverse. Reduced contact with vessel wall results in stimulation of platelet ADP release and activation of PGI-1, which reduces platelet activity. Prostaglandin-I2 (PGI2) is a vasodilator released by endothelial cells and inhibits platelet function through its action on adenylyl cyclase and its modulating effects on cAMP and calcium mobilization within platelets. Although several studies have shown increased production of PGI2 in

endothelium of uremic models, blockage of PGI2 production does not result in improved coagulation, thereby suggesting that there are other factors that are involved in platelet dysfunction in renal failure. Vasoactive substances like nitric oxide are increased in CKD, which can further inhibit platelet function.

Circulating uremic toxins may also play a role in uremic coagulopathy. Substances such as urea, creatinine, phenol, phenolic acids and guanidinosuccinic acid (GSA) have all been investigated for their potential effects on platelet function [24]. GSA inhibits the second wave of ADP-induced platelet aggregation. This is further supported by the observation that dialysis can partially correct these defects.

Finally, platelet number and volume are both reduced in uremia [30]. Platelet numbers are lower in uremia when compared to healthy controls, although they are rarely less than 80×10^9/L. The reduction in platelet volume can further reduce the amount of circulating platelet mass, resulting in ineffectual platelet contact with injured endothelium.

Treatment

Treatment for uremic coagulopathy in the past was based on its ability to normalize the prolonged bleeding time (BT) observed in uremic patients. However, BT has no in vivo correlation with risk of bleeding, so that treatment should only be directed towards active cases of bleeding in the setting of uremia. There is also limited evidence that prophylactic treatment reduces the bleeding risk [31]. Treatment should therefore be utilized if there is active bleeding and includes dialysis, erythropoietin, desmopressin (DDAVP), estrogens, and cryoprecipitate [27, 28, 32–34]. DDAVP should be the first line of therapy in a bleeding uremic patient [27]. Discussion here will focus around the use of DDAVP in settings where there is a significant history of clinical bleeding.

DDAVP was first utilized for its anti-diuretic properties until it was discovered in the 1970s to have hemostatic properties [35]. Infusions of DDAVP increase VWF, factor VIII coagulant activity, ristocetin co-factor and tissue plasminogen activator. The rise of coagulation factors is rapid, likely related to release of endogenous reserves rather than new synthesis. DDAVP may also promote the glycoprotein transmembrane proteins, including VWF and GP IIb/IIIa.

Administration of DDAVP:

- Administered via intravenous, subcutaneous, or intranasal routes.
- The maximal effect on clotting factor levels occurs at 30 min, lasting up to 6 h, with an intravenous dose of 0.3 μg/kg (to a maximum of 20 μg/dose infused in 20–50 mL of normal saline over 15–30 min).
- With subcutaneous dosing, the levels peak at 1–2 h.
- For intranasal administration, a dose of 300 μg is comparable to 0.2 μg/kg intravenous dose. Use one single spray in one nostril (150 μg) if <12 years/50 kg; 1 puff per nostril (300 μg) if ≥12 years/50 kg.
- Tachyphylaxis occurs after the first dose due to depletion of FVIII and vWF endothelial stores.
- Adverse effects of DDAVP include facial flushing, headache, hypotension, tachycardia, water retention, hyponatremia and seizures (uncommon but higher incidence in children less than 5 years of age). Hypotonic solutions should be administered with caution in children who have received DDAVP.
- DDAVP should not be used in children <3 years, or in cases of polydipsia, unstable angina, or congestive heart failure because of its antidiuretic effects.

Conjugated estrogens improve the BT in uremia by increasing platelet responsiveness [32]. There have been no reports of its use in children, although adult studies recommend a dosage of 0.6 mg/kg/day given intravenously daily for 4–5 days. Effects start within 6 h and can last for up to 2 weeks after an intravenous course. It can also be given as an oral dose, but the effect is shorter, lasting up to 5 days. Side effects include hypertension, fluid retention and raised liver transaminases.

Cryoprecipitate is rich in factor VIII, vWF, fibrinogen and factor XIII, but has the risks of blood borne infections and anaphylaxis [36].

Clotting in Renal Disease

Thrombotic Manifestations of Nephrotic Syndrome

Epidemiology

Nephrotic syndrome (NS) is a hypercoagulable state with a predisposition to the development of thromboembolic events [37–42]. With effective treatments for inducing remission, thromboembolism (TE) is much less common now in children as compared to adults with an overall incidence of 3% versus 25%, respectively [43–46]. TE seems to be more likely in adolescents [46], in children with congenital NS (incidence around 10%) [47, 48], and in secondary NS, such as NS associated with vasculitis (incidence around 17%) [46]. Membranous nephropathy or a histologically similar process (e.g. class V SLE nephritis) seems to confer the highest risk, where the incidence of TE approaches that seen in adults (25%) [46]. A correlation between infection and TE has also been observed [49].

Venous TE (VTE) is the predominate form of thromboembolic disease in children with NS, accounting for 97% of the cases in a large, retrospective study [46]. In that study, arterial disease was encountered in only 0.3% of subjects. Commonly affected areas for VTE include: deep venous system within the lower limbs, inferior vena cava, renal vein, hepatic veins, and sagittal and transverse sinovenous vessels. Arterial thromboses can involve any artery, including femoral, mesenteric and intracardiac [39, 41, 46]. Finally, TE tend to occur early in the course of the disease (usually <3 months from onset) [38].

Pathogenesis

The prothrombotic tendencies in nephrotic patients have been attributed to a number of factors including state of hydration and hyperviscosity, imbalance between clotting factors and thrombophilic proteins, increase in platelets and platelet activation, abnormalities of the fibrinolytic system and use of medications [38, 40].

Antithrombin (AT; formerly known as ATIII) is an endogenous anticoagulant that was first documented to be low in NS in 1976, with confirmation in subsequent studies [37–39]. In these studies, AT had a strong correlation with plasma albumin levels and a negative correlation with urinary protein excretion, suggesting that one of the mechanisms resulting in low AT levels is due to its loss in the urine [50]. Subsequent remission of the nephrotic syndrome results in normalization of AT levels. Data on other *in vivo* anticoagulants have not been conclusive, although the majority of studies suggest that PC, PS, and tissue factor pathway inhibitor are all elevated during acute nephrotic relapses, but functionality may be reduced [37, 42, 51–53]. This might exert a protective effect against thrombosis and might explain why children have fewer thromboembolic events than adults with NS.

Platelets have been found to be higher in number and more active in children with NS. Studies suggest improved platelet availability due to their higher numbers and increased exposure of the normally albumin-bound arachidonic acid leading to thromboxane A2 activation and subsequent platelet aggregation [42, 54]. Hyperlipidemia may also promote platelet aggregation, based on the simple observation that treatment with lipid lowering agents decreases platelet hyperaggregability in NS [55].

The fibrinolytic system is also speculated to influence the risk of thromboembolic events in NS. Lower levels of plasminogen and tissue-type plasminogen activator (tPA) results in diminished fibrinolytic activity. This may be accentuated by hypoalbuminemia since albumin is a cofactor for binding plasminogen to fibrin [37, 42, 46, 56].

The use of corticosteroids has been reported to be associated with hypercoagulability [57, 58]. Mechanisms responsible include increase in coagulation factors and reduction in fibrinolysis.

Finally, children with congenital nephrotic syndrome are at higher risk of TE, which may be explained in part by a disease-specific pathophysiology. In addition, they are unlikely to achieve NS remission and are thus hyperco-

agulable for a longer time-frame, and they more often require the use of a central venous catheter (CVC), which is a well-known risk factor for TE.

Prevention and Treatment

Non-pharmacologic strategies to limit the risk of VTE include regular ambulation, adequate hydration, and avoidance of CVCs whenever possible [59].

The use of prophylactic anticoagulation to prevent TE in children with NS is controversial, due in part to the lack of randomized trials to determine the efficacy and safety of such an approach [60]. Despite the lack of robust evidence, some authors have suggested prophylactic strategies for children based on risk factors, severity markers, or coagulation abnormalities. Among these strategies, some authors have recommended aspirin prophylaxis in some circumstances [59, 61, 62]. Others propose AT replacement by administration of AT concentrates [40, 63].

Once TE has developed, clinical management is similar to that utilized in patients without NS, starting with heparinization [64]. In this case (i.e., severe AT deficiency), treatment with AT concentrate may be necessary as heparinoids are dependent on AT for their mechanism of action. However, the optimal target level of AT to achieve an adequate anticoagulant effect in children with NS is unclear. Both plasma-derived and recombinant AT are clinically available [65].

Renal Vein Thrombosis

Although renal vein thrombosis (RVT) is the most common non-catheter related thrombosis in the newborn period, few long-term outcome studies have been carried out [66–71]. Most RVTs present in the first month, with 70% presenting in the first week of life. It affects twice as many males and there is a left sided predominance. The clinical features of RVT are variable and include hematuria, oliguria-anuria, hypertension, decreased renal function, palpable flank mass and thrombocytopenia. Doppler ultrasound may show a decrease in amplitude or absence of venous signal, abnormal flow patterns in a number of renal venous branches or evidence of venous collateral development. The etiology of RVT in most cases is unknown. There is speculation about decreased levels of naturally occurring anticoagulants and fibrinolytic compounds leading to the thrombotic event [71]. Risk factors reported for the development of RVT include prematurity, maternal diabetes mellitus (either type 1 or gestational), pathologic states associated with thrombosis (e.g., shock, dehydration, perinatal asphyxia, polycythemia, cyanotic heart disease), sepsis, umbilical venous catheterization, conjoined twins, and inherited prothrombotic abnormalities [69, 72, 73]. However, the prevalence of these disorders has not been studied in a cohort of patients with neonatal RVT.

The sequelae of RVT reported in the literature include death (5%), glomerular disease (3–100%), tubular dysfunction (9–47%), hypertension (9–100%), and evidence of renal scarring or atrophy (27–100) [66–71]. Performance of multicentre, randomized clinical trials is required to investigate the safety and efficacy of treatment for RVT and to determine the long-term outcomes.

Renal Artery Thrombosis

In neonates, renal artery thrombosis (RAT) occurs as a result of umbilical arterial cannulation, with a low incidence of symptomatic cases (1–3%) [74]. In older children, RAT is most commonly associated with renal transplant and occurs at the site of vascular anastomosis. It has also been reported in patients placed on ventricular assist devices [75].

Risk factors for RAT in kidney transplant recipients include cadaver donor source, pre-transplant peritoneal dialysis, more than five pre-transplant blood transfusions, cold ischemia time >24 h, type of immunosuppression, and prior renal transplant [76–79]. There are no studies on the treatment of RAT determining the safety and efficacy of embolectomy, fibrinolysis

or anticoagulation. If RAT associated with renal transplant is diagnosed, embolectomy or fibrinolytic/anticoagulation therapy should be considered in the absence of contraindications (bleeding) to attempt to save the graft [80].

Renal Vascular Thrombosis in Renal Transplantation

Graft failure secondary to renal vascular thrombosis in the most recent report of NAPRTCS is noted to be high at 10.7% in the transplant era of 2008–2017 [81].

Different from RAT predisposing factors, risk factors for renal vascular thrombosis include underlying renal disease, pre-existing thrombotic history, abnormal anatomy, young donor, recipient age (<5 years) and donor-recipient size mismatch [76, 82–85]. Thrombophilia may also be a potential risk factor for thrombosis and early graft loss, but conclusions are not definitive [86, 87]. Therefore, some programs screen all patients for genetic and acquired thrombophilic disorders before transplantation, while others selectively screen high risk patients, such as those with a personal or family history of thrombosis.

Outcomes of graft thrombosis are not favorable and preventative strategies are crucial, including a proper history and identification of risk factors for thrombosis. Evidence for routine heparinization of all patients post-transplantation is not conclusive. Some authors suggest selective heparinization only for higher risk groups, such as small patients (<20 kg) or those with confirmed inherited thrombophilia [87–93]. The type and duration of anticoagulation prophylaxis to prevent thrombosis is also unclear. Some centres use unfractionated heparin while others use low molecular weight heparin, sometimes followed by aspirin, for various durations of time [87, 91, 92]. The effect of using anticoagulation prophylaxis on the incidence of thrombosis and graft survival in the pediatric population is unknown. Any potential benefits of heparin must be closely balanced with the risks of bleeding, which was shown to be increased in some groups using that approach [87, 90, 91].

Hemolytic Uremic Syndrome and Coagulation

Although hemolytic uremic syndrome (HUS) is reviewed in detail elsewhere (Chaps. 24 and 25), because HUS is a pro-coagulant state, it is pertinent to review this feature of the disease here. The HUS triad of hemolytic anemia, thrombocytopenia and renal involvement underscore the primary abnormality with this entity, which is related to a procoagulant state initiated by endothelial injury [72]. In the presence of Shiga toxin, up-regulation occurs of the chemokine stromal cell-derived factor-1 (SDF-1), which is found in kidney, spleen, lung, liver, brain, heart, and muscle. This chemokine activates a pathway that enhances platelet activation induced by thrombin, thereby resulting in platelet aggregation [73, 94]. Shiga toxin also inhibits prostacyclin production and increase thromboxane A2 release from endothelial cells, thereby favoring platelet aggregation. Inhibition of SDF-1 normalized platelets in vivo and prevented formation of platelet strings [95].

The procoagulant state of HUS is evidenced by the formation of microthrombi throughout the systemic circulation. Subclinical thrombogenesis occurs prior to the clinical onset of HUS, with elevation of markers of thrombin activation (increase in prothrombin fragments 1 + 2 and thrombin-antithrombin complexes) [73]. In the normal state, levels of thrombin activation markers are negligible. Tissue factor (TF), expressed on mononuclear and endothelial cells and an initiator of the coagulation cascade leading to thrombin generation, has been found to be upregulated by Shiga toxin. Blockade of thrombin activity with lepirudin prevented lethal Shiga toxin effects in greyhounds, suggesting that Shiga toxin may mediate injury via thrombin activation.

The thrombocytopenia in HUS is also intertwined in this process and is due to a consumptive process with platelet deposition in the microthrombi [94]. The platelets are activated with degranulation as evidenced by reduction in intracellular levels of β-thromboglobulin and impaired aggregation in vitro. Other evidence of

platelet activation includes increase in platelet microparticles and platelet derived factors including platelet factor-4, β-thromboglobulin, and P-selectin. The resultant effect is the formation of platelet aggregates through binding of fibrinogen leading to thrombus formation.

Fibrinolysis has been suggested to be depressed in the setting of HUS, adding to the prothrombotic state [73, 94]. However, studies are conflicting as to whether indications of depressed fibrinolysis, such as elevated levels of plasminogen activator inhibitor type 1 (PAI-1), support this finding [95].

Finally, other evidence of vascular and complement activation includes increases terminal complement complex, *Fas*-ligand and soluble *Fas*, interleukin-1 receptor antagonist, transforming growth factor, platelet activating factor, degraded VWF multimers and numerous plasma factors as previously noted [95–97]. All of these changes support an enhanced thrombogenic state.

Diagnosis of Thromboembolism

Deep vein thrombosis (DVT) and pulmonary embolism (PE) are the two major categories of venous thrombotic events (VTE), both notably prevalent in the adult population [98]. In the USA, approximately 160,000–240,000 cases of DVT are diagnosed every year [99]. In children, VTE was initially thought to be extremely rare. International pediatric thrombosis registries were instrumental in changing this perception by characterizing the higher prevalence of VTE in hospitalized pediatric patients. Moreover, registries described peaks of thrombotic events during infancy and adolescence [100]. Pediatric VTE is commonly associated with several different underlying conditions (e.g., congenital heart defects, cancer, systemic lupus erythematosus), as well as treatment-related prothrombotic risk factors (e.g., CVCs, steroids, asparaginase). In comparison to adults, unprovoked VTE is uncommon in children. VTE occurs in 1:200 individuals admitted to pediatric tertiary care facilities [101, 102].

Importantly, thrombotic events in children are also associated with a significant thrombus-related morbidity and mortality, and affect children with a variety of underlying conditions [103]. For example, patients with nephrotic syndrome are at increased risk of developing DVT, particularly RVT and PE [50, 104]. RVT can be associated with several clinical complications including chronic renal tubular dysfunction and hypertension [105], whereas PE has an associated mortality rate of approximately 9% [106]. Therefore, prompt investigation of children, either under clinical suspicion or at risk for VTE development, is vital to decrease VTE-related short and long-term complications.

DVT can present with symptoms such as pain or swelling of the affected limb [107]. However, thrombotic events in children are commonly not accompanied by signs and symptoms, given that they are usually secondary to the placement of a CVC [108]. In those instances, their clinical presentation usually occurs in a sub-acute manner, when partial obstruction of the venous territory caused by the CVC and thrombus is counterbalanced by collateral vessel development. Moreover, CVC-related DVT in children is very prevalent in the upper venous system, where the mild findings of limb swelling can also be interpreted as line-related infection, leading to under-recognition of those events. Hence, to diagnose VTE in children imaging studies are required. A summary of the various imaging modalities used for the diagnosis of VTE in children is listed in Table 33.1.

The most common radiological modality utilized to diagnose DVT, the ultrasound Doppler (USD), is very sensitive for the detection of lower limb DVT. However, this imaging modality is not as sensitive for the diagnosis of upper limb DVT in children, especially for events located within the intrathoracic territory, as USD relies on vessel compressibility to confirm the presence of an intraluminal thrombus [109]. Therefore, a composite of USD and venogram has been suggested as the best way to diagnose upper extremity DVT in children. The role of computerized tomography (CT) and magnetic

Table 33.1 Summary of imaging studies for diagnosis of TE

Types of TE	Imaging	Advantages	Disadvantages
Deep vein thrombosis of limbs [95, 107–110]	Venography	Gold standard, ability to quantify venous obstruction and identify collateral veins	Invasive procedure, technical experience, cost, contrast media-related side effects, exposure to radiation, inter-radiologist interpretation discrepancy (up to 16%)
	Doppler ultrasound sensitivity 94% and specificity 98% (adults)	Noninvasive procedure, readily available, possible for bedside evaluation	Inter-variation between operators, difficulty to test in patients with obesity, edema, trauma, burns and casts, less sensitive for upper limb DVT especially for intrathoracic vessels
	CT venography sensitivity 100% and specificity 96% (adults)	Minimally invasive procedure, less radiation exposure, well tolerated contrast media	High technical demand, cost, not readily available, radiation and contrast exposure
	MR venography sensitivity 100% and specificity 96–100% (adults)	Minimally invasive procedure, well tolerated contrast media	High technical demands, cost, not readily available, requirement for anesthesia in young children
Pulmonary embolism [98, 107–111]	Pulmonary angiography	Gold standard	Same as venography for DVT of limbs; mortality of ~1% in adults
	Ventilation/perfusion (V/Q) scan sensitivity 31% and specificity 97% for high-probability scan (adults)	Less radiation exposure	Not convenient for young children due to the requirement of cooperation of patients
	CT pulmonary angiography sensitivity 69% and specificity 69% (adults)	Same as CT venography for DVT of limbs	Same as CT venography for DVT of limbs
	MR pulmonary angiography sensitivity 78% and specificity 99% (adults)	Same as MR venography for DVT of limbs	Same as MR venography for DVT of limbs
	Echocardiography for RV free wall hypokinesis sensitivity 77% and specificity 94% (adults)	Same as ultrasound for DVT of limbs	Unable to detect mild degree of PE, same as ultrasound for DVT of limbs
Renal vein thrombosis [107, 108, 111, 112]	Ultrasound high sensitivity	Same as ultrasound for DVT of limbs	Inter-variation between operators, bowel gas obscuring abdominal findings
	CT venography sensitivity almost 100% and specificity almost 100% (adults)	Findings not affected by bowel gas, same as CT venography for DVT of limbs	Same as CT venography for DVT of limbs
	MR venography sensitivity 94–96% and specificity 100% (adults)	Findings not affected by bowel gas, same as MR venography for DVT of limbs	Same as MR venography for DVT of limbs
Portal vein thrombosis [107, 108, 113–115]	Doppler ultrasound sensitivity 70–90%, specificity 99% and negative predictive value 98% (adults)	Same as ultrasound for DVT of limbs	Same as ultrasound of renal vein thrombosis
	CT venography	Able to show varices and hepatic parenchyma, same as CT venography for renal vein thrombosis	Same as CT venography for DVT of limbs
	MR venography	Able to show varices and hepatic parenchyma, same as MR venography for renal vein thrombosis	Same as MR venography for DVT of limbs

CT computerized tomography, *DVT* deep vein thrombosis, *MR* magnetic resonance
Data from: Young [107] and Monagle [108]

resonance imaging to diagnose upper extremity DVT in children is evolving.

Besides imaging studies, laboratory biomarkers have also been used in the diagnosis of adults with VTE. Most commonly, a normal D-dimer is used to rule out thrombotic events. To further improve the use of D-dimer testing, clinical predictive rules were instituted. They stratify patients into low, moderate or high clinical suspicion groups, which further improve the positive and negative pre-imaging predictive values of D-dimer.

The sensitivity of D-dimer for the diagnosis of VTE in adult patients is around 90% [116] and the specificity around 49–78% [117, 118]. For example, a normal D-dimer may have a negative predictive value as high as 99% to exclude VTE in patients who have a low pretest clinical likelihood [118]. Conversely, the low specificity of D-dimer testing for the diagnosis of VTE may be due to the fact that D-dimer levels are usually influenced by several factors, particularly underlying diseases such as recent major surgery, trauma, cancer, pregnancy, disseminated intravascular coagulation and end-stage liver disease [119, 120].

The three most comprehensive pediatric studies to date have shown disappointing results regarding the performance of D-dimer testing as a diagnostic tool for DVT in children. A retrospective chart evaluated children with suspected VTE that had D-dimer testing done within 72 h of imaging. The researchers identified 33 patients; 26 diagnosed with acute VTE, 6 unchanged chronic VTE, and 1 without VTE. D-dimer levels were significantly higher in patients with acute VTE compared to the remaining patients (77% sensitivity; 71% specificity) [121]. Conversely, another study evaluated 132 patients referred for CT pulmonary angiography to rule out PE: 88% of the patients with PE and 87% of those without PE had a positive D-dimer result, thus showing that D-dimer positivity did not show a significant relationship with the presence of PE [122]. The third study examined the role of the Wells score, which has been validated for the stratification of adults at risk for PE, as a potential tool to risk stratify children investigated for PE. The Wells score used in combination with D-dimer testing did not differentiate children with or without PE [123], illustrating that pediatric-specific tools will be required to improve the use of D-dimer.

Thrombophilia Work Up

Thrombus formation results from a dynamic balance between pro- and anticoagulant forces; more specifically, from several different pro- and anticoagulant factors involved in the generation or inhibition of thrombin formation.

Thrombophilia refers to conditions, either inherited or acquired, that increase the risk of thrombus formation. Patients identified with an inherited or acquired thrombophilia may be predisposed to sustain a thrombotic event. However, having one isolated thrombophilia trait rarely leads to an immediate VTE.

A little more than a decade ago, the International Society on Thrombosis and Haemostasis (ISTH) published a position statement suggesting that thrombophilia investigation should occur in a stratified manner in all pediatric patients with an objectively documented VTE. However, the epidemiology of VTE in children has evolved, demonstrating that underlying conditions and/or acquired risk factors other than thrombophilia are usually present in children with VTE, rendering the role of thrombophilia as a potential causal risk factor less relevant [124].

We now understand that laboratory investigation of a child with a recently diagnosed with VTE is rarely justified. Moreover, acute VTE may also affect the circulating levels of many of the natural anticoagulant factors [e.g., PC, PS, AT], adding to the reasoning of why thrombophilia evaluation should not be performed at the time of initial VTE diagnosis [124]. Furthermore, except in extremely rare instances, thrombophilia work-up results do not change the choice of antithrombotic intensity or duration [125, 126]. Those rare instances include newborns with purpura fulminans, as its recognition requires prompt PC or PS replacement in addition to anticoagulation [125]. Similarly, children with unprovoked VTE, who may have higher recurrence rates than provoked VTE, particularly if associated with lupus anticoagulant antibodies, PC, PS or AT

Table 33.2 The summary of thrombophilic risks in children

Thrombophilic risks	Prevalence in the general population [129]	OR (95% CI) for first onset VTE in children [128]	OR (95% CI) for recurrent VTE in children [128]	Acquired conditions related to abnormal thrombophilic tests [124]
Congenital thrombophilic risks				
Antithrombin deficiency—AT activity[a]	0.02%	8.73 (3.12–24.42)	3.37 (1.57–7.20)	Acute thrombosis, nephrotic syndrome, complex congenital heart disease, L-asparaginase therapy, liver disease, heparin therapy
Protein C deficiency—PC activity[a]	0.2%	7.75 (4.48–13.38)	2.53 (1.30–4.92)	Acute thrombosis, nephrotic syndrome, complex congenital heart disease, liver disease, warfarin therapy
Protein S deficiency—total and free PS antigen[a]	0.03–0.3%	5.77 (3.07–10.85)	3.76 (1.57–7.20)	Acute thrombosis, nephrotic syndrome, complex congenital heart disease, liver disease, warfarin therapy, inflammation, pregnancy
Factor V Leiden (G1691A)—genetic test[a]	3–7%	3.56 (2.57–4.93)	0.77 (0.40–1.45)	–
Prothrombin G20210A—genetic test[a]	0.7–4%	2.63 (1.61–4.29)	2.15 (1.12–4.10)	–
Lipoprotein (a)[a]	–	4.50 (3.19–6.35)	0.84 (0.50–1.40)	Inflammation, nephrotic syndrome
Acquired thrombophilic risks				
Antiphospholipid antibodies (persistent)[b] [33]—lupus anticoagulant[a]—anticardiolipin antibodies[a]—anti beta-2 glycoprotein I antibodies [33]	1–8% 5% 3.4%	4.9 (2.20–10.90)	–	Infection

AT antithrombin, *CI* confidence interval, *OR* odds ratio, *PC* protein C, *PS* protein S

[a]Level I laboratory testing for thrombophilia in pediatric patients on behalf of the Subcommittee for Perinatal and Pediatric Thrombosis of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis (ISTH) [130]

[b]Persistent means that at least one of the three tests were positive twice with at least 12 weeks between the repeated testing

deficiencies [127], may also benefit from an initial laboratory work-up [125].

The results of a meta-analysis enumerating the thrombotic risk for first onset and recurrent VTE of the most common thrombophilia traits in children [127, 128] are summarized in Table 33.2.

Treatment

When a patient is diagnosed with VTE, treatment with antithrombotic agents, including antiplatelet, anticoagulant or thrombolytic agents is usually considered. The goal of using anticoagulant drugs is to prevent progression of acute TE, whereas in thrombolytic therapy the goal is to lyse the thrombus in cases where the patient has a life-, limb-, or organ-threatening condition.

The guidelines of antithrombotic treatment in children published by the American College of Chest Physician [125], the British Committee for Standard in Haematology [126], and the American Society of Hematology [131] are the main available references regarding anticoagulant therapy in children. However, most recommendations are not based on randomized controlled trials due to the limitation in the number of studies in children. The following text summarizes those recommendations regarding the most commonly used agents.

Unfractionated heparin (*UFH*) *or heparin* is a glycosaminoglycan which forms a complex with AT, enhancing the inhibitory effect of AT against both activated factors X (FXa) and factor II (FIIa, e.g., thrombin) [132]. Moreover, this complex can also inhibit FIXa, FXIa and FXIIa [133]. Due to higher volume of distribution and physiologically low AT in infants [125, 126], the dose of heparin required for anticoagulation in infants is higher than the one required in children aged more than 1 year (Table 33.3). The anticoagulant effects of heparin can be monitored by the activated partial thromboplastin time (aPTT) or the anti-FXa assay [127]. Because of its short half-life, around 30 min in children, heparin is rapidly cleared from the body after discontinuation and its effect can be fully reversed by protamine sulfate [134].

Low molecular weight heparin (*LMWH*) is derived from unfractionated heparin after it is chemically fragmented into smaller molecular sizes. Whereas UFH contains polysaccharide chains from 5 to 40 kDa with at least 18 repeats of pentasaccharide sequences that bind to AT, conferring on the molecule its most potent anticoagulant effects (e.g., protease activity inhibition of activated coagulation FII (thrombin) and FX [anti-IIa and anti-Xa inhibition]), LMWH has chains with an average molecular weight between 4 and 5 kDa that still contain enough pentasaccharide sequences to retain anti-IIa and anti-Xa activity, depending on the LMWH length [132]. Overall, because of its reduced molecular size, LMWH inhibits FXa more effectively than thrombin. Similar to what occurs with unfractionated heparin, the dose requirements for LMWH are also age-dependent (Tables 33.4 and 33.5). Infants younger than 2 months need higher doses than older children. Because the kidney excretes LMWH, patients who have decreased

Table 33.3 Unfractionated heparin dosing

Loading dose: 50–75 units/kg, IV, over 10 min					
Maintenance dose:	≤1 year of age: 28 units/kg/h >1 year of age: 20 units/kg/h				
aPTT (s)	Anti-Xa (units/mL)	Bolus (units/kg)	HOLD (min)	Rate change	Repeat aPTT
<50	<0.1	50	0	Increase 10%	4 h
50–59	0.1–0.34	0	0	Increase 10%	4 h
60–85	0.35–0.7	0	0	0	24 h
86–95	0.71–0.89	0	0	Decrease 10%	4 h
96–120	0.9–1.20	0	30	Decrease 10%	4 h
>120	>1.20	0	60	Decrease 10%	4 h

Table 33.4 Low molecular weight heparin (enoxaparin) dosing

	Age ≤ 2 months	Age > 2 months–18 years
Initial treatment dose	1.75 mg/kg/dose SC q12h	1 mg/kg/dose SC q 12 h
Initial prophylactic dose	0.75 mg/kg/dose SC q12h or 1.5 mg/kg/dose SC q24h	0.5 mg/kg/dose SC q12h or 1 mg/kg/dose SC q24h

Table 33.5 Low molecular weight heparin (enoxaparin) adjustment

Anti-Xa (units/kg)	HOLD	Dose change	Repeat anti-Xa
<0.35	No	Increase 25%	4 h post next dose
0.35–0.49	No	Increase 10%	4 h post next dose
0.5–1.0	No	0	1×/week; 4 h post morning dose
1.01–1.5	No	Decrease 20%	4 h post morning dose
1.6–2.0	3 h	Decrease 30%	Trough level prior to next dose; and then 4 h post morning dose
>2.0	Yes (until level < 0.5)	Decrease 40%	Trough level prior to next dose, until Level < 0.5, and then 4 h post Morning dose

kidney function should be monitored with the anti-FXa assay carefully to prevent drug retention [132]. While there are several formulations of LMWH available, enoxaparin is the one most commonly used. Unlike heparin, LMWH is only partially reversed by protamine sulfate [134].

Warfarin is an oral vitamin-K antagonist that inhibits the carboxylation of the vitamin K-dependent FII, FVII, FIX and FX by blocking the activity of the enzyme vitamin K epoxide reductase complex subunit 1 (VKORC1) in the vitamin K cycle [135]. Therefore, the production of carboxylated factors, which are the active forms of these coagulation proteins, is depleted. The effect of warfarin can be reversed by vitamin K. To date, oral vitamin K inhibitors constitute the main class of oral anticoagulants widely used in children (Table 33.6). However, there are limitations for using warfarin in children: the drug level, which is monitored by international normalization ratio (INR), can be affected by many foods and other drugs; it takes a longer time than heparin or LMWH for patients to reach a therapeutic drug level; no liquid preparation is available; and its use is not recommended in infants [125, 126].

A summary of conventional anticoagulation in children is shown in Table 33.7.

Table 33.6 Warfarin loading doses (days 2–4)

	0.2 mg/kg PO, daily; maximum 5 mg	
Loading dose	0.1 mg/kg; with liver dysfunction, Fontan procedure, or severe renal impairment	
INR	1.1–1.3	Repeat initial loading dose
INR	1.4–3.0	50% of initial loading dose
INR	3.1–3.5	25% of initial loading dose
INR	>3.5	Hold until INR <3.5, then restart at 50% less than previous dose

Table 33.7 Conventional anticoagulant in children

	Heparin	Enoxaparin	Warfarin
Route of administration	Intravenous	Subcutaneous	Oral
Treatment dose	Bolus 75–100 units/kg/dose then: Age less than 1 year: 28 units/kg/dose Age 1 year and more: 20 units/kg/dose	Age less than 2 months: 1.75 mg/kg/dose Age 2 months and more: 1 mg/kg/dose	0.2 mg/kg/dose
Administration interval for treatment dose	Bolus followed by continuous infusion	Every 12 h	Once daily
Target range	Anti-FXa for heparin 0.35–0.70 U/mL APTT which correlates to anti-FXa at therapeutic level	Anti-FXa for enoxaparin 0.5–1.0 U/mL	INR 2.0–3.0
Half-life	30 min	6 h	42 h
Anti-thrombin dependence	Yes	Yes	No
Antidote	Protamine	Protamine (partial)	Vitamin K
Elimination	Renal	Renal	Liver
Bleeding risk	1.5–24%	0.8–5% (major bleeding)	0.05–12.2%/year (major bleeding)
Other complications	Heparin-induced thrombocytopenia (HIT) (0.3–1.0%) Osteoporosis (rare)	No report of HIT and osteoporosis in children	Warfarin- induced skin necrosis (0.01–0.1%) Hair loss (rare) Tracheal calcification (rare)

Data from Chalmers et al. [125]; Paul et al. [126]; and Young [134]

Table 33.8 Anticoagulation duration in children

VTE type	Neonates	Infants	Childhood	Level of evidence
CNS	0–6 months	0–6 months	3–12 months	2C
Non-CNS				
Provoked[a], symptomatic	6 weeks–3 months	6 weeks–3 months	3 months	2C
Unprovoked, symptomatic	6–12 months	6–12 months	6–12 months	2C

VTE venous thromboembolism, *CNS* central nervous system
Data from Monagle [80] and Monagle [64]
[a]Provoked includes central venous catheter (CVC)-related events. In those instances, if the CVC remains *in situ* after the end of anticoagulation, prophylaxis until the catheter is removed is recommended (please, see Table 33.4 for doses)

Adults with provoked VTE are treated for 3 months, while anticoagulation in unprovoked VTE is typically indefinite [136–138]. In children, anticoagulation duration is summarized in Table 33.8

Heparin-induced thrombocytopenia (HIT) is a clinical-laboratory entity where an immunological response against heparin and platelet factor-4 creates a hypercoagulable state. HIT is suspected when patients who receive heparin develop thrombocytopenia within 5–10 days after heparin treatment accompanied by a new episode or progression of TE.

Three additional major groups of anticoagulants are used in current adult practice with a high clinical suspicion of HIT: direct thrombin inhibitors (DTI, parenteral: bivalirudin and argatroban), indirect FXa inhibitor (parenteral: fondaparinux), and a direct FXa inhibitor (parenteral: danaparoid).

Bivalirudin, argatroban, and danaparoid can be used for children requiring hemodialysis who develop HIT, but most dosing recommendations have been extrapolated from the adult literature. There are only a few studies including children on hemodialysis or continuous renal replacement therapy with HIT [139–144]. The available pediatric doses that have been reported derive mostly from children undergoing surgery under cardiopulmonary bypass (CPB) who had also been diagnosed with HIT [145–150]. Of note, bivalirudin or argatroban can be monitored by the activated partial thromboplastin time (aPTT), which can be confounded in patients with disseminated intravascular coagulation (DIC), liver dysfunction, or a lupus anticoagulant. In such instances, danaparoid or fondaparinux may be preferred [151–154]. The summary of parenteral anticoagulants available for treatment of HIT in children requiring hemodialysis is shown in Table 33.9.

New oral anticoagulants (NOAC), or direct oral anticoagulants (DOAC), including the DTI dabigatran etexilate and the direct anti-Xa inhibitors apixaban, betrixaban, edoxaban, and rivaroxaban, have been approved in adult patients. Recently, rivaroxaban has also been approved by the Food and Drug Administration and Health Canada for the treatment of acute VTE in children, and dabigatran has been approved by the European Medicines Agency. A summary of the NOAC/DOAC under investigation in children is listed in Table 33.10.

Thrombolytic therapy is used when immediate thrombus lysis is required, such as life-, limb- or organ threatening scenarios. Pulmonary embolism accompanied by hypotension (massive PE), extensive or progressive DVT of a lower limb, bilateral RVT, or failure of treatment with conventional anticoagulants are examples of pediatric cases when this therapy should be considered [155, 156]. Tissue plasminogen activator (tPA) has been the drug of choice in children [155] and is the recommended agent by the American College of Chest Physician, the British Committee for Standard in Haematology [125, 126], and the American Society of Hematology to be used for thrombolytic therapy in neonates and children. However, the risk of major bleeding can be as high as 11–18% and intracerebral hemorrhage has been reported in up to 1.5% of pediatric patients who receive this therapy. Therefore, some patients might not be ideal candidates for this type of treatment. Contraindications for thrombolytic therapy

Table 33.9 Anticoagulants that can be used for treatment of HIT in children requiring hemodialysis

	Fondaparinux	Bivalirudin [145, 147, 148]	Argatroban [145, 148–150]	Danaparoid [144, 145]
Route of administration	Subcutaneous	Intravenous	Intravenous	Intravenous
Treatment dose	0.1 mg/kg/dose, x1, and reassess subsequent doses based on anti-Xa levels	Loading dose 0.5–1.0 mg/kg, then continuous infusion 2.5 mg/kg/h	Loading dose 75–250 μg/kg, then continuous infusion with 0.1–24 μg/kg/min (average dose 1–5 μg/kg/min)	1000 U plus 30 U/kg in patients aged <10 years and 1500 plus 30 U/kg in patients aged 10–17 years, then subsequent dose adjusted by anti-Xa
Half-life	17–21 h	25–34 min	39–60 min	Approximately 25 h
Target range	0.5–1 U/mL peak level 3 h post-dose	ACT >200–400 s or APTT ratio 1.5–2 times of baseline	ACT >200–400 s or APTT ratio 1.5–2 times of baseline	Anti-Xa <0.3 U/mL pre-dialysis If anti-Xa 0.3–0.5 U/mL, then decrease dose by 250 U If anti-Xa >0.5 U/mL, then hold next dose
Elimination	Renal	Intravascular proteolysis	Liver	Renal
Antidote	None	None	None	None

Table 33.10 Direct oral anticoagulant use in children

	Dabigatran	Rivaroxaban	Apixaban	Edoxaban	Betrixaban
Evidence in pediatrics	Yes (phase 3)	Yes (phase 3)	No (study ongoing)	No (study ongoing)	No
Mechanism of action	Direct thrombin inhibitor	Xa inhibitor	Xa inhibitor	Xa inhibitor	Xa inhibitor
Bioavailability	3–7%	Almost complete when administered with a meal	~50%	62%	34%
Time to peak concentration	1 h; delayed by food	2–4 h	3–4 h	1–2 h	3–4 h
Protein binding	35%	92–95%	87%	55%	60%
Half-life	12–17 h (prolonged in renal dysfunction)	5–9 h	8–15 h	10–14 h	19–27 h
Renal clearance (unchanged)	80%	36%	27%	50%	11%
PGP substrate	Yes	Yes	Yes	Yes	Yes
CYP3A4 substrate	No	Yes (~18%)	Yes (~25%)	No (<4%)	No (<1%)
Administration considerations	Do not open capsules; swallow whole	Administer with a meal (or up to 2 h after)	N/A	N/A	Administer with food
Usual effect on coagulation parameters at therapeutic doses	Increased dilute thrombin time and often increased aPTT. May increase PT	Increased anti-Xa and often increased PT. May increase aPTT	Increased anti-Xa. May increase PT and aPTT	Increased anti-Xa and PT. May increase aPTT	Increased anti-Xa. May increase PT and aPTT
Reversal agent	Idarucizumab (no published pediatric data; study ongoing). Partially removed by dialysis.	Prothrombin complex concentrate (limited pediatric data) or andexanet alfa (no pediatric data)	Prothrombin complex concentrate (very limited pediatric data) or andexanet alfa (no pediatric data)	Prothrombin complex concentrate (very limited pediatric data) or andexanet alfa (no pediatric data)	Prothrombin complex concentrate (very limited pediatric data) or andexanet alfa (no pediatric data)

include any type of previous operation within 10 days prior to therapy, severe asphyxia within 7 days prior to therapy, an invasive procedure within 3 days of therapy, seizures within 48 h of therapy, preterm newborns with gestational age less than 32 weeks and patients who are bleeding and are unable to maintain a platelet count >50–100 × 10^9/L and fibrinogen >1.0 g/L [155, 156].

There are two methods to administer thrombolytic therapy in children: systemic thrombolysis and catheter-directed thrombolysis. Two types of dosage of systemic thrombolysis have been published in children: high dose tPA, with doses ranging between 0.1 and 0.6 mg/kg/h for 6 h, and low dose tPA, with doses ranging between 0.01 and 0.06 mg/kg/h for 4–48 h. Even though lower doses have been claimed to have a lower incidence of therapy-associated bleeding [155, 156], the American College of Chest Physician recommends a dose of 0.5 mg/kg/h for 6 h [126].

For catheter-directed tPA therapy, there have been no randomized trials and very few prospective pediatric series in children [157]. Even though the risk of major bleeding is potentially smaller and the efficacy higher in patients who are treated with this modality (dose reduction of tPA to 0.015–0.2 mg/kg/h), there have been no comparisons regarding efficacy and safety in children [155, 156]. Currently, the use of catheter-directed thrombolysis in children depends on center availability, local protocol, and level of complexity of care delivered.

In summary, in the last decade, there has been tremendous progress in the recognition and care of children affected by VTE, which includes children with underlying renal conditions.

Conclusion

Children with renal disease may have disordered hemostasis, resulting in a risk of either bleeding or clotting. Normal hemostasis in children must be understood by the clinician in order to determine whether, in a child with renal disease, therapeutic intervention to prevent abnormal bleeding or clotting is prudent. Unfortunately, there are few properly designed studies in children with renal disease providing guidelines for best practice relating to diagnosis and treatment of disordered hemostasis.

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Part VII

Renal Tubular Disorders

34

Differential Diagnosis and Management of Fluid, Electrolyte and Acid-Base Disorders

Giacomo D. Simonetti, Sebastiano A. G. Lava, Gregorio P. Milani, and Mario G. Bianchetti

Introduction

In this chapter, the disturbances of fluid, electrolyte and acid-base balance will be addressed in different subchapters that deal with water, salt, K^+, acid-base, Ca^{++}, Mg^{++}, and phosphate. This traditional presentation is didactically relevant. It is worth mentioning, however, that more than one disturbance in fluid, electrolyte and acid-base homeostasis often concurrently occurs in the same subject.

Overall, the etiology of fluid, electrolyte and acid-base disorders is straightforward, since the most commonly occurring causes are easily recognized on clinical grounds. In some cases, however, the cause is not readily apparent, and a comprehensive systematic approach is recommended. The diagnostic approach to initially unexplained "isolated" disturbances involving the fluid, electrolyte and the acid-base balance should include both very careful history and clinical examination as well as the concurrent assessment of an extended "electrolyte spectrum". In the setting of initially unclassified and apparently "isolated" disturbances involving the fluid, electrolyte and acid-base balance, the concomitant measurement in blood of pH, pCO_2, HCO_3^-, Na^+, K^+, Cl^-, Ca^{++} (either total or ionized), Mg^{++}, inorganic phosphate, alkaline phosphatase, total protein level (or albumin), uric acid, urea and creatinine is advised.

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Water and Salt

Introduction

Body Fluid Compartments

Water accounts for ≈50–75% of the body mass. The most significant determinants of the wide range in water content are age and gender: (a) the

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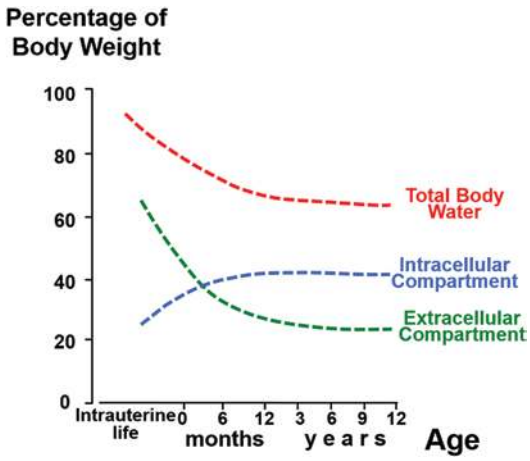

Fig. 34.1 Influence of age on the subdivision of total body water, intracellular fluid and extracellular fluid. For clinical purposes, "the rule of 3" is suggested: (1) total body water makes up 2/3 of the body mass; (2) the intracellular compartment contains 2/3 of the total body water; (3) the extracellular compartment is further subdivided into the interstitial and the intravascular compartments (blood volume), which contain 2/3 and 1/3 of the extracellular fluid, respectively

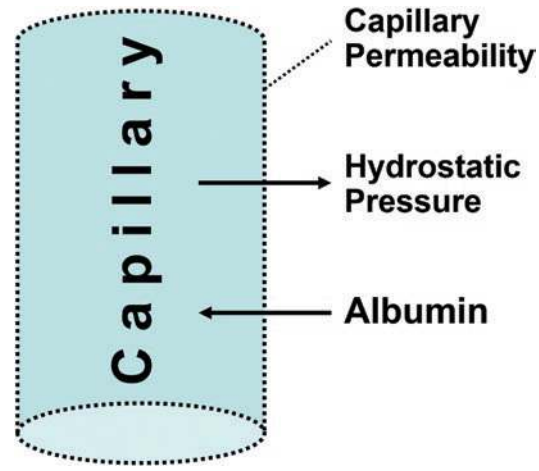

Fig. 34.2 Distribution of ultrafiltrate across the capillary membrane. The barrel-shaped structure represents a capillary. A high hydrostatic pressure or increased capillary permeability causes fluid to leave the vascular space. By contrast, an increased intravascular albumin concentration (and, therefore, an increased oncotic pressure) causes fluid to enter the vascular space

water content of a newborn, an adolescent and an elderly man are ≈75, ≈60 and ≈50%; (b) after puberty males generally have 2–10% higher water content than females (Fig. 34.1). The intracellular compartment contains about two-third of the total body water and the remaining is held in the extracellular compartment. The solute composition of the intracellular and extracellular fluid differs considerably because the sodium pump (=Na^+-K^+-ATPase) maintains K^+ in a primarily intracellular and Na^+ in a primarily extracellular location. Consequently, K^+ largely determines the intracellular and Na^+ the extracellular compartment [1–4]. The extracellular compartment is further subdivided into the interstitial and the total intravascular compartments (total blood volume), which contain ≈2/3 and ≈1/3 of the extracellular fluid [1–4], respectively (the transcellular fluid compartment, which comprises the digestive, cerebrospinal, intraocular, pleural, peritoneal and synovial fluids, will not be further addressed in this review).

The size of the total intravascular compartment is determined by the overall size of the extracellular fluid compartment and by the Starling forces. Three major forces control the distribution of fluids across the capillary membrane (Fig. 34.2): (a) the hydrostatic pressure, which causes fluids to leave the vascular space, and; (b) the higher concentration of proteins in the intravascular compartment as compared with that in interstitial fluid, which causes fluids to enter the vascular space. This force, which is called oncotic pressure, is due both to the concentration gradient of albumin (blood proteins other than albumin account for 50% of the weight of proteins in blood but only for 25% of the oncotic pressure) as well to the fact that albumin is anionic and therefore attracts cations (largely Na^+) into the vascular compartment (Gibbs-Donnan effect). (c) Capillary permeability, which is a further modulator of the distribution of fluids across the capillary membrane (and can be increased, for example, during inflammatory states like infection, post-operatively or in the context of an idiopathic capillary leak syndrome).

Recent data suggest that an extrarenal system might also contribute to sodium homeostasis. Sodium might be stored on negatively charged glycosaminoglycans in the skin interstitium,

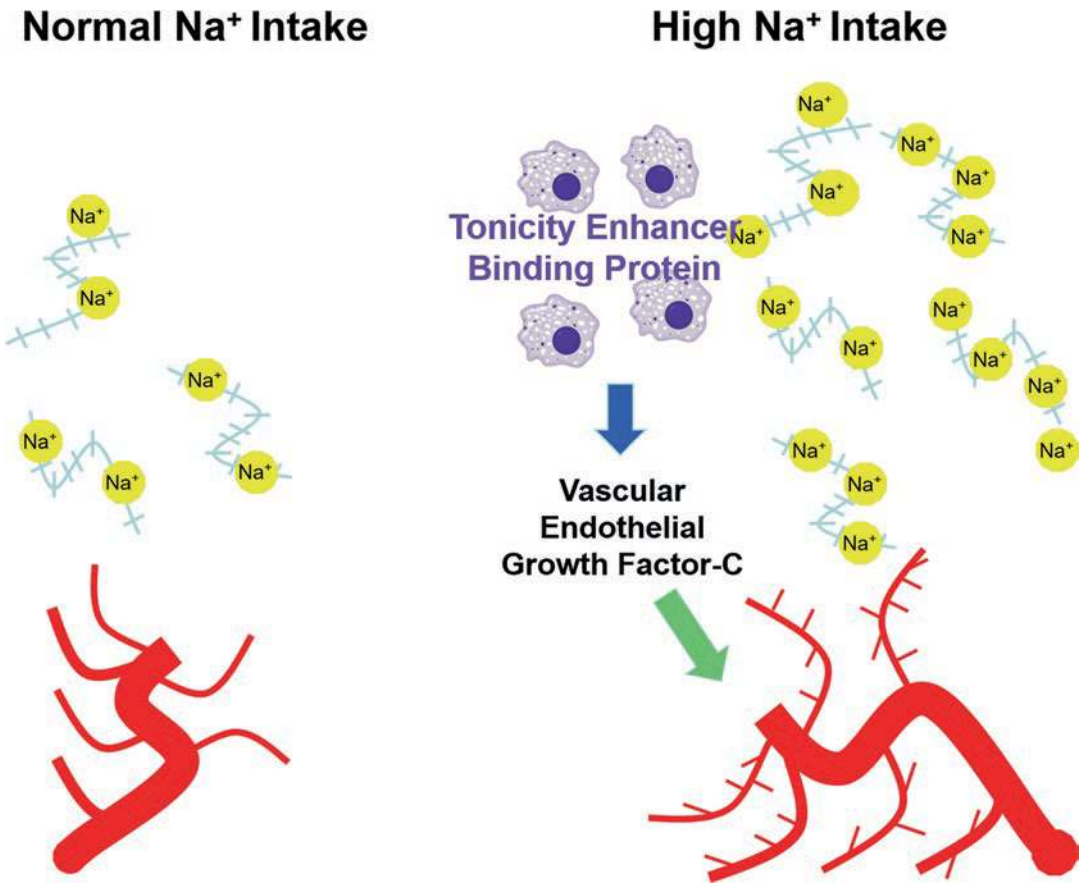

Fig. 34.3 Sodium is stored on negatively charged glycosaminoglycans in the skin interstitium. Excess sodium modulates lymphangiogenesis, and osmotically inactive sodium accumulates in the skin interstitium, binding proteoglycans. Excess sodium recruits macrophages, and subsequently activates within subcutaneous macrophages (cells with blue nucleus) a transcription factor, tonicity-enhanced binding protein, which in turn induces the production of the angiogenic protein vascular endothelial growth factor-C. Vascular endothelial growth factor-C stimulates lymphatic vessel (red) growth and creates a new fluid compartment, which buffers the increased body sodium (yellow) and ameliorates the tendency to excess body fluid linked with excess salt intake [5–7]

becoming osmotically inactive [5]. The skin interstitium might therefore represent a sort of fluid-buffering system, able to store sodium without commensurate water retention and potentially limiting accumulation of excess body fluid following high salt intake (Fig. 34.3) [6, 7].

Effective Circulating Volume

The total intravascular compartment is subdivided into the effective (≈ arterial) and the ineffective (≈ venous) compartment. Effective circulating volume denotes the part of the total intravascular compartment that is in the arterial system and is effectively perfusing the tissues. The effective circulating volume is biologically more relevant than the total intravascular (respectively the ineffective) compartment and usually varies directly with the extracellular fluid volume. As a result, the regulation of extracellular fluid balance (by alterations in urinary Na^+ excretion) and the maintenance of the effective circulating volume are intimately related. Na^+ loading will tend to produce volume expansion, whereas Na^+-loss (e.g., due to vomiting, diarrhea, or diuretic therapy) to volume depletion. The body responds to changes in effective circulating volume in two steps: (a) the change is sensed by the volume receptors that are located in the cardio-

pulmonary circulation, in the carotid sinuses and aortic arch, and in the kidney; (b) these receptors activate effectors that restore normovolemia by varying vascular resistance, cardiac output, and renal water and salt excretion. In brief, the non-renal receptors primarily govern the activity of the sympathetic nervous system and natriuretic peptides, whereas the renal receptors affect volume balance by modulating the renin-angiotensin II-aldosterone system [1–4].

In some settings the effective circulating volume is independent of the extracellular fluid volume. For example, among patients with heart failure the extracellular fluid volume is increased but these patients are effectively volume depleted due to the low volume of blood pumped by the heart [1–4].

Blood Osmolality: Measurement of Sodium

Osmolality is the concentration of all solutes in a given weight of water (the similar concept of osmolarity denotes the concentration of all of the solutes in a given volume of water). The total (or true) blood osmolality is equal to the sum of the osmolalities of the individual solutes in blood. Most osmoles in blood are Na^+ salts, with lesser contributions from other ions, glucose, and urea. However, under normal circumstances, the osmotic effect of the ions in blood can usually be estimated as two times the Na^+ concentration. *Blood osmolality* (in mosm/kg H_2O) can be measured directly (via determination of freezing point depression) or estimated from circulating Na^+, glucose and urea (in mmol/L[1]) as follows [3, 4, 8–12]:

$$\left(Na^+ \times 2\right) + \text{glucose} + \text{urea}$$

The *effective blood osmolality*, known colloquially as blood tonicity, is a further clinically significant entity, which denotes the concentration of solutes impermeable to cell membranes (Na^+, glucose, mannitol) and are therefore restricted to the extracellular compartment (osmoreceptors sense effective blood osmolality rather than the total blood osmolality). These solutes are effective because they create osmotic pressure gradients across cell membranes leading to movement of water from the intracellular to the extracellular compartment. Solutes that are permeable to cell membranes (urea, ethanol, methanol) are ineffective solutes because they do not generate osmotic pressure gradients across cell membranes and therefore are not associated with such water shifts. Glucose is a unique solute because, at normal concentrations in blood, it is actively taken up by cells and therefore acts as an ineffective solute, but under conditions of impaired cellular uptake (like diabetes mellitus) it becomes an effective extracellular solute.

Since no direct measurement of effective blood osmolality (which is biologically more important than the total or true blood osmolality) is possible, the following equations are used to calculate this entity [3, 4, 8–12]:

$$\left(Na^+ \times 2\right) + \text{glucose}$$

$$\text{measured total blood osmolality} - \text{urea}$$

Plasma normally consists of about 93% water and 7% solids (proteins and lipids). Electrolytes are dissolved exclusively in the water portion of plasma. Blood Na^+ (normal range between 135 and 145 mmol/L) can be determined either by indirect or direct potentiometry. The two techniques show good agreement as long as protein and lipid concentrations are normal. However, while assessed by indirect potentiometry in diluted samples, increased protein or lipid concentrations result in spuriously low Na^+ (pseudo-hyponatraemia) whereas decreased protein or lipid concentrations result in spuriously high Na^+ (pseudo-hypernatraemia). A spuriously normal Na^+ value (pseudonormonatraemia) might also occur [13]. Direct potentiometry measures Na^+ in undlitued samples and avoids this problem. Therefore, it is the currently recommended technique [14].

Flame photometry, the traditional assay for circulating Na^+, measures the concentration of Na^+ per unit volume of solution, with a normal

[1]To obtain glucose in mmol/L divide glucose in mg/dL by 18. To obtain urea in mmol/L divide urea nitrogen in mg/dL by 2.8 or urea in mg/dL by 6.0.

range between 135 and 145 mmol/L. In fact, Na^+ is dissolved in plasma water, which accounts for 93% of the total volume of plasma. Ion selective electrodes, that have replaced flame photometry in most laboratories, determine the activity of Na^+ in plasma water, which ranges between 145 and 155 mmol/L (that, multiplied by 0.93, gives the traditional range of 135–145 mmol/L). For convenience, laboratories routinely apply a correction factor so that the reported values still correspond to the traditional normal range of 135–145 mmol/L. A kind of "pseudohyponatremia" caused by expansion of the non-aqueous phase of plasma—for example, due to hyperlipidemia or paraproteinemia—is no longer seen because determination by selective electrodes in undiluted samples is not affected by this (the recommended name for this quantity is ionized sodium). Although, strictly speaking, a Na^+ concentration outside the range of 135–145 mmol/L denotes dysnatremia, clinically relevant hypo- or hypernatremia is mostly defined as a concentration outside the range of 130–150 mmol/L [3, 4].

Dehydration and Extracellular Fluid Volume Depletion

Although dehydration[2] semantically and in general usage means loss of water, in physiology and medicine the term denotes both a loss of water and salt. Depending on the type of pathophysiologic process, water and salts (primarily Na^+Cl^-) may be lost in physiologic proportion or lost disparately, with each type producing a somewhat different clinical picture, designated as normotonic (mostly isonatremic), hypertonic (mostly hypernatremic), or hypotonic (always hyponatremic) dehydration. Dehydration develops when fluids are lost from the extracellular space at a rate exceeding intake. The most common sites for extracellular fluid loss are (1) the intestinal tract (diarrhea, vomiting, or bleeding), (2) the skin (fever, excessive sweating or burns), and (3) the urine (osmotic diuresis, diuretic therapy, diabetes insipidus, or salt losing renal tubular disorders). More rarely, dehydration results from prolonged inadequate intake without excessive losses [3, 4, 8–12].

The risk for dehydration is high in children and especially infants for the following causes: (a) infants and children are more susceptible to infectious diarrhea and vomiting than adults; (b) there is a higher proportional turnover of body fluids in infants compared to adults (it is estimated that the daily fluid intake and outgo, as a proportion of extracellular fluid, is in infancy more than three times that of an adult, Fig. 34.4); (c) young children do not communicate their need for fluids or do not independently access fluids to replenish volume losses.

Dehydration reduces the effective circulating volume, therefore impairing tissue perfusion. If not rapidly corrected, ischemic end-organ damage occurs.

Three groups of symptoms and signs occur in dehydration: (a) those related to the manner in which fluids loss occurs (including diarrhea, vomiting or polyuria); (b) those related to the electrolyte and acid-base imbalances that sometimes accompany dehydration; and (c) those directly due to dehydration. The following discussion will focus on the third group.

When assessing a child with a tendency towards dehydration, the clinician needs to address the degree of extracellular fluid volume depletion. More rarely the clinician will address the laboratory testing and the type of fluid lost (extracellular or intracellular fluid).

Degree of Dehydration

It is crucial to correctly assess the degree of dehydration since severe extracellular fluid volume depletion calls for rapid isotonic fluid resuscita-

[2]The terms dehydration and extracellular fluid volume depletion are mostly used interchangeably. However, these terms denote conditions resulting from different types of fluid losses. Volume depletion refers to any condition in which the effective circulating volume is reduced. It is produced by salt and water losses (as with vomiting, diarrhea, diuretics, bleeding, or third space sequestration). Strict sense dehydration refers to water loss alone. The clinical manifestation of dehydration is often hypernatremia. The elevation in serum Na^+ concentration, and therefore effective blood osmolality, pulls water out of the cells into the extracellular fluid. However, much of the literature does not distinguish between the two terms.

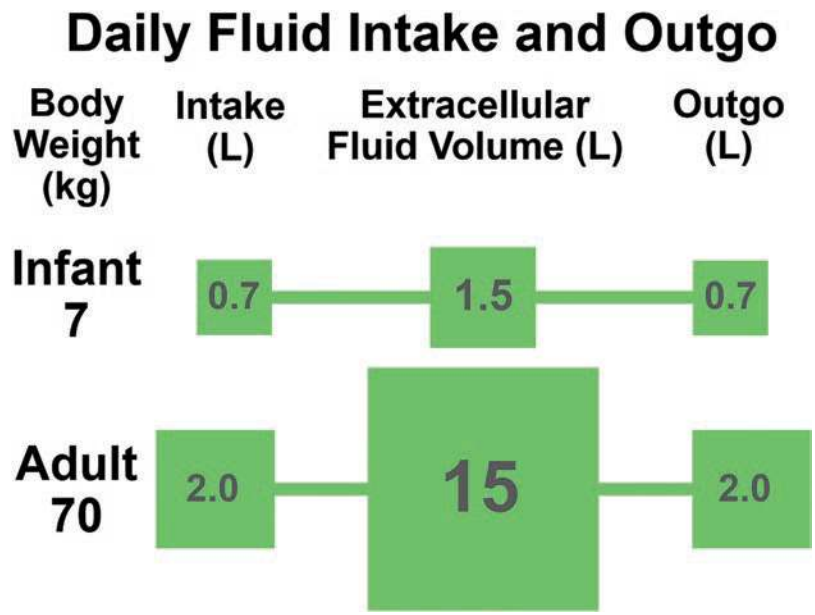

Fig. 34.4 Fluid turnover in infancy and adulthood. There is a proportionally greater turnover of fluids and solutes in infants and children as compared with adults. The figure depicts fluid intake, extracellular fluid volume and fluid outgo (diuresis and perspiratio insensibilis) in a healthy 6-month-old infant weighing 7.0 kg (input represents ≈50% of extracellular fluid volume) and in a healthy adult weighing 70 kg (input represents ≈15% of extracellular fluid volume)

tion. Dehydration is most objectively measured as an acute change in weight from baseline (acute loss of body weight reflects the loss of fluid, not lean body mass; thus, a 1.3 kg weight loss reflects the loss of 1.3 liters of fluid). In most cases, however, an accurate recent weight is unavailable.

As a result, a pertinent history and a number of findings on physical examination are used to help assess dehydration. The signs and symptoms of dehydration include static (reduced general appearance, dry mucous membranes, reduced skin turgor, sunken eyes and fontanelle, colder extremities) and dynamic signs (delayed capillary refill, deep respiration with or without increased respiratory rate, tachycardia, weak peripheral pulses, reduced blood pressure, reduced urine output, reduced or absent tears' production). Skin turgor, sometimes referred to as skin elasticity, is a sign commonly used to assess the degree of hydration (the skin on the back of the hand, lower arm, or abdomen is grasped between two fingers, held for a few seconds and then released: skin with normal turgor snaps rapidly back to its normal position but skin with decreased turgor remains elevated and returns slowly to its normal position). However, decreased skin turgor is a late sign in dehydration that is associated with moderate or, more frequently, severe dehydration. Like decreased skin turgor, arterial hypotension is a late sign in hypovolemia (in children with minimal to mild dehydration blood pressure is often slightly increased).

Several attempts have been made to determine a measure of dehydration by using combinations of clinical findings. In children ≤4 years of age with a diagnosis of acute diarrhea or vomiting, four clinical items ((a) general appearance, (b) eyes, (c) mucous membranes, (d) tears), which may be summed up to a total score ranging from 0 to 8, accurately estimate dehydration (Table 34.1) [15].

Laboratory Testing and the Type of Fluid Lost

Laboratory testing can confirm the presence of dehydration. The *fractional clearance of* Na^+ (which measures the amount of filtered Na^+ that is excreted in the urine)

$$\frac{\text{Urinary } Na^+ \times \text{Circulating creatinine}}{\text{Circulating } Na^+ \times \text{Urinary creatinine}}$$

is $<0.5 \times 10^{-2}$ (or <0.5%) and the *urine spot* Na^+ *concentration* <30 mmol/L (unless the disease underlying dehydration is renal).

Furthermore, in dehydration, the urine is concentrated with an *osmolality* >450 mosm/kg

Table 34.1 "4-item 8-point rating scale" clinical dehydration scale. The score consists of 4 clinical items, which may be summed for a total score ranging from 0 to 8. The final 3 categories are no or minimal dehydration (<3%; score of 0), mild dehydration (≥3% to <6% dehydration; score of 1–4), and moderate to severe dehydration (≥6% dehydration; score of 5–8)

	Score		
Characteristic	0	1	2
General appearance	Normal	Thirsty, restless or lethargic but irritable when touched	Drowsy, limp, cold, or sweathy; comatose or not
Eyes	Normal	Slightly sunken	Very sunken
Mucous membranes (tongue)	Moist	Sticky	Dry
Tears	Tears	Decreased tears	Absent tears

H_2O. The urinary concentration is measured with an osmometer or fairly estimated, in the absence of proteinuria and glucosuria, from the specific gravity, as determined by refractometry (dipstick assessment of specific gravity is unreliable), as follows:

$$(\text{specific gravity} - 1000) \times 40$$

Furthermore, laboratory testing can detect associated electrolyte and acid-base abnormalities but determination of circulating electrolytes and acid-base balance is typically limited to children requiring intravenous fluids. These children are more severely volume depleted and are therefore at greater risk for dyselectrolytemias. Laboratory testing is less useful for assessing the degree of volume depletion.

- *Bicarbonatemia* ≤17.0 mmol/L is considered by some a useful laboratory test to assess dehydration.
- The *blood urea* level might be a further good biochemical marker of dehydration because it reflects both the decreased glomerular filtration rate and the enhanced Na^+ and water reabsorption in the proximal tubule. Unfortunately, this test is of limited usefulness since it can be increased by other factors such as bleeding or tissue breakdown (on the other side the rise can be minimized by a concomitant decrease in protein intake).
- The *serum* Na^+ concentration varies with the relative loss of solute to water. Changes in Na^+ concentration play a pivotal role in determining the type of fluid depletion (Fig. 34.5):
  - Hyponatremic (and hypotonic) dehydration: Here, hyponatremia reflects net solute loss in excess of water loss. This does not occur directly, as fluid losses such as diarrhea are not hypertonic. Usually solute and water are lost in proportion, but water is taken in and retained in the context of hypovolemia-induced secretion of antidiuretic hormone. Since body water shifts from extracellular fluid to cells under these circumstances, and since signs of dehydration mostly depend on effective intravascular volume and tissue perfusion, signs of dehydration easily become profound.
  - Normonatremic (and isotonic) dehydration: in this setting, solute is lost in proportion to water loss.
  - Hypernatremic (and hypertonic) dehydration: this setting reflects water loss in excess of solute loss. Since body water shifts from intracellular to extracellular fluid under these circumstances, these children have less signs of dehydration for any given amount of fluid loss than do children with normonatremic (or normotonic) dehydration and especially those with hyponatremic dehydration. Clinical assessment can therefore underestimate the degree of dehydration in these children.
- *Bioimpedance devices* can also be helpful in determing hydration status in children as they measure fluid deficit in relation to extracellular water content or body weight, or consider absolute resistance [16].

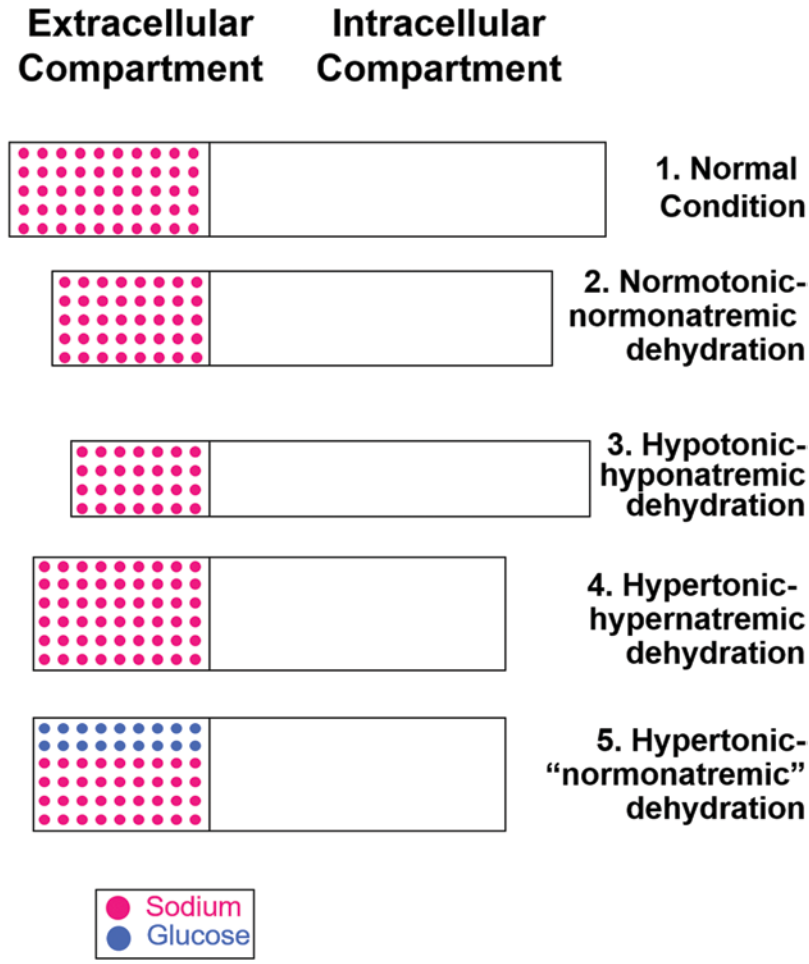

Fig. 34.5 Extracellular and intracellular compartments in children with dehydration. Normally, the extracellular compartment makes up approximately 20% and the intracellular 40% of the body weight (panel 1 of the figure). The second, third and fourth panels depict the relationship between extracellular and intracellular compartment in three children with dehydration in the context of an acute diarrheal disease: dehydration is normotonic-normonatremic in the first (panel 2), hypotonic-hyponatremic (mainly extracellular fluid losses) in the second (panel 3), and hypernatremic (mainly intracellular fluid losses) in the third child (panel 4). The lower panel depicts the relationship between extracellular and intracellular compartment (mainly intracellular fluid losses) in a child with dehydration in the context of diabetic ketoacidosis (hypertonic-"normonatremic" dehydration; the brackets indicate that in the context of diabetic ketoacidosis the concentration of circulating sodium is normal or even reduced). In each panel the red circles denote sodium and blue circles impermeable solutes that do not move freely across cell membranes (in the present example glucose). For reasons of simplicity, no symbols are given for potassium, the main intracellular cation

Dysnatremia

Consequences, Symptoms and Diagnostic Work Up

Under normal conditions, blood Na^+ concentration is maintained within the narrow range of 135–145 mmol/L despite great variations in water and salt intake. Na^+ and its accompanying anions Cl^- and HCO_3^- account for 90% of the extracellular effective osmolality. The main determinant of the Na^+ concentration is the plasma water content, itself determined by water intake (thirst or habit), "insensible" losses (such as metabolic water, sweat and respiration), and urinary dilution. The last of these is under most circumstances crucial and predominantly determined by anti-diuretic hormone. In response to this hormone, concentrated urine is produced. Dysnatremias produce central nervous system dysfunction. While hyponatremia may induce brain swelling, hypernatremia may induce brain shrinkage, yet the clinical features elicited by opposite changes in tonicity are remarkably similar [3, 4, 8–12].

Hyponatremia

Introduction

Hyoponatremia is classified (Fig. 34.6) according to the extracellular fluid volume status, as either "hypovolemic" (= depletional) or "normo-hypervolemic" (= dilutional). Vasopressin is released both in children with low effective circulating volume, the most common cause of hyponatremia in everyday clinical practice, as well as

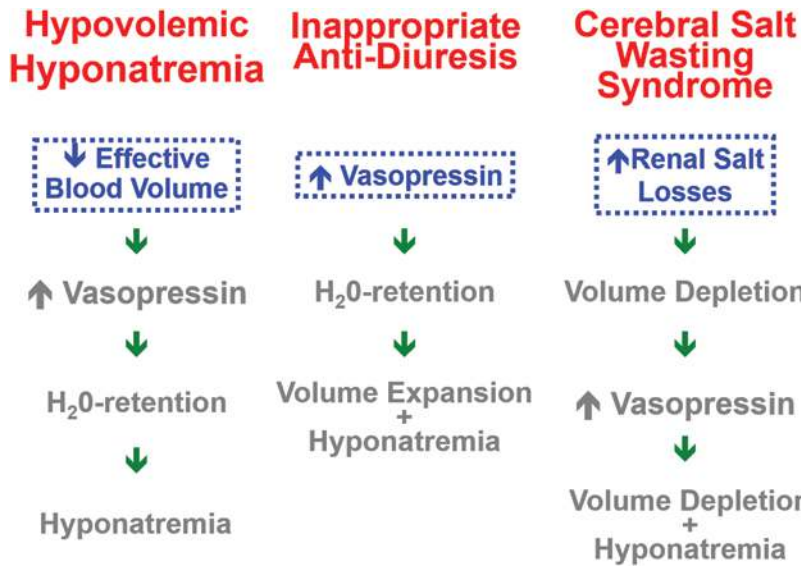

Fig. 34.6 Extracellular fluid volume status in children with hyponatremia. Hyoponatremia is classified according to the extracellular fluid volume status, as either hypovolemic (= depletional) or normo-hypervolemic (= dilutional). In most cases (left panel) hyponatremia results from a low effective arterial blood volume and is termed hypovolemic hyponatremia (the term appropriate anti-diuresis is sometimes used in this circumstance). The true syndrome of inappropriate anti-diuresis results from persistently high levels of vasopressin or, more rarely, activation of its renal receptor (middle panel). Cerebral salt-wasting syndrome is a further form of hyponatremia that sometimes develops in patients with intracranial disorders (right panel). In this condition, renal salt-wasting is the primary defect, which is followed by volume depletion leading to a secondary rise in vasopressin

in those with normo-hypervolemic hyponatremia. In hypovolemic hyponatremia, vasopressin release is triggered by the low effective arterial blood volume (this condition is also referred to as 'syndrome of appropriate anti-diuresis'). In dilutional hyponatremia, the primary defect is a euvolemic, inappropriate increase in circulating vasopressin levels (this condition is also termed 'syndrome of inappropriate anti-diuresis').

Assessing the cause of hyponatremia may be straightforward if an obvious cause is present (for example in the setting of vomiting or diarrhea) or in the presence of a clinically evident extracellular fluid volume depletion [3, 4, 8, 11, 12]. Sometimes, however, assessing the volume status and distinguishing hypovolemic from normo-hypervolemic hyponatremia may not be straightforward. In such cases, the urine spot Na^+ and the fractional Na^+ clearance are helpful, as patients with dilutional hyponatremia have a urinary Na^+ >30 mmol/L (and fractional Na^+ clearance >0.5%), whereas those with extracellular fluid volume depletion (unless the source is renal) will have a urinary Na^+ <30 mmol/L (and fractional Na^+ clearance <0.5%). Since effective blood osmolality is mostly low in hyponatremia, and urine is less than maximally dilute (inappropriately concentrated), blood and urine osmolalities, although usually measured, are rarely discriminant.

A decrease in Na^+ concentration and effective blood osmolality causes movement of water into brain cells and results in cellular swelling and raised intracranial pressure. Nausea and malaise are typically seen when the Na^+ level acutely falls <125–130 mmol/L. Headache, lethargy, restlessness, and disorientation follow, as its concentration falls <115–120 mmol/L. With severe and rapidly evolving hyponatremia, seizures, coma, permanent brain damage, respiratory arrest, brain stem herniation, and death may occur. In more gradually evolving hyponatremia, the brain self regulates to prevent swelling over hours to days by transport of, firstly, Na^+, Cl^-, and K^+ and, later, solutes like glutamate, taurine, myoinositol, and glutamine from intracellular to extracellular compartments. This induces water loss and ameliorates brain swelling, and hence leads to few symptoms in subacute and chronic hyponatremia.

Evaluating the Cause

In normovolemic subjects, the primary defense against developing hyponatremia is the ability to dilute urine and excrete free-water [3, 4, 8, 11, 12]. Rarely is excess ingestion of free-water alone the cause of hyponatremia. It is also rare to develop hyponatremia from excess urinary Na^+ losses in the absence of free-water ingestion. In order for hyponatremia to develop, both a relative excess of free-water as well as an underlying condition that impairs the ability to excrete free-water are typically required. Renal water handling is primarily under the control of vasopressin, which is released from the posterior pituitary and impairs water diuresis (hence the synonymous name "anti-diuretic hormone") by increasing the permeability to water in the collecting tubule.

There are osmotic, hemodynamic and non-hemodynamic stimuli for release of vasopressin. In most cases, hyponatremia develops when the body attempts to preserve the extracellular fluid volume at the expense of circulating Na^+ (therefore, a hemodynamic stimulus for vasopressin production overrides an inhibitory effect of hyponatremia). However, there are further stimuli for production of vasopressin in hospitalized children that make virtually any hospitalized patient at risk for hyponatremia (Table 34.2).

Some specific causes of hypotonic hyponatremia deserve further discussion.

- **Hospital-acquired hyponatremia** is most often seen in the postoperative period or in association with a reduced effective circulating volume.
- Postoperative hyponatremia is a serious problem in children, which sometimes is caused by a combination of nonosmotic stimuli for release of anti-diuretic hormone, such as pain, nausea, stress, narcotics, and edema-forming conditions. Subclinical depletion of the effec-

Table 34.2 Causes of hypotonic hyponatremia in childhood

Hypovolemic	Normovolemic (or hypervolemic)
Intestinal salt loss – Diarrheal dehydration – Vomiting, gastric suction – Fistulae – Laxative abuse	*Increased body water* – Parenteral hypotonic solutions – Exercise-associated hyponatremia – Habitual (and psychogenic) polydipsia
Transcutaneous salt loss – Cystic fibrosis – Endurance sport	*Non osmolar release of antidiuretic hormones*[a] – Cardiac failure – Sever liver disease (mostly cirrhosis) – Nephrotic syndrome – Glucocorticoid deficiency – Drugs causing renal water retention – [Hypothyroidism][b]
Renal sodium loss – Mineralocorticoid deficiency (or resistance) – Diuretics – Salt wasting renal failure – Salt wasting tubulopathies (including Bartter syndromes) – Gitelman syndrome, and De Toni-Debré–Fanconi syndrome) – Cerebral salt wasting	*Syndrome of inappropriate anti-diuresis* – Classic syndrome of inappropriate secretion of antidiuretic – hormone – Hereditary nephrogenic inappropriate antidiuresis
Perioperative (e.g.: preoperative fasting, vomiting, third space losses)	*Reduced renal water loss* – Chronic renal failure – Oliguric acute renal failure

[a] Effective arterial blood volume mostly reduced
[b] Evidence supporting this association is rather poor

tive blood volume and administration of hypotonic fluids are currently considered the most important causes of postoperative hyponatremia.

– More rarely, hospital-acquired hyponatremia is seen in association with the syndrome of inappropriate anti-diuresis, which is caused either by elevated activity of vasopressin or, less commonly, by hyperfunction of its renal (= V2) receptor, independently of increased effective blood osmolality and hemodynamic stimulus (i.e.: reduced effective circulating volume). It is currently assumed that this condition results not only from dilution of the blood by free-water but also from inappropriate natriuresis. The syndrome of inappropriate anti-diuresis (Fig. 34.6) should be suspended in any child with hyponatremic hypotonia, a urine osmolality >100 mosmol/kg H_2O, a normal fractional Na^+ clearance (>0.5%), low normal or reduced uric acid level, low blood urea level and normal acid-base and K^+ balance.
– The longstanding assumption that hypontremia associated with meningitis or respiratory infections is caused by inappropriate antidiuresis is not substantiated by reports that adequately assessed the volume status. On the other hand, dysnatremia is frequently observed in many other community-acquired infections. Hyponatremia develops in approximately 50% of acute moderate gastroenteritis cases, often associated with metabolic acidosis and potassium disturbances. Up to 60% of infants with bronchiolitis tend to develop an isolated hyponatremia, while 70% of infants with pyelonephritis might develop hyponatremia. In these patients, metabolic acidosis and hyperkalemia are also often present, suggesting silent renal resistance to aldosterone [17].
– **Desmopressin**, a synthetic analogue of the natural anti-diuretic hormone, is used in central diabetes insipidus, in some bleeding disorders, in diagnostic urine concentration testing and especially in primary nocturnal enuresis with nocturnal polyuria. Desmopressin is generally regarded as a safe drug and adverse effects are uncommon. Nonetheless, hyponatremic water intoxication leading to convulsions has been reported as a rare side effect of desmopressin therapy in enuretic children. This complication mostly develops in subjects managed with the intranasal formulation ≤14 days after starting the medication, following excess fluid intake and during intercurrent illnesses (Table 34.3) [18].

Table 34.3 Drug-induced hyponatremia

Diuretics (thiazides more commonly than loop diuretics)
Drugs blocking the renin-angiotensin-aldosterone system (converting enzyme inhibitors, sartans or renin inhibitors)
Antidiuretic drugs
• ↑ **water permeability of the renal collecting tubule:** arginine-vasopressin, vasopressin analogues like desmopressin and oxytocin
• ↑ **antidiuretic hormone release,** ↑ **antidiuretic hormone action:** carbamazepine, lamotrigine, valproate, barbiturates, antidiabetic drugs (chlorpropamide, tolbutamide), clofibrate, colchicine, nicotine, vincristine, cyclophosphamide
• ↓ **synthesis of prostaglandins:** nonsteroidal anti-inflammatory drugs including salicylates, paracetamol
• **Mechanism unknown:** haloperidol, tricyclic antidepressants, selective serotonin-reuptake inhibitors, monoamine oxidase inhibitors, narcotics like morphine

– Male infants have been described with hyponatremia and laboratory features consistent with release of vasopressin but who had no measurable circulating levels of this hormone. This rare condition results from gain-of-function mutations of the X-linked receptor gene that mediates the renal response to vasopressin, resulting in persistent activation of the receptor. The condition, which has been termed **hereditary nephrogenic syndrome of inappropriate anti-diuresis**, represents a kind of mirror image of the X-linked nephrogenic diabetes insipidus, the result of loss-of-function genetic defects in the aforementioned renal receptor.
– **Cerebral salt wasting syndrome** is a peculiar form of depletional hyponatremia that sometimes occurs in patients with cerebral disease (Fig. 34.6). It mimics the syndrome of inappropriate anti-diuresis, except that salt-wasting is the primary defect with the ensuing volume depletion leading to a secondary release of vasopressin. Salt wasting of central origin might result from increased secretion of a natriuretic peptide with subsequent suppression of aldosterone synthesis. The distinction between cerebral salt wasting and inappropriate activity of vasopressin is not always simple since the true volume status is sometimes difficult to ascertain.
– **Endurance athletes** sometimes replace their diluted but Na^+-containing sweat losses with excessive amounts of severely hypotonic solutions: the net effect is a reduction in the circulating Na^+ level. The effect is likely compounded by a reduced renal function during exercise (such individuals may also be taking non-steroidal anti-inflammatory drugs, which can impair the excretion of free water).
– A tendency towards low normal blood Na^+ level is sometimes seen in children who drink excessively and present with polyuria and polydipsia. Usually the problem is simply one of habit, particularly in infants who are attached to a bottle (= **habitual polydipsia**). In childhood, polydipsia is rarely a symptom of significant psychopathology (= **psychogenic polydipsia**).
– Diuretics (thiazides more frequently than loop diuretics) and **drugs** that block the renin-angiotensin-aldosterone system (converting enzyme inhibitors, sartans or direct renin inhibitors) make up a common cause of hyponatremia (Table 34.3). More rarely, other drugs sometimes cause renal retention of fluids and therefore dilutional hyponatremia.

Hypernatremia

Introduction

Hypernatremia (Na^+ >145 mmol/L) reflects a net water loss or a hypertonic Na^+ gain, with inevitable hypertonicity [3, 4, 8–10]. Severe symptoms are usually evident only with acute and large increases in Na^+ concentration ≥160 mmol/L. Importantly, the sensation of thirst protecting against the tendency towards hypernatemia is absent or reduced in patients with altered mental status or with hypothalamic lesions and in infancy.

The cause of hypernatremia is almost always evident from the history. Determination of urine osmolality in relation to the effective blood osmolality and the urine Na^+ concentration helps

if the cause is unclear. Patients with diabetes insipidus present with polyuria and polydipsia (and not hypernatremia unless thirst sensation is impaired). Central diabetes insipidus and nephrogenic diabetes insipidus may be differentiated by the response to water deprivation (failure to concentrate urine) followed by desmopressin, causing concentration of urine uniquely in patients with central diabetes insipidus.

Non-specific symptoms such as anorexia, muscle weakness, restlessness, nausea, and vomiting tend to occur early. More serious signs follow, with altered mental status, lethargy, irritability, stupor, or coma. Acute brain shrinkage can induce vascular rupture, with cerebral bleeding and subarachnoid hemorrhage.

Evaluating the Cause

Two mechanisms protect against developing hypernatremia or increased effective blood osmolality: the ability to release vasopressin (and therefore to concentrate urine) and a powerful thirst mechanism. Release of vasopressin occurs when the effective blood osmolality exceeds 275–280 mosmol/kg H_2O and results in maximally concentrated urine when the effective blood osmolality exceeds 290–295 mosmol/kg H_2O. Thirst, the second line of defense, provides a further protection against hypernatremia and increased effective osmolality. If the thirst mechanism is intact and there is unrestricted access to free-water, it is rare to develop sustained hypernatremia from either excess Na^+ ingestion or a renal concentrating defect (Table 34.4).

Hypernatremia is primarily a hospital-acquired condition occurring in children who have restricted access to fluids. Most children with hypernatremia are debilitated by an acute or chronic disease, have neurological impairment, are critically ill or are born premature. Hypernatremia in the intensive care setting is common as these children are typically either intubated or moribund, and often are fluid restricted, receive large amounts of Na^+ as blood products or have renal concentrating defects from diuretics or renal dysfunction. The majority of hypernatremia results from the failure to administer sufficient free-water to children who are unable to care for themselves and have restricted access to fluids.

Two special causes of hypernatremia deserve some further discussion.

- A frequent cause of hypernatremia in the outpatient setting is currently **breastfeeding-associated hypernatremia**, which should more properly be labeled "not-enough-breastfeeding-associated hypernatremia" [19]. This condition occurs between days 7 and 15 in otherwise healthy term or near-term newborns of first-time mothers who are exclusively breast-fed. In all cases feeding had been

Table 34.4 Causes of hypernatremia in childhood

Hypovolemic	Normovolemic	Hypervolemic
Inadequate intake – Breast feeding hypernatremia – Poor access to water – Altered thirst perception (uncosciousness, mental impairment) **Intestinal salt loss** (diarrheal dehydration) **Renal water and salt loss** – Postobstructive polyuria – Diuretics – Diabetes insipidus (either primary or, more rarely, secondary[a]) – Medullary renal damage	**Hypodypsia** (essential hypernatremia) **Hyperventilation** **Fever**	**Inappropriate intravenous fluids** (e.g.: hypertonic saline, $NaHCO_3$) **Salt poisoning** (accidental, deliberate) **Primary aldosteronism** (and other conditions that cause low-renin hypertension)

[a] Secondary nephrogenic diabetes insipidus may develop as a complication of inherited renal diseases such as nephropathic cystinosis, Bartter and Gitelman syndromes and nephronophthisis

difficult to establish and the volume of milk ingested was likely to have been low. The underlying problem is water deficiency: Na^+ concentration raises predominantly as a result of low volume intake and a loss of water, demonstrating that inadequate feeding is the cause of hypernatremic dehydration. Monitoring postnatal weight loss provides an objective assessment of the adequacy of nutritional intake allowing targeted support to those infants who fail to thrive or demonstrate excessive weight loss (≥10% of birth weight).
– Diarrhea or vomiting are a further reason of hypernatremia in the outpatient setting, but are much less common than in the past, presumably due to the advent of low solute infant formulas and the increased use and availability of oral rehydration solutions.

Management

The discussion will exclusively focus on some features of parenteral hydration, and the management of hyponatremia with V2 anti-diuretic hormone receptor antagonists [3, 4, 8–12].

Maintenance and Perioperative Fluids

Intravenous maintenance fluids are designed to provide water and electrolyte requirements in a fasting patient. The prescription for intravenous maintenance fluids was originally described by Holliday (Table 34.5), who rationalized a daily H_2O requirement of 1700–1800 mL/m^2 body surface area and the addition of 3 and 2 mmol/kg body weight of Na^+ and K^+ respectively (approximating the electrolyte requirements and urinary excretion in healthy infants). This is the basis for the traditional recommendation that hypotonic intravenous maintenance solutions are ideal for children. This approach has subsequently been questioned because of the potential for these hypotonic solutions to cause hyponatremia. Surgical patients appear as the subgroup of children with the highest risk to develop severe hyponatremia with the use of hypotonic intravenous solutions, likely because they tend to be hypovolemic. Furthermore, traditional maintenance fluid recommendations may be much greater than actual water needs in children at risk of hyponatremia.

Most authors currently suggest (Table 34.5) that hyponatremia should be prevented by (a) using isotonic (usually normal saline, which contains NaCl 9 g/L) or near isotonic (usually Ringer's lactate) solutions and (b) reducing by ≈20% the daily volume of maintenance fluid to 1400–1500 mL/m^2 body surface area. Considering the potential for hypoglycemia in infancy, ringer's lactate or normal saline in 5% glucose in water (which contains glucose 50 g/L) is considered the safest fluid composition for most children.

Table 34.5 Intravenous maintenance fluids designed to provide water and electrolyte requirements in a fasting patient. Both the recommendation originally described by Holliday and the most recent recommendation are given. The addition of KCl 2 mmol/kg body weight is also recommended

	Holliday's recommendation	Current suggestion
Solution	5% dextrose in water supplemented with NaCl 3 mmol/kg body weight daily	Isotonic saline in 5% dextrose in water
Amount (mL/m^2 body surface area[a] daily)	1700–1800	1400–1500
Clinical practice	100 mL/kg body weight for a child weighing less than 10 kg[b] + 50 mL/kg for each additional kg up to 20 kg + 20–[25] mL/kg for each kg in excess of 20 kg	80 mL/kg body weight for a child weighing less than 10 kg[b] + 40 mL/kg for each additional kg up to 20 kg + 15–[20] mL/kg for each kg in excess of 20 kg

[a] The Mosteller's formula may be used to calculated the body surface area (in m^2): $\sqrt{\frac{\text{height (cm)} \times \text{body weight (kg)}}{3600}}$

[b] In children weighing ≤5.0 kg the daily parenteral water requirement is 120 mL/kg body weight

Dehydration

Oral rehydration therapy is currently the treatment of choice for children with minimal, mild or moderate dehydration due to diarrheal diseases. However, in the practice of pediatric emergency medicine, mainly because of rapidity, intravenous rehydration is a commonly used intervention for these children.

Treatment approaches to parenteral rehydration in the hospitalized child vary. There are numerous ways to estimate the degree of dehydration (the "4-item 8-point rating scale" is currently widely recommended; Table 34.1), to calculate fluid and electrolyte deficits, and to deliver the deficits to the patient. For many years, the recommendation was to accomplish 100% (or even less) replacement of the volume deficit during the first 24 h of treatment. More recently, the aim of treatment has generally been to accomplish a more rapid full repletion within ≤6 h. In many children with mild to moderate dehydration, especially those resistant to initial oral rehydration therapy, and in children with severe dehydration, we currently administer intravenous isotonic (or near isotonic) crystalloid solutions such as normal saline or lactate Ringer's as repeated boluses of 10–20 mL/kg body weight (administered over 20–60 min).

In children with diarrhea or vomiting, reduced carbohydrate intake leads to free fatty acid breakdown, excess ketones, and an increased likelihood of continued nausea and vomiting. Consequently, some authorities have suggested the use of a glucose containing normal solution (mostly the lactate Ringer's in 5% glucose in water), which will stimulate insulin release, reduce free fatty acid breakdown, and therefore reduce treatment failure due to persisting nausea and vomiting (owing to hyperketonemia) [20].

The child with hypovolemic circulatory shock presents with (a) increased heart rate and weak peripheral pulses, (b) cold, pale and diaphoretic skin, and (c) delayed capillary refill. The initial management recommended by the American Academy of Pediatrics includes the administration of a high concentration of O_2 (ensuring that 100% of the available arterial hemoglobin is oxygenated). Common errors in the child with hypovolemic circulatory shock secondary to a diarrheal disease are delayed or inadequate (i.e. with hypotonic crystalloid solution) fluid resuscitation.

Children with hypernatremic dehydration are also hydrated parenterally with isotonic crystalloid solutions until diagnosis of the dyselectrolytemia, followed by slightly hypotonic solutions (e.g.: half-saline) in order to slowly correct the circulating Na^+ concentration (abruptly correcting hypernatremia using a Na^+ free glucose solution creates an increased risk of brain edema; Fig. 34.7). In acute dysnatremic dehydration, Na^+ should be corrected slowly at a rate not exceeding 0.5 mmol/L per hour (or more than by 12 mmol/L per day). Subacute or chronic hypernatremia should be corrected even more slowly.

Hydration in Infectious Diseases Associated with a Tendency Towards Hyponatremia

Fluid restriction has been widely advocated in the initial management of infectious diseases such as meningitis, pneumonia or bronchiolitis, which are often associated with a low Na^+ level. However, there is no evidence that fluid restriction is useful. Furthermore, hyponatremia results from appropriate, volume-dependent antidiuresis in these disease conditions. In clinical practice, initial restoration of the intravascular space with an isotonic crystalloid followed by isotonic maintenance fluids 1400–1500 mL/m^2 body surface area per day (Table 34.5, right panel) are currently advised. In cases presenting with overt hyponatremia, frequent monitoring of electrolytes is also required with adjustments to be made according to laboratory findings.

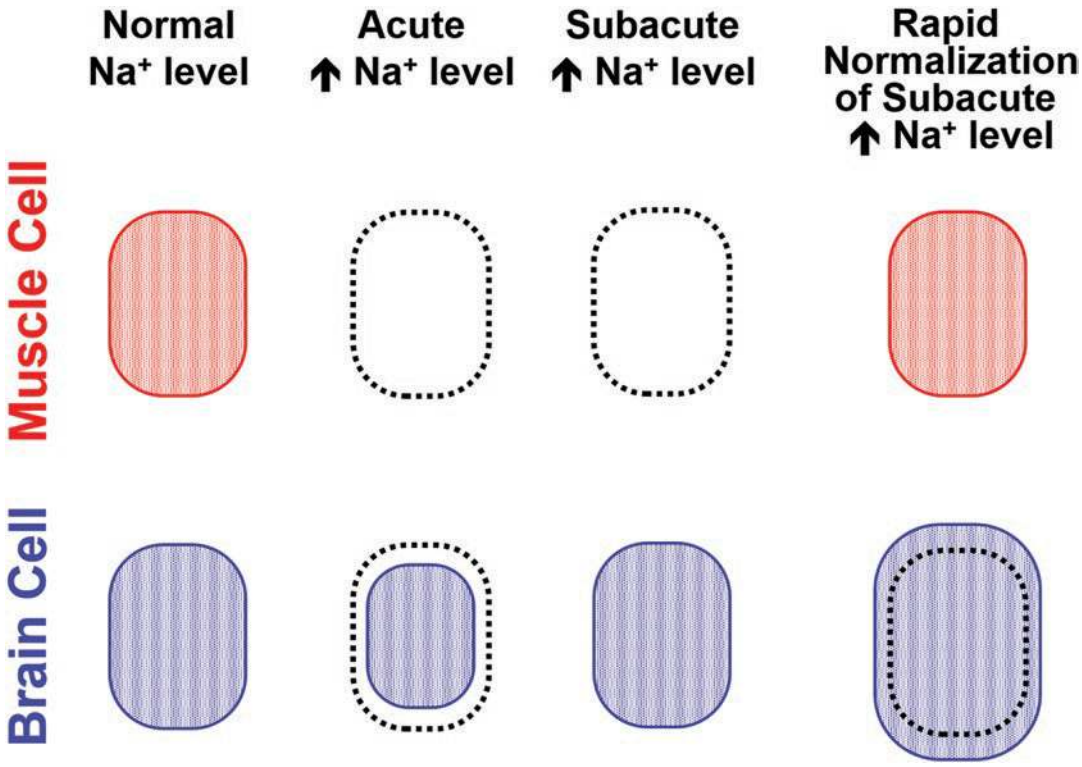

Fig. 34.7 Cell volume in acute or subacute hypernatremia and after rapid correction of hypernatremia. When hypernatemia develops acutely, all cells are reduced in size (the degree of cell volume reduction reflects the degree of hypernatremia). When hypernatremia is present for 36–48 h or more (= subacute hypernatremia), cell volume reduction persists in most cells, including muscle cells (upper panel). However, brain cells and red blood cells tend to restore their normal cell volume (lower panel). A rapid normalization of sodium level in children affected with subacute or chronic hypernatremia pathologically increases the volume of brain cells (and red blood cells). Swelling of cells, which does not have serious consequences when it occurs in most organs, may have damaging consequences when it occurs in the brain (lower panel, right)

Hyponatremia

Chronic normovolemic (or hypervolemic) hyponatremia is usually managed either by restricting water intake or by giving salt. An alternative may be the use of nonpeptide vasopressin receptor antagonists. There are three receptors for vasopressin: the $V1_a$ receptors that mediate vasoconstriction, the $V1_b$ receptors that mediate adrenocorticotropin release, and the V2 receptors that mediate the anti-diuretic response. Vaptans, oral V2 receptor antagonists, have been approved for the management of normovolemic and hypervolemic hyponatremia: these agents produce a selective water diuresis (without affecting Na^+ and K^+ excretion) that raises the circulating Na^+ level. Only limited information is currently available with these agents in childhood.

Vaptans do not correct hyponatremia in patients affected by nephrogenic syndrome of inappropriate childhood anti-diuresis. In these patients, a way to enhance water excretion is the oral administration of urea (dosage in adults: 30 g per day). This regimen, which may be effective because it causes simultaneously water diuresis and renal Na^+ retention, is well tolerated, and has been used chronically in pediatric outpatients.

Potassium

Balance

Most (98%) of the K^+ in the body (40–50 mmol/kg) is within cells. The maintenance of distribution of K^+ across cells is largely dependent on the activity of the sodium pump (= Na^+-K^+-ATPase). In healthy humans, the extracellular K^+ concentration is maintained between 3.5 and 5.0 mmol/L. K^+ balance, like that of other ions, is a function of intake and urinary excretion. In adults, the daily K^+ intake averages 0.5–2 mmol/kg body weight (Fig. 34.8). The homeostasis goal of the adult is to remain in zero K^+ balance. Thus, ≈90–95% of the typical daily intake of 1 mmol/kg is ultimately eliminated from the body in the urine (the residual 5–10% is lost through the stool). Infants maintain a positive K^+ balance (the estimated requirement for growth is 1.2 mmol/day during the first 3 months of life, 0.8 mmol/day up to 1 year and 0.4 mmol/day thereafter). The net accretion of K^+ ensures the availability of adequate substrate for incorporation into cells newly formed during periods of somatic growth. Postnatal growth is associated with an increase in total body K^+ from ≈8 mmol/cm body height at birth to >14 mmol/cm body height by 18 years of age. The rate of accretion of body K^+ per kg body weight in the infant is more rapid than in the older child, reflecting both an increase in cell number and K^+ concentration, at least in skeletal muscle, with advancing age [21–23].

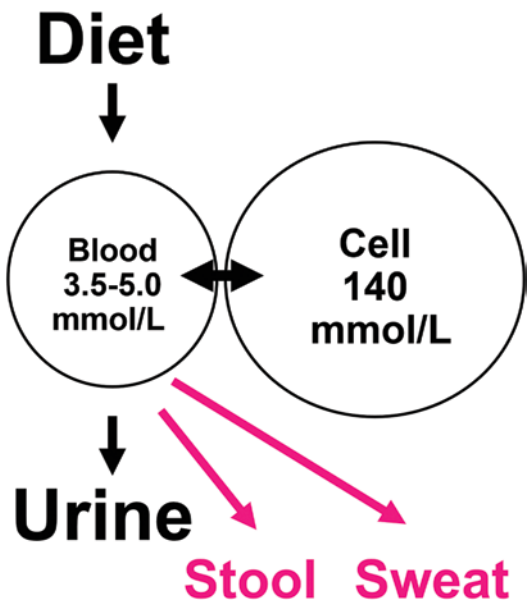

Fig. 34.8 Major factors governing the homeostasis of potassium. Most of the potassium in the body resides within cells. The maintenance of distribution of potassium across cells is largely dependent on the activity of the sodium pump. Potassium balance, like that of other ions, is a function of intake and urinary excretion. Since only small amounts of potassium are normally lost in the sweat and in the stool, they are depicted in a different color. However, substantial intestinal potassium and therefore potassium depletion can be seen with vomiting, diarrhea or other intestinal disease conditions or when sweat production is chronically increased

Regulation of Circulating Potassium

Circulating K^+ concentration is regulated by the total body K^+ content, which depends upon (a) the external balance, i.e. the difference between intake and excretion in the urine, feces and sweat, and (b) the internal balance, which represents the relative distribution of K^+ between the intracellular and the extracellular spaces [21–23].

External (≈ Renal) Potassium Homeostasis

Virtually all regulation of urinary K^+ excretion and therefore of external K^+ homeostasis occurs in the **renal cortical collecting tubule**. Indeed, almost all of the filtered K^+ is reabsorbed in the proximal tubule and the loop of Henle, so that <10% of the filtered load is delivered to the cortical collecting tubule. This tubular segment adjusts the external homeostasis of K^+ by modulating its secretion.

- The major physiologic regulators of K^+ secretion within the cortical collecting tubule are "hyperkalemia" and aldosterone, which act in concert to promote the tubular secretion and therefore the urinary excretion of this ion.
- Increasing the flow rate traversing the cortical collecting tubule is a further factor that may increase the K^+ excretion. This response is most prominent in the presence of hyperkale-

mia, since the concurrent elevations in aldosterone and circulating K^+ concentration produce a high level of K^+ secretion within the cortical collecting tubule.

Internal Potassium Homeostasis

The main modulators of the distribution of K^+ between the intracellular and the extracellular spaces are insulin, the sympathetic nervous system (via β_2-adrenergic receptors) and the acid-base balance.

Prenatal and Neonatal Potassium Balance

During fetal life K^+ is actively transported across the placenta from the mother to the fetus (indeed, the fetal K^+ concentration is maintained at levels ≥5.0 mmol/L even in the face of maternal K^+ deficiency). The tendency to retain K^+ early in postnatal life is reflected by the observation that infants, especially premature newborns, tend to have higher circulating K^+ levels than children. Furthermore, in infancy the ability to increase urinary K^+ excretion is blunted (see: non-oliguric hyperkalaemia of the premature infant).

Symptoms, Signs, and Consequences of Hypokalemia and Hyperkalemia

Excess or deficient K^+ in the extracellular space impairs cardiovascular, neuromuscular, renal and endocrine-metabolic body functions [21, 22]. The most dangerous clinical consequence of these dyselectrolytemias is the predisposition to life-threatening cardiac arrhythmias. The manifestations of hypokalemia are outlined in Table 34.6, those of hyperkalemia in Table 34.7.

The severity of hypokalemic manifestations is proportionate to the degree and duration of hypokalemia and symptoms generally do not become manifest until the K^+ concentration is below 2.5–3.0 mmol/L. Hypokalemia causes characteristic

Table 34.6 Body functions impaired by hypokalemia

• Cardiovascular abnormalities
Cardiac arrhythmias (premature atrial and ventricular beats, sinus bradycardia, paroxysmal atrial or junctional tachycardia, atrioventricular block, and ventricular tachycardia or fibrillation)
Increased systemic vascular resistance
• Neuromuscular disturbances
Skeletal muscle weakness (usually beginning with the lower extremities and progressing to the trunk and upper extremities; sometimes involvement of the respiratory muscles)
Muscle cramps, rhabdomyolysis
Smooth muscle dysfunction (intestinal and urinary system)
• Renal effects
Decreased urinary concentrating ability (decreased expression of the antidiuretic hormone-sensitive water channel aquaporin-2)
Increased renal ammonium production (and therefore ↑ generation of HCO_3)
Hypokalemic nephropathy[a] (interstitial fibrosis, tubular atrophy, cyst formation in the renal medulla)
• Endocrine and metabolic effects
Negative nitrogen balance (causing growth retardation)
Glucose intolerance (with tendency towards diabetes mellitus)
Decreased aldosterone release (direct adrenal action), increased renin secretion
Hepatic encephalopathy (in susceptible individuals)

[a] Following prolonged hypokalemia

Table 34.7 Body functions impaired by hyperkalemia

• Cardiovascular abnormalities
Cardiac arrhythmias (ventricular fibrillation or standstill are the most severe consequences)
Reduced systemic vascular resistance
• Neuromuscular disturbances
Skeletal muscle weakness (usually beginning with the lower extremities and progressing to the trunk and upper extremities; rarely involvement of the respiratory muscles)
Smooth muscle dysfunction (intestinal and urinary system)
• Renal effects
Reduced renal ammonium production (and therefore reduced generation of HCO_3)
• Endocrine and metabolic effects
Increased aldosterone release (direct adrenal action), reduced renin secretion

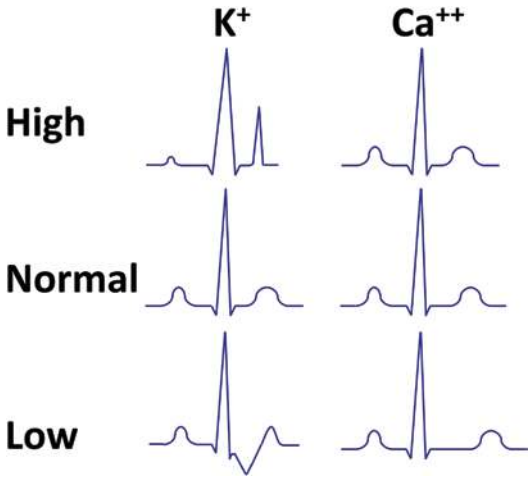

Fig. 34.9 Diagram illustrating some electrocardiographic consequences of hyperkalemia, hypokalemia, hypercalcemia and hypocalcemia. The electrocardiographic changes of hyperkalemia include tall and "peaked" T waves, PR prolongation, QRS widening and, at very high K^+ concentrations, ventricular arrhythmias (not shown). Hypokalemia produces a decreased amplitude of the T wave, a depression of the ST segment and an increased amplitude of U waves, which occur at the end of the T wave. Hypercalcemia manifests with a shortened, hypocalcemia with a prolonged QTc interval

electrocardiographic changes (Fig. 34.9): depression of the ST segment, a decreased amplitude of the T wave, and an increased amplitude of U waves which occur at the end of the T wave.

There are very few symptoms or signs of hyperkalemia, and these tend to occur only with very high levels. Symptoms generally do not become manifest until the K^+ concentration exceeds 7.0 mmol/L, unless the rise in concentration has been very rapid. Hyperkalemia produces the following elecrocardiographic changes: peaked T wave with shortened QT interval is the first change, followed by progressive lengthening of the PR interval and QRS duration. The severity of hyperkalemia is classified as follows: (a) mild: K^+ between 5.1 and 6.0 mmol/L and absent or equivocal electrocardiographic changes; (b) moderate: K^+ between 6.1 and 7.0 mmol/L and definite eletrocardiographic changes in reploarization ("peaked" T waves); (c) severe: K^+ ≥7.1 mmol/L and severe definite eletrocardiographic changes including atrial standstill, advanced atrio-ventricular heart block, QRS widening or ventricular arrhythmia (usually associated with weakness of skeletal muscles). The electrocardiographic signs of hyperkalemia are given in Fig. 34.9.

Evaluating the Causes of Hypokalemia and Hyperkalemia ("Diagnostic Tests")

The following tests have been developed to evaluate and distinguish the various causes of hypo- or hyperkalemia in childhood [21–23].

(a) The **t**rans**t**ubular **K+** concentration **g**radient, colloquially referred to as TTKG, measures the K^+ secretion within the cortical collecting tubule and represents an estimate of the aldosterone activity. This parameter can be easily calculated assuming (a) that the urine osmolality at the end of the cortical collecting tubule is similar to that of blood, and (b) that no K^+ secretion or reabsorption takes place in the medullary collecting tubule. If these assumptions are accurate, then the K^+ concentration in the final urine will rise above that in the cortical collecting tubule due to reabsorption of water in the medullary collecting duct. This effect can be accounted for by dividing the urine K^+ concentration by the ratio of the urine to blood osmolality. If, for example, this ratio is 2, then 50% of the water leaving the cortical collecting tubule has been reabsorbed in the medulla, thereby doubling the luminal K^+ concentration. This parameter is calculated as follows:

$$\frac{\text{Urinary } K^+ \,/\, \text{Urinary Osmolality}}{\text{Blood Osmolality} \,/\, \text{Circulating } K^+}$$

Blood osmolality (in mosm/kg) can be measured with an osmometer or very reasonably estimated from circulating Na^+, glucose, and urea (in mmol/L) as follows: (Na^+ × 2) + glucose + urea. On the other hand, urinary osmolality (in mmol/kg H_2O) can be measured with an osmometer or esti-

mated, in the absence of proteinuria and glucosuria, from the specific gravity, as determined by refractometry, as follows: (specific gravity − 1000) × 40.

(b) The fractional clearance of K^+ (which measures the amount of filtered K^+ that is excreted in the urine)

$$\frac{\text{Urinary } K^+ \times \text{Circulating Creatinine}}{\text{Circulatory } K^+ \times \text{Urinary Creatinine}}$$

and the molar urinary potassium/creatinine ratio (mol/mol)

$$\frac{\text{Urinary } K^+}{\text{Urinary Creatinine}}$$

are two further frequently used tests, which are strongly correlated.

(c) The fractional clearance of Cl^- (which measures the amount of filtered Cl^- that is excreted in the urine)

$$\frac{\text{Urinary } Cl^- \times \text{Circulating Creatinine}}{\text{Circulating } Cl^- \times \text{Urinary Creatinine}}$$

and especially the molar urinary chloride/creatinine ratio (mol/mol; see above)

$$\frac{\text{Urinary } Cl^-}{\text{Urinary Creatinine}}$$

are two further, closely correlated tests that have been suggested.

The molar urinary potassium/creatinine and the urinary chloride/creatinine indices are based on a near-constant creatinine excretion rate and consequently have a limited significance in patients with a very low body mass index.

(d) The 24-h K^+-excretion is a further useful diagnostic test. The use of this traditional diagnostic test is not generally advised considering that 24-h urine collections are troublesome and difficult to obtain (and often imprecise) in children who are not hospitalized, are not practical in a medical emergency, and almost impossible without invasive techniques such as bladder catheterization in infants.

In our experience, the following three "urinary tests" are useful to distinguish the various causes of hypokalemia and hyperkalemia:

- In normotensive children with hypokalemia the transtubular K^+ gradient and the urinary potassium/creatinine ratio (and perhaps the fractional clearance of K^+) easily help distinguish hypokalemia due to a short-term shift of the ion into cells (transtubular K^+ gradient <2.5; urinary potassium/creatinine <2.5 mol/mol) from hypokalemia resulting from a deficit of this ion, including renal K^+ losing conditions and hypokalemia complicating intestinal diseases (in patients experiencing diarrhea, secondary hyperaldosteronism caused by circulating volume depletion leads to an increased urine K^+ excretion).
- In normotensive children with hypokalemia and metabolic alkalosis the urinary excretion of Cl^- helps distinguish renal (urinary chloride/creatinine ratio >10 mol/mol) from non-renal causes (urinary chloride/creatinine ratio <10 mol/mol), as explained in the subchapter metabolic alkalosis.
- It has sometimes been incorrectly assumed that, when hypokalemia occurs in the context of non-renal conditions, the fractional clearance of K^+, the molar urinary potassium/creatinine ratio and the 24 h K^+-excretion are very low, allowing discrimination of non-renal and renal conditions. However, in most children with non-renal hypokalemia extracellular volume depletion is present also, leading to secondary activation of the renin-angiotensin II-aldosterone system, and therefore to an increased urinary K^+ excretion. As a consequence, the urinary K^+ excretion sometimes does not discriminate between non-renal and renal conditions associated with hypokalemia.

- In hyperkalemia the molar urinary potassium/creatinine ratio and the transtubular K^+ gradient help distinguish impaired from unimpaired urinary K^+ excretion. In subjects with unimpaired urinary K^+ excretion the molar urinary potassium/creatinine ratio is expected to be >20 mol/mol, the transtubular K^+ gradient >7.

Hypokalemia

The clinician evaluating a child with hypokalemia (<3.5 mmol/L) should consider five groups of causes [21–26]: (a) spurious hypokalemia; (b) redistribution; (c) true K^+ depletion due to non-renal (mostly intestinal) conditions; (d) true K^+ depletion due to renal conditions; and (e) hypokalemia associated with an expanded "effective" circulating volume and therefore with systemic hypertension due to enhanced mineralocorticoid activity (Table 34.8).

The total K^+ stores are reduced only in subjects with hypokalemia due to non-renal or renal conditions. On the other side, the body K^+ content is normal in children with spurious hypokalemia, in those with an increased shift of K^+ into cells and in those with hypokalemia associated with an expanded "effective" circulating volume.

Table 34.8 Causes of hypokalemia

• **Spurious hypokalemia** (cells take up potassium after blood has been drawn)
• **Hypokalemia associated with normal or low blood pressure**
– **Increased shift of potassium into cells (total body K^+ content normal)**
Activation of β_2-adrenergic receptors
Endogenous: stress, hypothermia
Exogenous: β_2-adrenergic agonists (e.g.: albuterol), xanthines
Hormones
Insulin
Endogenous: anabolism (e.g.: refeeding syndrome)
Exogenous: treatment of diabetic ketoacidosis
Aldosterone (possibly)
Alkalosis (metabolic)
Rare causes
Hypokalemic periodic paralysis
Congenital (autosomal dominant inheritance)
Complicating thyrotoxicosis (particularly in Chinese males)
Barium-induced hypokalemia, acute chloroquine intoxication
Maturation of red cell precursors after treatment of megaloblastic anemia with vitamin B_{12} or folic acid
Paraneoplastic hypokalemia secondary to increased cell synthesis in acute myeloid leukemia[a]
– **True potassium depletion (= total K^+ body content reduced)**
Extrarenal "conditions"
Prolonged poor potassium intake, protein-energy malnutrition
Gastrointestinal conditions
Gastric (associated with alkalosis): Vomiting, nasogastric suction
Small bowel
Associated with acidosis: biliary drainage, intestinal fistula, malabsorption, diarrhea (including diarrhea associated with AIDS), radiation enteropathy
Associated with alkalosis: Congenital chloride diarrhea
Large bowel
Associated with acidosis: uretero-sigmoidoscopy
Acid-base balance unpredictable: bowel cleansing agents, laxatives, clay ingestion, potassium binding resin ingestion
Sweating, full thickness burns

(continued)

Table 34.8 (continued)

Cystic fibrosis
Dialysis
Renal "conditions"
Interstitial nephritis, post-obstructive diuresis, recovery from acute renal failure
With metabolic acidosis: renal tubular acidosis (type I or II), carbonic anhydrase inhibitors (e.g.: acetazolamide and topiramate), amphotericin B, outdated tetracyclines
With metabolic alkalosis
Inherited conditions: Bartter syndromes, Gitelman syndrome and related syndromes
Acquired conditions: normotensive primary aldosteronism**,** loop and thiazide diuretics, high dose antibiotics (penicillin, naficillin, ampicillin, carbenicillin, ticarcillin), magnesium depletion
Acid-base balance unpredictable: cetuximab
• **Hypokalemia associated with high blood pressure (often linked with metabolic alkalosis; total K^+ body content normal)**
Low renin: primary aldosteronism (either hyperplasia or adenoma), apparent mineralocorticoid excess (= defect in 11-β-hydroxysteroid-dehydrogenase), Liddle syndrome (congenitally increased function of the collecting tubule sodium channels), dexamethasone-responsive aldosteronism (synthesis of aldosterone promoted not only by renin but also by adrenocorticotropin), congenital adrenal hyperplasia (11-β-hydroxylase or 17-α-hydroxylase deficiency), Cushing disease, exogenous mineralocorticoids, licorice-ingestion (= 11-β-hydroxysteroid-dehydrogenase blockade)
Normal or high renin: renal artery stenosis, malignant hypertension, renin producing tumor

[a] The pathogenic mechanism includes also hyperkaluresis due to activation of the renin-angiotensin II system

Occasionally, metabolically active cells (in the blood sample) take up K^+ after blood has been drawn and before it has been tested in the laboratory. This condition, which has been called **spurious hypokalemia**, has been noted in patients with acute myeloid leukemia associated with a very high white blood cell count and in hot weather. The problem of spurious hypokalemia, which is much less common than spurious hyperkalaemia, can be avoided if plasma (or serum) is rapidly separated from the cells or if the blood is stored at 4 °C.

Normal total body K^+ content with hypokalemia results from an **increased shift of this ion into cells**. Metabolic alkalosis, increased endogenous secretion or exogenous administration of insulin, sympathetic activation and exogenous administration of β_2-adrenergic receptors are the main causes of hypokalemia caused by cellular uptake of K^+. Hypokalemic periodic paralysis is an uncommon form of hypokalemia resulting from an increased shift of K^+ into cells, which is characterized by recurrent episodes of hypokalemia (associated with hypophosphatemia and mild hypomagnesemia) and muscular weakness or paralysis that occurs primarily in males of Asian descent. The hypokalemic episodes are precipitated by rest after exercise, a carbohydrate meal or the administration of insulin or β-adrenergic agonists.

The K^+ stores may be depleted when **dietary K^+ is very low** and therefore fails to counterbalance the obligatory K^+ losses and, in infancy and childhood, the required K^+ accretion. Since the kidney is able to lower K^+ excretion to very low figures in the presence of K^+ depletion, decreased intake alone will cause hypokalemia only in rare cases. However, it contributes to the severity of K^+ depletion when another problem is superimposed. Under normal circumstances the net fluid loss from the skin and the gastrointestinal tract is small, therefore preventing the development of K^+ depletion. Sometimes, however, in cases such as prolonged exertion in hot, dry environment, in cystic fibrosis (in these patients sweat contains large amounts of Na^+, K^+ and Cl^-), and especially in the context of various gastrointestinal conditions (Table 34.8) K^+ loss occurs. In most of these cases extracellular volume depletion is present also, leading to secondary activation of the renin-angiotensin II-aldosterone system, and further worsening the K^+ deficiency. It has even been noted that in some patients with diarrheal states, an increased urinary K^+ excretion plays a more important role than intestinal losses in the development of K^+ deficiency. Hypokalemia is mostly

associated with metabolic alkalosis after poor dietary K^+ intake, in the context of "upper" gastrointestinal conditions or in conditions associated with increased sweating and with acidosis in "lower" gastrointestinal conditions. Finally, **renal K^+ losses** occur either associated with acidosis or, more frequently, with alkalosis.

Excessive mineralocorticoid activity is the main cause of hypokalemia associated with metabolic alkalosis and arterial hypertension. The underlying mechanism will be discussed elsewhere.

Clinical Work Up

The clue to the diagnosis of spurious hypokalemia is a normal electrocardiogram without the characteristic changes (Fig. 34.9). Considering that the great majority of children with true hypokalemia have either a gastrointestinal condition or take drugs associated with renal K^+ wasting, the causes of hypokalemia can almost always be discerned clinically. When the data obtained from the clinical history fail to establish a presumptive diagnosis, the following simple steps are suggested:

- Repeated measurement of blood pressure;
- Concurrent determination of the acid-base balance, Na^+, Cl^-, Ca^{++}, Mg^{++}, inorganic phosphate, alkaline phosphatase, uric acid, and especially urea and creatinine;
- In normotensive subjects the molar urinary potassium/creatinine ratio distinguishes hypokalemia due to a short-term shift of K^+ into cells (ratio <2.5 mol/mol) from hypokalemia resulting from a deficit of the ion;
- In normotensive subjects with hypokalemia and metabolic alkalosis the urinary chloride/creatinine ratio discriminates renal (ratio >10 mol/mol) from non-renal causes (ratio <10 mol/mol).

Management

Considering the numerous origins of hypokalemia, this section will focus exclusively on the urgency and the mode of substitution in patients with normotensive hypokalemia [24–26].

- The urgency for substitution is dictated by following factors: (a) Conditions that increase the likelihood of dangerous cardiac arrhythmias (and/or concurrent cardiac pathologies reducing the hemodynamic tolerance of arrhythmias); (b) The possibility that K^+ will shift into cells (e.g.: during recovery from diabetic ketoacidosis); (c) Severe muscle weakness in a child who must intensively hyperventilate because of metabolic acidosis, (d) Magnitude of the ongoing K^+ losses (e.g.: during severe diarrhea); and (e) Degree of hypokalemia.
- Potassium preparations
  - Potassium chloride: preferred in metabolic alkalosis due to diuretic therapy or vomiting;
  - Potassium citrate or potassium bicarbonate: prescribed in hypokalemia and metabolic acidosis (typically renal tubular acidosis);
  - Potassium phosphate: administered in the recovery from diabetic ketoacidosis, in subjects at risk of refeeding syndrome and during total parenteral nutrition.
- The concurrent intravenous administration of K^+Cl^- with glucose or bicarbonate is not advised in patients with severe hypokalemia, because glucose and bicarbonate cause a shift of K^+ into cells and transiently reduce circulating K^+ concentration.
- Route of administration. The safest way to administer K^+ is by mouth. Intestinal conditions that limit intake or absorption of K^+, severe hypokalemia (<2.5 mmol/L), characteristic electrocardiogram abnormalities (with or without cardiac arrhythmias) or respiratory muscle weakness and an anticipated shift of K^+ into cells mandate intravenous substitution.
- Intravenous K^+Cl^-
  - Bolus of K^+Cl^- is recommended exclusively in very severe degree of hypokalemia and abnormal electrocardiogram. The aim will be to raise K^+ to 3.0 mmol/L in

1–2 min.[3] The amount of intravenous K^+Cl^- (in mmol) will be chosen from measured K^+ (in mmol/L) and body weight (in kg) using the formula: (3.0 − measured K^+) × body weight × 0.04. Following this bolus, the rate of infusion of K^+ should be reduced to 0.015 mmol/kg body weight per minute, and measurement of K^+ concentration should be repeated each 5–10 min.

N.B.: The K^+Cl^- supplementation should be minimal if hypokalemia is due exclusively to an abnormal distribution of the K^+ stores (e.g.: exogenous administration of β_2-adrenergic receptors or hypokalemic periodic paralysis).

- In conditions demanding intravenous K^+ but without any acute emergency the rate of infused K^+ should not exceed 0.5–1.0 mmol/kg body weight hourly. Furthermore the K^+ concentration in intravenous solutions should be less than 40 mmol/L for use in peripheral veins because higher concentrations lead to local discomfort, venous spasm and sclerosis.
- Parenteral supplemental K^+ administration is the most common cause of severe hyperkalemia. Consequently, the safest route to give K^+ is by mouth. A traditional approach to minimizing hypokalemia is to ensure adequate dietary K^+ intake (unfortunately the K^+ contained in foods that have a high K^+ content is almost entirely coupled with phosphate rather than with Cl^- and therefore is not effective in repairing K^+-loss associated with Cl^--depletion, including use of diuretics, vomiting or nasogastric drainage). In most circumstances, oral replacement with K^+Cl^- (1–3 mmol/kg body weight daily in divided doses) is effective in correcting hypokalemia.

[3]The basis for this decision is as follows: the total blood volume approximates 7% of body weight (this volume circulates each minute, cardiac output being at least 70 mL/min/kg body weight) and plasma volume 60% of blood volume, i.e. approximately 4% of body weight. Consequently a 50.0-kg adolescent with a very severe hypokalemia of 1.5 mmol/L will be given (3.0–1.5) × 50 × 0.04 = 3.0 mmol of K^+Cl^- in 1–2 min. Considering that infused K^+ will mix with interstitial fluid (approximately 3–4 times the plasma volume) before reaching cell membrane, there will be a much smaller increase in K^+ concentration near cell membranes.

Hyperkalemia

The clinician evaluating a child with hyperkalemia (>5.5 mmol/L) will initially consider the possible diagnosis of **spurious hyperkalemia** (Table 34.9) [21–23, 27–29]. The term refers to those conditions in which the elevation in the measured K^+ is due to K^+ movement out of the cells during or after the blood specimen has been drawn. The major cause of this common problem is mechanical trauma during venipuncture, resulting in the release of K^+ from red cells and a characteristic reddish tint of the serum (or plasma) due to the release of hemoglobin. A normal electrocardiogram without the characteristic signs (Fig. 34.9) is the initial clue to this diagnosis.

In very rare instances, however, red serum (or red plasma) represents severe intravascular hemolysis rather than a hemolyzed specimen.

Furthermore, spurious hyperkalemia can also occur in hereditary spherocytosis and in familial pseudohyperkalemia, a rare autosomal dominant disorder recognized as a laboratory artifact. In the circulation, the Na^+ and K^+ content of red cells is normal. However, the measured plasma or serum K^+ concentration is elevated because of an abnormally high rate of efflux of K^+ from the red cells when the temperature is lowered below 22 °C. The in vitro K^+ efflux can be reversed by incubation at 37 °C.

K^+ also moves out of white cells and platelets after clotting has occurred. Thus, the serum K^+ concentration normally exceeds the true value in the plasma by 0.1–0.5 mmol/L. Although in normal individuals this difference is clinically insignificant, the measured serum K^+ concentration may be as high as 9 mmol/L in patients with marked leukocytosis or thrombocytosis. Spurious hyperkalemia is suspected whenever there is no apparent cause for the elevation in the serum K^+ concentration in an asymptomatic patient.

True hyperkalemia (Table 34.9) occurs rarely in healthy subjects, because cellular and urinary adaptations prevent substantial extracel-

Table 34.9 Causes of hyperkalemia (drugs associated with hyperkalemia appear in Table 34.10)

• **Spurious hyperkalemia** (potassium movement out of the cells during or after blood has been drawn)
– Mechanical trauma during venipuncture
– Hereditary spherocytosis
– Familial pseudohyperkalemia
• **True hyperkalemia**
– **Increased potassium load**[a]
– **Increased shift of potassium out of cells**
Normal anion gap metabolic acidosis
Insulin deficiency
Extracellular hypertonicity
Increased tissue catabolism (severe hemolysis, rhabdomyolysis, tumor lysis syndrome, immediately after cardiac surgery)
Severe exercise
Familial hyperkalemic periodic paralysis (= Gamstorp disease)
Hyperkalaemia of the premature infant
– **Impaired renal potassium excretion**
Global renal failure: acute or chronic
Hyperreninemic hypoaldosteronism: adrenal insufficiency (= Addison disease), salt-losing congenital adrenal hyperplasia (21-hydroxylase deficiency)
Hyporeninemic hypoaldosteronism: idiopathic, complicating acute glomerulonephritis or mild-to-moderate renal failure)
Pseudohypoaldosteronism
Type 1 (cortical collecting tubule)
Primary
Autosomal recessive[b]: reduced sodium channel activity
Autosomal dominant: mutations in the gene for the mineralocorticoid receptor, phenotype mild and transient
Secondary: complicating obstructive uropathy (with or without urinary tract infection), systemic lupus erythematosus, sickle cell disease, renal transplantation, renal amyloidosis
Type 2 (= Familial hyperkalemic hypertension or Gordon syndrome[c])

[a] Not a cause of hyperkalemia, unless very acute (and important) or occurring in subjects with impaired potassium excretion (due, for example, to underlying kidney disease)
[b] Autosomal recessive pseudohypoaldosteronism type 1 is opposite to Liddle syndrome
[c] The clinical phenotype of Gordon syndrome is opposite to Gitelman syndrome

lular K^+ accumulation. Furthermore, the efficiency of K^+ handling is increased if K^+ intake is enhanced, thereby tolerating what might be a fatal K^+ load. These observations lead to the following conclusions concerning the development of hyperkalemia:

(a) **Increasing K^+ load** is not a cause of hyperkalemia, unless very acute or occurring in a patient with impaired urinary K^+ excretion. In special conditions, acute hyperkalemia can be induced (primarily in infants because of their small size) by the intravenous administration of unusual large doses of K^+ or the use of stored blood for transfusions.
(b) The net **release of K^+ from the cells** can cause a transient elevation in the circulating K^+ concentration (in the presence of normal or even low total body K^+ stores). Four causes of hyperkalemia resulting from release of the ion from cells will be discussed: (a) extracellular hypertonicity; (b) tumor lysis syndrome; (c) hyperkalemic familial periodic paralysis; and d. non-oliguric hyperkalaemia of the premature infant.

An *elevated extracellular tonicity* results in water movement from the cells into the extracellular fluid. This is linked with K^+ movement out of the cells by two mechanisms. First, the loss of water raises the intracellular K^+ level, creating a gradient for passive K^+ exit. Second, the friction forces between water and solute result in K^+ being carried along with water. Hypertonicity-induced hyperkalemia occurs in

hyperglycemia, in hypernatremia, and following administration of mannitol.

The phenotype of acute *tumor lysis syndrome* is opposite to refeeding syndrome. The term denotes the metabolic abnormalities that occur either spontaneously or immediately after initiation of cytotoxic therapy in neoplastic disorders. The findings include hyperuricemia, hyperkalemia, hyperphosphatemia, hypocalcemia (due to precipitation of calcium phosphate), and acute renal failure. The syndrome has been noted in children with a tumor characterized by rapid cell turnover such as lymphomas (particularly non-Hodgkin lymphoma) and some leukemias.

Hyperkalemic familial periodic paralysis, or Gamstorp disease, is a rare inherited autosomal dominant disease that causes patients to experience episodes of flaccid weakness[4] associated with increased K^+ levels. In this syndrome, hyperkalemia occurs with increased K^+ intake, cold weather, exercise or at rest. The attacks of paralysis, however, are not always linked with hyperkalemia.

Non-oliguric hyperkalemia (>6.5 mmol/L) of the premature infant is a common and serious condition. The features are a rapid rise of K^+ concentration to excessively high values at 24 h after birth, a tendency towards cardiac arrhythmia, and occurrence only within 72 h after birth exclusively in premature infants. This peculiar condition mainly results from a K^+ loss from the intra- into the extracellular space. Moreover, renal K^+ excretion that is dependent on both glomerular filtration rate and urinary output is slightly decreased in this setting. Finally, aldosterone unresponsiveness, rather than a decreased concentration of aldosterone, also contributes to the degree of hyperkalemia. However, since there is no significant K^+ intake during the first days of life of premature infants, even total absence of renal K^+ excretion cannot increase K^+ concentration, if there is no intra- to extracellular K^+ shift.

(c) Persistent hyperkalemia requires an **impaired urinary K^+ excretion** (the total body K^+ stores are increased in this condition). Two factors modulate renal K^+ homeostasis in the cortical collecting tubule: "hyperkalemia" and aldosterone, which act in concert to promote the tubular secretion and therefore the excretion of K^+, and the flow rate traversing the cortical collecting tubule. Consequently, a decreased aldosterone release or effect or a decreased renal tubular flow rate are the major conditions impairing urinary K^+ excretion.

- *Reduced aldosterone secretion or effect*: Any cause of decreased aldosterone release or effect can diminish the efficiency of K^+ secretion and lead to hyperkalemia. The ensuing tendency towards hyperkalemia directly stimulates K^+ secretion, partially overcoming the relative absence of aldosterone. The net effect is that the rise in the K^+ concentration is generally small in patients with normal renal function, but can be clinically important in the presence of underlying renal insufficiency or with multiple insults.
- *Decreased renal tubular flow rate*: The ability to maintain K^+ excretion at near normal levels is habitually preserved in advanced renal disease as long as both aldosterone secretion and distal flow are maintained. Thus, hyperkalemia generally develops in oliguria or in the presence of an additional problem including high K^+ diet, increased tissue breakdown or reduced aldosterone bioactivity. Impaired cell uptake of K^+ also contributes to the development of hyperkalemia in advanced renal failure. Decreased distal tubular flow rate due to marked effective volume depletion, as in heart failure or "salt-losing" nephropathy, can also induce hyperkalemia.
- Acute and chronic renal failure, the most recognized causes of impaired urinary K^+ excretion, will be discussed elsewhere (See Chaps. 51 and 58). Hyperkalemia

[4]Please note that hyperkalemic skeletal muscle paralysis results from hyperkalemia of any cause, rather than this rare inherited disorder.

and a tendency towards hyponatremia and metabolic acidosis characteristically occur in children with hyperreninemic hypoaldosteronism (including classic congenital adrenal hyperplasia), hyporeninemic hypoaldosteronism, and end-organ resistance to aldosterone, mostly referred to as pseudohypoaldosteronism. In North-America, Japan, and most European countries neonatal screening (measurement of 17-hydroxy-hydroxyprogesterone in filter paper blood) identifies children affected by classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency before salt-losing crises with hyperkalemia develop. Consequently, in these countries classic congenital adrenal hyperplasia is nowadays an uncommon cause of hyperkalemia. In our experience, secondary type 1 pseudohypoaldosteronism is, together with advanced renal failure, a common cause of true hyperkalemia, at least in infancy. Secondary type 1 pseudohypoaldosteronism develops in infants with urinary tract infections, in infants (but not older children) with urinary tract anomalies (either obstructive or vesicoureteral reflux), and especially in infants with both urinary tract infections and urinary tract anomalies [30].

– Finally, the syndrome of (acquired) *hyporeninemic hypoaldosteronism*, which is characterized by mild hyperkalemia and metabolic acidosis, is due to diminished renin release and, subsequently, decreased angiotensin II and aldosterone production. The syndrome, which mostly occurs in subjects with mild renal failure, has been first reported in subjects with overt diabetic kidney disease and has been occasionally noted in children with acute glomerulonephritis or mild-to-moderate chronic renal failure.
– Prescribed medications, over-the-counter drugs, and nutritional supplements may disrupt K^+ balance and promote the development of hyperkalemia, as shown in Table 34.10. Although most of these prod-

Table 34.10 Drugs that have been associated with hyperkalemia

Medication	Mechanism of action
Increased K^+ input	K^+ ingestion or infusion
K^+ supplements (and salt substitutes)	
Nutritional and herbal supplements	
Stored packed red blood cells	
Potassium containing penicillins	
Transcellular K^+ shifts	
β-adrenergic receptor antagonists	↓ β_2-driven K^+ uptake
Intravenous amino acids (Lysine, Arginine, Aminocaproic acid)	↑ K^+ release from cells
Succinylcholine	Depolarized cell membranes
Digoxin intoxication	↓ Na^+-pump
Impaired renal excretion	
Potassium sparing diuretics	
Spironolactone, eplerenone	Aldosterone antagonists
Triamterene, amiloride	Na^+ channels blocked (collecting tubule)
Trimethoprim[a], pentamidine	Na^+ channels blocked (collecting tubule)
Nonsteroidal anti-inflammatory drugs	↓ Aldosterone synthesis, ↓ glomerular filtration rate, ↓ renal blood flow
Blockers of the renin-angiotensin II-aldosterone system (converting enzyme inhibitors, angiotensin II-antagonists, renin inhibitors)	↓ Aldosterone synthesis
Heparins (both unfractionated and low molecular weight heparins)	↓ Aldosterone synthesis
Calcineurin inhibitors (e.g.: cyclosporineand tacrolimus)	↓ Aldosterone synthesis, ↓ Na^+-pump, ↓ K^+-channels

[a] Including cotrimoxazole, the fixed combination of trimethoprim with sulfomethoxazole

ucts are well tolerated, drug-induced hyperkalemia may develop in subjects with underlying renal impairment or other abnormalities in K^+ handling or with concurrent, combined use of multiple drugs potentially inducing hyperkalemia. Their hyperkalemic action is less evident in children than in elderly subjects.

Clinical Work Up

The clue to the diagnosis of spurious hyperkalemia, the most common cause of elevated K^+ levels in clinically asymptomatic infants and children, is a normal electrocardiogram without the characteristic changes.

Considering that the great majority of children with true hyperkalemia have renal failure (either acute or chronic), secondary type 1 pseudohypoaldosteronism or take drugs that can cause hyperkalemia, the cause of hyperkaemia can mostly be discerned clinically. When the data obtained from the clinical history fail to establish a presumptive diagnosis, the following simple steps are suggested [21–23, 27–29]:

- Repeated measurement of blood pressure;
- Concurrent determination of the acid-base balance, Na^+, Cl^-, Ca^{++}, Mg^{++}, inorganic phosphate, alkaline phosphatase, uric acid, and especially urea and creatinine;
- In subjects with true hyperkalemia unrelated to an impaired urinary K^+ excretion, the expected molar urinary potassium/creatinine ratio is >20 mol/mol and the transtubular potassium gradient >10.

Management

Because many conditions account for true hyperkalemia, there is no universal therapy for this dyselectrolytemia. The following measures deserve consideration upon recognition of hyperkalemia with increased total body K^+ stores: (a) Interruption of excessive dietary K^+ intake; (b) Discontinuation of drugs that may cause hyperkalemia; (c) Increasing renal K^+ excretion (for this purpose children without end-stage renal failure or physical signs of fluid overload must have substantial salt intake[5] via oral or parenteral routes; the use of a loop diuretic, less frequently a thiazide diuretic, also increases renal K^+ excretion); (d) Increasing gastrointestinal K^+ excretion using cation exchange resins; and (e) Institution of dialysis in children with end-stage renal failure.

Emergencies: Because of the serious deleterious cardiac effects, severe hyperkalemia (≥7.1 mmol/L) with electrocardiographic abnormalities requires emergency intervention. The following measures, which are listed according to their rapidity of action, have been recommended:

(a) Intravenous Ca^{++}, which directly antagonizes the membrane actions of hyperkalemia;
(b) Intravenous insulin (and glucose), which lowers extracellular K^+ level by driving K^+ into cells;
(c) Intravenous or nebulised $ß_2$-adrenergic agonists, which, like insulin, drive K^+ into cells; and
(d) Intravenous $NaHCO_3$, which results in H^+ ion release from cells (as part of the buffering reaction), a change that is accompanied by K^+ movement into cells to preserve electroneutrality.

Available data indicate (1) that nebulized (or inhaled) albuterol, a $ß_2$-adrenergic agonist, or intravenous insulin (and glucose) are the best supported recommendations; (2) that their combination may be more effective than either alone; (3) that although there are no properly conducted studies assessing the efficacy of Ca^{++}, there remains little doubt of its effectiveness in treating or preventing arrhythmias; and (4) that evidence for the use of intravenous $NaHCO_3$ is equivocal. For practical purposes, the emergency interventions given in Table 34.11 are advised.

[5] A low salt intake and extracellular fluid volume depletion are the most commonly observed contributing factors in the development of hyperkalemia in children with renal failure. In patients with a salt-retaining disease, proper management is achieved by avoiding severe restriction of dietary salt while concurrently administering diuretics.

Table 34.11 Currently recommended emergency intervention for severe hyperkalemia (≥7.1 mmol/L) with electrocardiogram abnormalities. Albuterol and intravenous glucose (with insulin) lowers extracellular potassium level by driving potassium into cells, while calcium directly antagonizes the membrane actions of hyperkalemia but does not modify extracellular potassium concentration. None of the recommended emergency interventions modifies the total body potassium content

Medication	Dosage	Onset (min)	Length of effect (h)	Comments—Cautions
Nebulized albuterol	10–20 mg (diluted in 4 mL of saline) over 10 min	15–30	4–6	May increase heart rate
Intravenous albuterol	10 μg/kg body weight over 15 min	15–30	4–6	May increase heart rate
Glucose and insulin	Glucose 0.5–1.0 g/kg body weight and insulin 0.1 U/kg body weight intravenously over 15 min	15–30	4–6	Tendency towards hypoglycemia (monitor blood glucose level)
Intravenous calcium	20–50 mg/kg body weight over 1–3 min	Immediate	½–1	Does not lower potassium level

Acid-Base Balance

Introduction

Maintenance of acid-base balance within narrow limits (i.e. pH 7.00–7.30 within the cell depending on the cell type and tissue of origin and pH 7.35–7.45 in extracellular fluids) is a crucial function of the living organism, largely because of its effects on body proteins. The "pCO2-HCO_3^--pH"-based approach, popularized by Relman and Schwartz in the 1960s, relies upon the definition of pH in blood as a function of the ratio between the partial pressure of carbon dioxide (pCO_2; mmHg or kPa) and the bicarbonate (HCO_3^-; mmol/L or meq/L) concentration, as indicated by the (simplified) Henderson-Hasselbalch equation [31, 32]:

$$pH = pK + \frac{HCO_3^-}{pCO_2}$$

Arterial blood is the standard sample for the determination of acid-base balance but arterial blood sampling, which can be painful, is often unavailable in childhood. In this age group, acid-base balance is mostly assessed in arterialized capillary blood samples (from the finger pulp following hand warming during ≥10 min or the earlobe following spreading the lobe with a vasodilating cream during ≥10 min) or in venous blood samples. Normal values for peripheral venous blood differ from those of arterial blood due to the uptake and buffering of metabolically produced CO_2 in the capillary circulation. If a tourniquet is used to facilitate phlebotomy, it should be released about one minute before blood is drawn to avoid changes induced by ischemia. The peripheral venous pH range is ≈0.02–0.04 pH units lower than in arterial blood, the pCO_2 ≈3–8 mmHg higher and the HCO_3^- concentration ≈1–2 mmol/L lower. Automated blood gas analyzers measure pH and pCO_2, while the HCO_3^- concentration is calculated from the Henderson-Hasselbalch equation. Most currently available blood gas analyzers determine circulating L-lactate as well (the assay does not detect D-lactate). Abnormalities of blood pH result from a deviation in circulating bicarbonate (HCO_3^-; mmol/L) or in partial pressure of carbon dioxide (pCO_2; mmHg[6]). There are four primary disturbances of acid-base balance (Fig. 34.10). Since alveolar ventilation regulates pCO_2, any disturbance in pH that results from a primary change in pCO_2 is called respiratory acid-base disorder: retention of CO_2 leads to a reduction in pH (<7.35) called respiratory acidosis, a fall in pCO_2 leads to a rise in pH (>7.45) called respiratory alkalosis. On the other side, primary changes in the concentration of HCO_3^- are called metabolic acid-base disorders: a primary reduction in

[6]To obtain SI units (kPa) divide by 7.5.

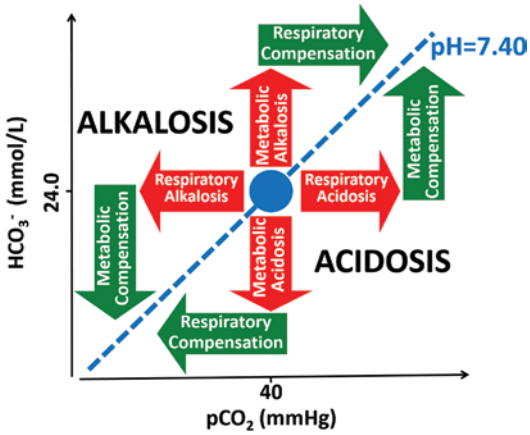

Fig. 34.10 Common sense diagram depicting the four primary disturbances of acid-base balance with the expected compensation. There are four primary disturbances (red arrows) of acid-base balance. Since ventilation modulates pCO_2, any disturbance in pH that results from a primary change in pCO_2 is called respiratory acid-base disorder: retention of CO_2 leads to a reduction in pH (<7.35) called respiratory acidosis, while a fall in pCO_2 leads to a rise in pH (>7.45) called respiratory alkalosis. On the other side, primary changes in the HCO_3^- concentration are called metabolic acid-base disorders: a primary reduction in HCO_3^- is termed metabolic acidosis and a primary increase in HCO_3^- is called metabolic alkalosis. Since blood pH is determined by the ratio between HCO_3^- and pCO_2, and not either one alone, primary respiratory (primary changes in pCO_2) disturbances invoke compensatory metabolic (secondary changes in HCO_3^-) responses (green arrows), and primary metabolic (primary changes in HCO_3^-) disturbances elicit compensatory respiratory (secondary changes in pCO_2) responses (green arrows)

HCO_3^- is termed metabolic acidosis and a primary increase in HCO_3^- is called metabolic alkalosis.

Blood pH is determined by the ratio between HCO_3^- and pCO_2, not either one alone. Thus, primary respiratory disturbances (primary changes in pCO_2) invoke compensatory metabolic responses (secondary changes in HCO_3^-), and primary metabolic disturbances (primary changes in HCO_3^-) elicit compensatory respiratory responses (secondary changes in pCO_2). For instance, metabolic acidosis due to an increase in endogenous acids (e.g., ketoacidosis) lowers extracellular fluid HCO_3^- and decreases extracellular pH. This stimulates the medullary chemoreceptors to increase the ventilation in an attempt to return the ratio of HCO_3^- to pCO_2, and thus pH, towards normal. The physiologic metabolic and respiratory compensations to simple primary acid-base disturbances can be guessed from the relationships displayed in Table 34.12.

"Base excess" and "standard HCO_3^-" are *in vitro* generated parameters of the acid-base balance that are of little value and often even misleading [31, 32].

Systemic Effects of Metabolic Acid-Base Abnormalities

The systemic effects of acid-base abnormalities will be briefly addressed below [31, 32].

Respiratory System

Primary metabolic acid-base balance disturbances (primary changes in HCO_3^-) elicit compensatory respiratory responses (secondary changes in pCO_2). Metabolic acidosis (=primary reduction in HCO_3^-) stimulates the ventilation to correct the ratio of HCO_3^- to pCO_2, and thus pH, towards normal (Fig. 34.10). The rise in ventilation occurs within minutes but may take several hours to reach its fullest expression. The increase is more the result of increased tidal volume than respiratory rate. This degree of hyperventilation, called Kussmaul respiration, may cause some dyspnea and be appreciated on physical examination. In a child with simple metabolic acidosis, the pCO_2 is expected to decrease by 1.3 mmHg for each mmol per liter decrease in HCO_3^- (Table 34.12), reaching a minimum of 10–15 mmHg. Thus, a patient with metabolic acidosis and HCO_3^- of 16.0 mmol/L would be expected to have a pCO_2 of ≈30 mmHg, i.e. between 27 and 33 mmHg. Values for pCO_2 <27 or >33 mmHg define a mixed disturbance (metabolic acidosis and respiratory alkalosis or metabolic acidosis and respiratory acidosis, respectively).

Primary metabolic alkalosis (= primary increase in HCO_3^-) may lead to compensatory hypoventilation and consequent CO_2 retention. Uncomplicated metabolic alkalosis is usually not associated with profound alveolar hypoventilation. Metabolic alkalosis should be repaired

Table 34.12 Predicted compensations to simple primary acid-base disturbances

Disorder	Primary change	Compensatory response
Metabolic acidosis	↓ HCO_3^-	↓ pCO_2 by 1.3[a] mmHg for ↓ 1.0 mmol/L[b] in HCO_3^-
Metabolic Alkalosis	↑ HCO_3^-	↑ pCO_2 by 0.6[a] mmHg for ↑ 1.0 mmol/L[b] in HCO_3^-
Respiratory Acidosis	↑ pCO_2	
Acute		↑ 1.0 mmol/L[c] in HCO_3^- for ↑ 10 mmHg[d] in pCO_2
Chronic		↑ 3.5 mmol/L[c] in HCO_3^- for ↑ 10 mmHg[d] in pCO_2
Respiratory Alkalosis	↓ pCO_2	
Acute		↓ 2.0 mmol/L[c] in HCO_3^- for ↓ 10 mmHg[d] in pCO_2
Chronic		↓ 5.0 mmol/L[c] in HCO_3^- for ↓ 10 mmHg[d] in pCO_2

[a] Range approximately ±3 mmHg
[b] From 25 mmol/L
[c] Range approximately ±2.0 mmol/L
[d] From 40 mmHg

before surgery (e.g.: before pyloromyotomy in infantile hypertrophic pyloric stenosis) because this acid-base abnormality predisposes to respiratory depression in the immediate postoperative course.

Potassium Balance

There are major interactions between the internal K^+ balance and acute metabolic acid-base changes. In patients with normal anion gap metabolic acidosis the excess hydrogen ions are buffered in the cell and electroneutrality is maintained by movements of intracellular K^+ into the extracellular fluid. Interestingly, metabolic acidosis is much less likely to raise the extracellular K^+ concentration in patients with high anion gap acidosis like L-lactate acidosis or ketoacidosis. The underlying mechanisms are briefly explained in Fig. 34.11 (upper panel). For similar reasons, some tendency towards hypokalemia is noted in metabolic alkalosis (Fig. 34.11, lower panel). Respiratory acidosis and alkalosis do not significantly modulate K^+ balance.

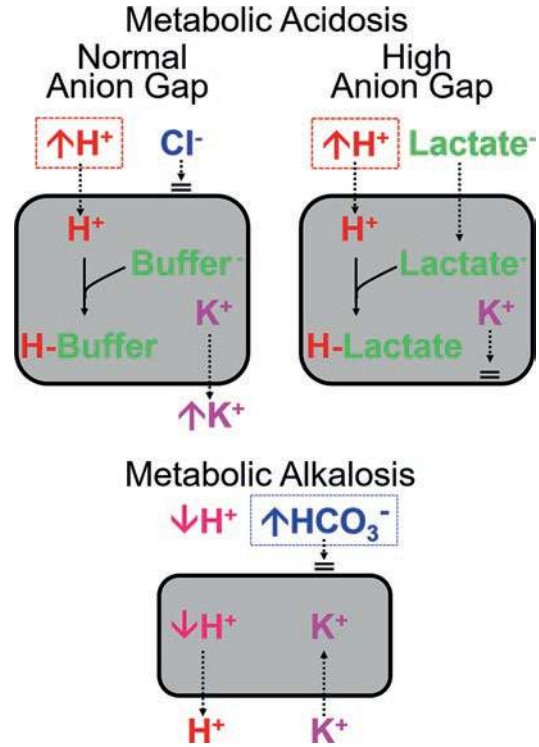

Fig. 34.11 Effect of metabolic acidosis or alkalosis on circulating potassium level. Both in normal (= hyperchloremic) and high (= normochloremic) anion gap metabolic acidosis some extracellular H^+ shifts into the intracellular fluid volume (the squares denote the cell membrane). In normal anion gap (left upper panel) metabolic acidosis, Cl^- remains largely in the extracellular fluid volume. On the contrary, in high anion gap (right upper panel) metabolic acidosis (e.g.: L-lactate acidosis) some organic anions enter the intracellular fluid. Hence, a tendency towards hyperkalemia, the consequence of a shift of K^+ from the intracellular to the extracellular fluid volume, occurs almost exclusively in normal anion gap metabolic acidosis only. Please note that hyperkalemia is followed by a stimulated aldosterone release and results in the urinary excretion of the extra K^+. No tendency towards hyperkalemia occurs in respiratory acidosis. In metabolic alkalosis (lower panel) some intracellular H^+ shifts into the extracellular fluid volume. Hence, a tendency towards hypokalemia, the consequence of a shift of K^+ from the extracellular to the intracellular fluid volume, occurs. No tendency towards hypokalemia occurs in respiratory alkalosis

Ca^{++} (and Mg^{++}) Balance

Acid-base disorders affect circulating Ca^{++}, Mg^{++}, inorganic phosphate, and K^+. Acidemia increases calcium phosphate dissociation, increasing free (ionized) Ca^{++}. Acidemia also allows greater dis-

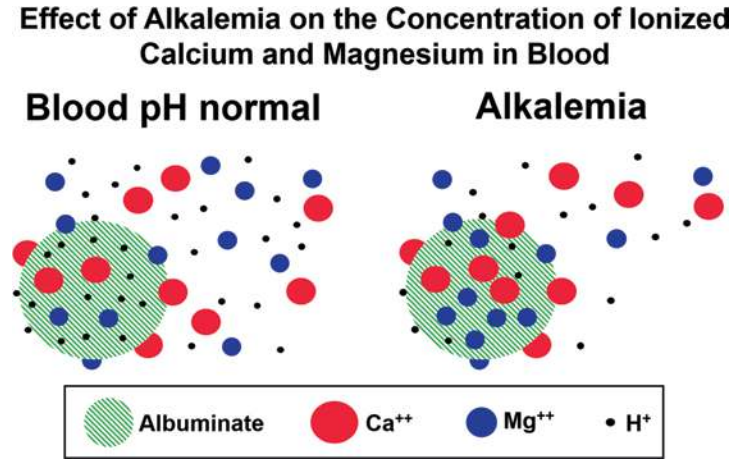

Fig. 34.12 Effect of Alkalemia on the concentration of ionized calcium and magnesium in blood. In the context of alkalemia (e.g.: hyperventilation) blood H^+ concentration decreases. As a consequence, freely ionized calcium and magnesium concentrations decrease. The opposite is observed in the context of acidemia

sociation of Ca^{++} and Mg++ from plasma protein. The effect of acidosis on Ca^{++} salt dissociation extends to the bone. Alkalemia might increase calcium phosphate precipitation and lowers ionized Ca^{++} and Mg^{++} (the underlying mechanisms are explained in Fig. 34.12).

Hemoglobin Oxygen Affinity

Blood pH alters hemoglobin oxygen binding and tissue oxygen delivery. Acidemia decreases hemoglobin oxygen affinity, shifts the oxygen dissociation curve "to the right" and increases tissue delivery of oxygen (Bohr effect). On the contrary, alkalemia shifts the curve to the left, increasing the oxygen binding to hemoglobin and tending to decrease tissue delivery.

Cardiovascular System

Acidemia impairs cardiovascular function in four ways: (a) it depresses vascular tone; (b) it alters the release of, and the response to, catecholamines; (c) it depresses myocardial contractility inducing diastolic dysfunction; and (d) it induces arrhythmias (in mild to moderate acidemia, increased catecholamines produce sinus tachycardia; when acidemia is severe, vagal activity increases and bradycardia ensues; there is also an increased risk of ventricular fibrillation). Alkalemia exerts fewer effects on the cardiovascular system. The predominant clinical problem is an increase in myocardial irritability. Alkalemia reduces the free Ca^{++} and Mg^{++} inside the cell and out, and most alkalemic patients are also hypokalemic. Changes in both ions contribute to the increased potential for arrhythmias.

Alkalemia has significant effects on vascular tone in the cerebral circulation: hypocarbia constricts the cerebral vasculature as indicated by the fact that subjects with respiratory alkalosis develop lightheadedness and lack of mental acuity, but coma does not occur.

Central Nervous System

Acidosis and alkalosis impair central and peripheral nervous system function. Alkalemia increases seizure activity. If pH is $\geq$7.60, seizures may occur in the absence of an underlying epileptic diathesis. Acidosis depresses the central nervous system (this most frequently occurs in respiratory acidosis). Early signs of impairment include tremors, myoclonic jerks, and clonic movement disorders. At pH $\leq$7.10, there is generalized depression of neuronal excitability. Central effects of severe hypercarbia include lethargy and stupor at pCO_2 60 mmHg or more, coma occurs at pCO_2 $\geq$90 mmHg. Metabolic acidosis causes central nervous system depression less commonly. Fewer than 10% of diabetics with ketoacidosis develop coma (hyperosmolarity and the presence of acetoacetate may be more important than acidosis per se).

Metabolism

A final aspect of acid-base pathophysiology is the effect of pH on metabolism. The most often

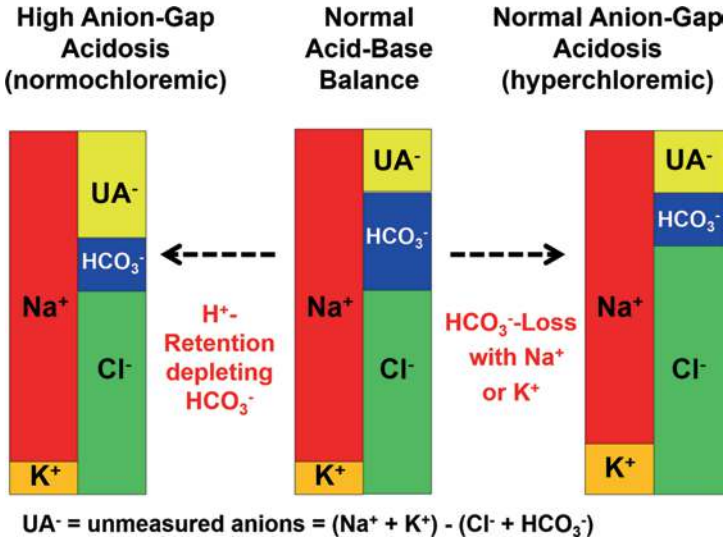

Fig. 34.13 High anion gap (= normochloremic) and normal anion gap (= hyperchloremic) metabolic acidosis. Calculation of the blood anion gap, the difference between the major measured cations (Na^+ and K^+; mmol/L) and the major measured anions (HCO_3^- and Cl^-; mmol/L), is a crucial laboratory diagnostic tool in patients with metabolic acidosis. The blood anion gap separates two major types of metabolic acidosis. High anion gap (= normochloremic) metabolic acidosis results from retention of fixed acids, which deplete HCO_3^- stores by releasing their protons (most cases develop following excessive endogenous or exogenous acid load). Most cases of normal anion gap (= hyperchloremic) metabolic acidosis result from an intestinal or a renal loss of HCO_3^- (accompanied either by Na^+ or K^+). The figure also emphasizes the tendency towards hyperkalemia (the result of a K^+-shift from the intracellular to the extracellular fluid volume) in normal anion gap (hyperchloremic) metabolic acidosis (as explained in Fig. 34.11)

cited example of pH control of enzyme activity is the pH regulation of phosphofructokinase, which catalyzes a rate-controlling step in carbohydrate metabolism. Glycolysis terminates in lactic and pyruvic acid; and accumulation of these acids reduces pH. This is but one example of pH feedback. Most enzymes operate most effectively at a specific optimum pH. As pH varies from the optimum, enzyme activity changes. The integrated response of the individual enzyme alterations may serve to maintain or restore normal pH.

Metabolic Acidosis

Primary hypobicarbonatemia and, therefore, metabolic acidosis mostly occurs when endogenous acids are produced faster than they can be excreted, when HCO_3^- is lost from the body, or when exogenous acids are administered [31–35].

The main laboratory tool in metabolic acidosis is the calculation of the blood anion gap (Fig. 34.13), the difference between the major measured cations (Na^+ and K^+; mmol/L) and the major measured anions (bicarbonate and Cl^-; mmol/L) by means of the equation:

$$AG = \left(Na^+ + K^+\right) - \left(Cl^- + HCO_3^-\right)$$

Because electroneutrality must be maintained, the anion gap results from the difference between the unmeasured anions (primarily albumin, which is largely responsible for the normal anion gap, but also phosphate, sulfate, and organic anions such as lactate) and the remaining cations (Ca^{++} and Mg^{++}).

The calculation of blood anion gap (reference: ≤18 mmol/L[7]) allows separation of the two major types of metabolic acidosis: one type has an increased anion gap (>18 mmol/L; high anion

[7]The blood anion gap sometimes does not include the blood concentration of K^+: $Na^+ - (HCO_3^- + Cl^-)$. The approximate upper value of this anion gap is lower by 4 mmol/L: 14 mmol/L.

Table 34.13 Causes of metabolic acidosis

• **Metabolic acidosis with increased anion gap**
– Excessive acid load
Endogenous sources of acid (due to abnormal metabolism of substrates)
Ketoacidosis (largely β-hydroxybutyric acid)
Congenital organic acidemias (e.g.: methylmalonic acidemia and propionic acidemia)
L-lactate acidosis
Type A (impaired tissue oxygenation; e.g.: sepsis, hypovolemia, cardiac failure)
Type B (altered metabolism of L-lactate with normal tissue oxygenation in the context of a mitochondrial impairment)
Inherited metabolic diseases: either altered production of glucose from lactate or altered degradation of pyruvate derived from pyruvate
Thiamine deficiency
Drugs (e.g.: biguanides, antiretroviral agents)
Toxins (e.g.: ethanol)
Chronic diseases (mostly hepatic)
Overproduction of organic acids in the gastrointestinal tract (D-lactate)
Conversion of alcohols (methanol, ethylene glycol) to acids and poisonous aldehydes
– Defective renal excretion of acids due to generalized renal failure ("uremic acidosis")
• **Metabolic acidosis with normal anion gap**
– Losses of bicarbonate (HCO_3^-)
Intestinal: diarrhea, surgical drainage of the intestinal tract, gastrointestinal fistulas resulting in losses of fluid rich in HCO_3^-, patients whose ureters have been attached to the intestinal tract (the alkali of intestinal secretion is lost by titration with acid urine)
Urinary: carbonic anhydrase inhibitors (e.g.: acetazolamide), proximal renal tubular acidosis (= type 2)
– Failure to replenish HCO_3^- stores depleted by the daily production of fixed acids
Distal renal tubular acidosis (either classic, also called type 1, or type 4)
Diminished mineralocorticoid (or glucocorticoid) activity (adrenal insufficiency, selective hypoaldosteronism, aldosterone resistance)
Administration of potassium sparing diuretics (spironolactone, eplerenone, amiloride, triamterene)
– Exogenous infusions
Amino acids like L-arginine and L-lysine (during parenteral nutrition)
HCl or NH_4Cl
– Rapid administration of normal saline solution (= "dilutional" metabolic acidosis)

gap metabolic acidosis) and the other does not (normal anion gap metabolic acidosis or hyperchloremic metabolic acidosis), as shown in Fig. 34.13 and Table 34.13.

High Anion Gap Metabolic Acidosis

The HCO_3^- deficit observed in high anion gap metabolic acidosis results from retention of fixed acids, which deplete HCO_3^- stores by releasing their protons. Two mechanisms lead to this form of metabolic acidosis (Fig. 34.13 and Table 34.13): (a) excessive acid load (endogenous or exogenous) overwhelming the normal capacity to decompose or excrete the acid; and (b) diminished capacity to excrete the normal load of fixed acids in the context of renal failure.

In health, the blood anion gap is predominantly due to the net negative charge of albumin. Abnormally low serum albumin levels influence acid-base interpretation as calculated by the anion gap. For example, in a patient with increased production of endogenous acids, elevation of the anion gap may be masked by concurrent hypoalbuminemia. In this condition the anion gap corrected for albumin may be calculated by means of the following formula (albumin in g/L):

$$\left(Na^+ + K^+\right) - \left(Cl^- + HCO_3^-\right) + \frac{1}{4}\left(40 - \text{Albumin}\right)$$

Considering that many currently available blood gas analyzers determine circulating L-lactate, the determination of the albumin and lactate corrected anion gap has been recently suggested (upper reference: 15 mmol/L):

$$\left(Na^{+}+K^{+}\right)-\left(Cl^{-}+HCO_3^{-}+\text{Lactate}\right)+\frac{1}{4}\left(40-\text{Albumin}\right)$$

Normal Anion Gap Metabolic Acidosis (= "Hyperchloremic")

This form of metabolic acidosis develops (Fig. 34.13 and Table 34.13): (a) from a primary loss of HCO_3^-, (b) from the failure to replenish HCO_3^- stores depleted by the daily production of fixed acids (H^+: 1–3 mmol/kg body weight) in subjects with normal glomerular filtration rate, or (c) from the administration of exogenous acids (including the rapid administration of large volumes of normal saline solution and other Cl^- rich fluids).

The following factors account for the metabolic acidosis that is observed after administration of normal saline solution, which is called "dilutional" (or "chloride overload") acidosis: (a) Volume expansion, which results from infusion of normal saline, reduces the renal threshold for HCO_3^- leading to bicarbonaturia; (b) The infusion of normal saline with a Na^+ level almost identical to that of blood results in a relatively stable Na^+ level in blood. By contrast, the concentration of Cl^- in the infused solution, which is much higher than that of normal blood, leads to progressive hyperchloremia and hypobicarbonatemia.

Urine Net Charge

The kidney prevents the development of metabolic acidosis by modulating the HCO_3^- concentration in blood. This is done by (a) preventing loss of large amounts of filtered HCO_3^- (primarily a task of the proximal tubule, which may reclaim the filtered HCO_3^-) and (b) generating HCO_3^- (primarily a task of the distal tubule). The main mechanism by which the distal tubule generates HCO_3^- is the conversion of glutamine to NH_4^+, which is excreted in the urine, plus HCO_3^-, which is added to the blood. As a consequence, the urinary NH_4^+ excretion reflects the renal HCO_3^- generation, and the renal NH_4^+ excretion can be equated with HCO_3^- regeneration on a 1:1 basis. In a child with normal anion gap metabolic acidosis and normal renal mechanisms of acidification a very low urinary concentration of HCO_3^- and, more importantly, a large concentration of NH_4^+ will result. The measurement of these parameters, which is complicated by the need to avoid significant changes in urine composition after voiding,[8] is usually unavailable in clinical practice. In the context of metabolic acidosis, a urinary pH significantly <6.2 indicates a very low urinary concentration of HCO_3^- and argues against an altered renal mechanism of urinary acidification. Furthermore, and more importantly, the crucial concept of urinary net charge or urine anion gap[9] (which results from urinary Na^+, K^+ and Cl^-) was developed as an indirect assessment of urinary NH_4^+ concentration. Usually, because ammonium (an unmeasured cation) accompanies Cl^- in the context of metabolic acidosis, the concentration of Cl^- should be greater than the sum of Na^+ and K^+, and the net charge negative ($Na^+ + K^+ < Cl^-$). A positive net charge ($Na^+ + K^+ > Cl^-$) indicates impaired ammonium secretion and, therefore, impaired distal acidification of renal tubule. For instance, in the aforementioned context of metabolic acidosis with normal renal mechanisms of acidification (e.g. a child with normal anion gap metabolic acidosis

[8] The changes are due to bacterial overgrowth, especially at room temperature, as well as to open exposure to the atmosphere, which produces gas loss.

[9] The term urine anion gap is a misnomer for what should have been named urine cation gap.

Table 34.14 Indirect assessment of urinary excretion of NH_4^+ by means of the urinary net charge in subjects with normal anion gap metabolic acidosis

Distal acidification of the renal tubule	Urinary NH_4^+	Urinary net charge
Normal	↑ NH_4^+	$Na^+ + K^+ < Cl^-$
Impaired	↓ NH_4^+	$Na^+ + K^+ > Cl^-$[a]

[a] The urine osmolal charge is a more precise estimate of the urinary NH_4^+ concentration in this setting: $\frac{\text{Measured Osmolality} - \left[2 \times (\text{Na} + \text{K}) + \text{Urea} + \text{Glucose}\right]}{2}$

due to mild diarrhea) the enhanced urinary NH_4^+ excretion will result in a large urinary level of urinary NH_4Cl and consequently the measured urinary cations (= $Na^+ + K^+$) will have a concentration lower than that of the measured anion Cl^-: $Na^+ + K^+ < Cl^-$. On the contrary, in a child with an impaired renal acidification, the urine net charge will be as follows: $Na^+ + K^+ > Cl^-$ (Table 34.14).

When the urine net charge is positive ($Na^+ + K^+ > Cl^-$) and it is unclear whether increased excretion of unmeasured anions is responsible, the urinary NH_4^+ concentration can be estimated from calculation of the urine osmolal gap (Table 34.14). This calculation requires measurement of the urine osmolality (in mosm/kg) and the urine Na^+, K^+, urea, and, if the dipstick is positive, glucose concentrations[10] (in mmol/L). In the context of metabolic acidosis, an estimated urinary NH_4^+ concentration of <20 mmol/L indicates an impaired NH_4^+ excretion.

Metabolic Acidosis During the First Months of Life

During the first months of life bicarbonatemia is lower by 2–4 mmol/L than in older children, and it is even lower in preterm infants. This is the consequence of a lower renal threshold for bicarbonate. In addition, in preterm infants and in growing children the daily production of H^+ is higher by 50–100% than that noted in adults (this is mainly explained by the fact that the growing skeleton releases 20 mmol of H^+ for each 1 g of Ca^{++} that is incorporated). The clinical implications of these data are that, as compared with older children, newborns and infants have a relatively limited capacity to compensate for hypobicarbonatemia. In this age, the tendency towards metabolic acidosis is compensated for by the large intake of milk, whose alkali content is high. Infants are therefore more prone to develop metabolic acidosis in conditions associated with a decreased milk intake.

Symptoms, Signs, Consequences

The signs and symptoms of acute metabolic acidosis include (1) high respiratory rate (in young children and infants, the increase in depth of respiration, as observed in classic Kussmaul type deep breathing, may not be as apparent as in adults and the response to metabolic acidosis may be tachypnea alone); (2) abdominal pain and vomiting; (3) irritability and lethargy.

The gastrointestinal absorption and excretion of dietary base plays a major role in acid-base homeostasis in infants in whom the predominantly milk-based diet supplies a considerable amount of alkali. Infants are therefore more vulnerable to developing metabolic acidosis in illnesses associated with decreased milk intake.

Since an excessive chronic acid burden interferes with Ca^{++} deposition in the bone and Ca^{++} intestinal absorption, metabolic acidosis of any form can impair growth in children. Other signs and symptoms are abdominal pain, vomiting, irritability, lethargy, seizures and coma. However, the latter manifestations are primarily due to the underlying disease (e.g.: organic acidemias or hyperosmolality in diabetic ketoacidosis) and not primarily to the acidosis itself.

Clinical Work Up

The causes of metabolic acidosis, which appear in Table 34.13, can almost always be discerned clinically. A careful history and physical exami-

[10] The obtain urea and glucose in mmol/L divide blood urea nitrogen (in mg/dL) by 2.8 and glucose (in mg/dL) by 18.

nation and the determination of the blood anion gap direct an accurate evaluation. For the initial diagnostic approach to metabolic acidosis of unknown origin, the following initial steps are taken [31–35]:

- Confirm the diagnosis of metabolic acidosis
- Confirm that the respiratory response is appropriate
- Distinguish high from normal anion gap metabolic acidosis:
  - Normal anion gap: consider intestinal loss of HCO_3^-
  - High anion gap: assess urinary ketones, blood glucose and blood L-lactate

The major causes of high anion gap acidosis are L-lactate acidosis, which results from impaired tissue oxygenation (type A acidosis) or from an altered metabolism of L-lactate with normal tissue oxygenation in the context of a mitochondrial impairment (type B acidosis), diabetic ketoacidosis, which mainly results from the accumulation of ß-hydroxybutyrate, and "uremic" metabolic acidosis, which is characterized by the accumulation of phosphate, sulfate, and organic anions.

In children, normal anion gap metabolic acidosis mostly results from intestinal bicarbonate losses due to diarrhea. Renal bicarbonate wasting is much less common. In children with normal anion gap acidosis but without history of diarrhea, the concurrent determination of urinary Na^+, K^+, Cl^- will provide information on the renal mechanisms of acidification.

Sometimes there is overlap between the causes of a normal and high anion gap metabolic acidosis. Diarrhea, for example, is most often associated with a normal anion gap. However, severe diarrhea and hypovolemia can result in an increase in the anion gap due to hypoperfusion-induced lactic acidosis and starvation ketosis.

Management

The management of metabolic acidosis includes the following four points:

- Emergency measures:
  - Avoidance of further production of H^+ including measures to ensure a proper airway, adequate peripheral perfusion and O_2 delivery. For instance, in a child with type A L-lactate acidosis in the context of severe dehydration, delivery of O_2 and the rapid administration of normal saline will regenerate adenosine triphosphate. On the other hand, in a child with accidental methanol intoxication the administration of ethanol might stop the production of toxins leading to acidosis.
  - Increasing pH level by lowering the pCO_2, ensuring an adequate degree of hyperventilation, if necessary by mechanical ventilation.
- Correction of the underlying condition. For example, the administration of insulin, in addition to normal saline, in diabetic ketoacidosis.
- Administration of $NaHCO_3$. The use of $NaHCO_3$ is controversial, considering the possible benefits ((a) metabolic advantage of faster glycolysis with better availability of adenosine triphopsphate in vital organs; (b) improved cardiac action) and the risks ((a) extracellular fluid volume expansion; (b) tendency towards hypernatremia; (c) development of hypokalemia and hypocalcemia; (d) worsening of intracellular acidosis). The following guidelines have been suggested for administration of $NaHCO_3$:
  - Diabetic ketoacidosis: $NaHCO_3$ should be considered when hyperkalemia persists despite insulin therapy, when acidemia worsens despite insulin therapy (suggesting insulin resistance as a result of acidemia) and perhaps when HCO_3^- is <5.0 mmol/L. We are very reluctant to use bicarbonate in diabetic ketoacidosis because the administration of $NaHCO_3$ is a risk factor for cerebral edema.
  - Type A L-lactate metabolic acidosis: In this form of acidosis the primary effort should be directed at improving delivery of O_2. $NaHCO_3$ should be given when HCO_3^- is <5.0 mmol/L.

– Since the "HCO_3^- space" is ≈0.5 L/kg body weight the dose of $NaHCO_3$ in severe metabolic acidosis may be calculated from body weight (in kg), current blood HCO_3^-, and desired blood HCO_3^- (both in mmol/L), using the equation:

$$\text{Body weight} \times 0.5\left(\text{desired } HCO_3^- - \text{current } HCO_3^-\right)$$

– Hence, a child weighing 20.0 kg with a severe hypobicarbonatemia of 3.5 mmol/L will be given 40 mmol of $NaHCO_3$ over several minutes (i.e. 2.0 mmol/kg body weight) if the "desired" blood HCO_3^- level is 7.5 mmol/L. In most cases, however, the initial dosage of $NaHCO_3$ is 1.0 mmol/kg body weight, a dosage that is expected to increase blood HCO_3^- by 2.0 mmol/L.

- Correction of metabolic acidosis tends to decrease circulating K^+ level. Hence, one must avoid a severe degree of hypokalemia when $NaHCO_3$ is given. K^+ depletion and metabolic acidosis are associated in three settings: classic distal renal tubular acidosis, acute diarrheal disease and diabetic ketoacidosis, as shown in Table 34.15. The management of renal tubular acidosis will be discussed in the chapter "Renal tubular acidosis", that of uremic acidosis in the sections discussing renal replacement therapy.

Table 34.15 Conditions associating metabolic acidosis and potassium depletion

Condition	Basis of potassium depletion
Classic distal renal tubular acidosis	Renal loss
Diarrhea	Intestinal and renal (secondary hyperaldosteronism due to circulating volume depletion) loss
Diabetic ketoacidosis	Renal loss (osmotic diuresis)[a], cellular shift following insulin therapy

[a] Circulating potassium is often initially normal in diabetic ketoacidosis

Metabolic Alkalosis

Primary hyperbicarbonatemia and, therefore, alkalemia, are the hallmarks of metabolic alkalosis [31, 32, 36, 37]. In this peculiar acid-base disorder (Table 34.16) hyperbicarbonatemia, alkalemia and the compensatory hypoventilation (resulting in a rise of the pCO_2) are almost always associated with hypokalemia (see: systemic effects of acid-base abnormalities).

With the constraints of electroneutrality, the ways to add HCO_3^- to extracellular space are loss of the anion Cl^- or retention of Na^+. Hence circulating HCO_3^- may be raised either (1) associated with a normal or contracted "effective" circulating volume (blood pressure normal or low) or with (2) an expanded "effective" circulating volume (blood pressure increased).

- **Metabolic alkalosis associated with normal or contracted "effective" circulating volume (= "unaccompanied" Cl^- deficiency syndrome or normotensive hypokalemic metabolic alkalosis) = chloride depletion metabolic alkalosis**
- In this clinical-laboratory entity Cl^- is lost from the extracellular space "not accompanied" by the major cations Na^+ and K^+ but "accompanied" by H^+ or NH_4^+. Since a loss of H^+ or NH_4^+ is equivalent to a gain of HCO_3^-, the final effect is loss of Cl^- and gain of HCO_3^-.
- Two further steps complete the development of metabolic alkalosis:
  – "Extra" HCO_3^-, which is filtered by the kidney, is mostly reabsorbed and only a little HCO_3^- is excreted.

Table 34.16 Causes of metabolic alkalosis (linked with hypokalemia)

• Associated with normal (or contracted) "effective" circulating volume (and therefore with normal or even low blood pressure)
(a) Nonrenal causes (low urine chloride excretion: chloride/creatinine <10 mol/mol)
Intestinal causes
Low dietary chloride intake (e.g.: soybean formula with a low chloride content in infancy, "tea and toast diet")
Loss of gastric secretions (vomiting, nasogastric suction)
Posthypercapnia
Congenital chloridodiarrhea (uncommon), villous adenoma (uncommon)
Cutaneous cause
Cystic fibrosis
Excessive sweating (uncommon, associated with low dietary chloride intake)
"Posthypercapnia" (= posthypercapnic alkalosis)
Refeeding syndrome
Transient neonatal metabolic alkalosis in infants of mothers affected by chloride deficiency (eating disorders associated with chloride deficiency, Bartter syndromes, Gitelman syndrome)
(b) Renal causes (high urine chloride excretion: chloride/creatinine >10 mol/mol)
Primary chloride losing tubulopathies (Bartter syndromes, Gitelman syndrome)
Secondary chloride losing tubulopathies (some cases of chronic cisplatin tubulopathy)
Current diuretic use (including surreptitious use)[a]
• Associated with an expanded "effective" circulating volume (and therefore with high blood pressure)
Enhanced mineralocorticoid activity
Primary aldosteronism (either hyperplasia or adenoma)
Apparent mineralocorticoid excess (= defect in 11-β-hydroxysteroid-dehydrogenase), Liddle syndrome (congenitally increased function of the collecting tubule sodium channels), dexamethasone-responsive aldosteronism (synthesis of aldosterone promoted not only by renin but also by adrenocorticotropin), congenital adrenal hyperplasia (11-β-hydroxylase or 17-α-hydroxylase deficiency), Cushing disease
Secondary hyperaldosteronism (including renal artery stenosis, malignant hypertension, and renin producing tumor)
Exogenous mineralocorticoids, licorice-ingestion (= 11-β-hydroxysteroid-dehydrogenase blockade)
Reduced renal function plus a source of HCO_3^-: alkali ingestion, ingestion of ion-exchange resin plus nonreabsorbable alkali

[a] The urinary chloride excretion is low in subjects with remote use of diuretics

  - Contraction of the circulating volume activates the renin-angiotensin II-aldosterone system resulting in urinary K^+ excretion, which further aggravates hyperbicarbonatemia.
- Secondary hyperaldosteronism resulting in urinary K^+ excretion is the main cause of hypokalemia that accompanies this form of metabolic alkalosis.
- This clinical-laboratory entity, termed in the past volume contraction hypokalemic alkalosis, is currently termed Cl^- depletion hypokalemic alkalosis because balance and clearance studies indicate that Cl^- repletion in the face of persisting alkali loading, volume contraction, and K^+ and Na^+ depletion repairs alkalosis. During the first months of life metabolic alkalosis is often not associated with hypokalemia (alternatively it is associated with mild hypokalemia) because the ability of the kidney to excrete K^+ is reduced early in life.
- Maternal Cl^- depletion, deficient Cl^- intake, gastrointestinal Cl^- losses, cutaneous Cl^- losses in the setting of cystic fibrosis, diuretics and renal tubular disturbances are the most important causes of normotensive hypokalemic metabolic alkalosis (Table 34.16). The urinary excretion of chloride is low in patients with non-renal and normal or high in subjects with renal causes of this peculiar form of metabolic alkalosis. In our experience the determination of the molar urinary chloride/

creatinine ratio in spot urine samples from patients with normotensive metabolic alkalosis distinguishes between renal (urinary chloride/creatinine ratio largely >10 mol/mol) and non-renal causes (urinary chloride/creatinine ratio <10 mol/mol). In clinical practice this simple parameter is useful in patients in whom the etiology of metabolic alkalosis with normal or low normal blood pressure is not obtainable from the history. Please note that the urinary chloride/creatinine ratio is also usually >10 mol/mol in patients with metabolic alkalosis associated with expanded effective circulating volume (see below).

- **Posthypercapnic alkalosis (= "posthypercapnia").** Chronic respiratory acidosis is associated with a compensatory hyperbicarbonatemia. In those patients with a tendency towards a contracted circulating volume when pCO_2 falls to normal, there will be a stimulus for persistently increased HCO_3^- levels and hypokalemia. In addition, a rapid correction of chronic respiratory acidosis (e.g.: mechanical ventilation) results in an acute rise in cerebral pH that can produce serious neurologic sequelae or even death. Consequently, pCO_2 should be lowered slowly and carefully in chronic hypercapnia.
- **Metabolic alkalosis associated with an expanded "effective" circulating volume (= hypertensive metabolic alkalosis)**
- The second way to add HCO_3^- to the circulating volume and preserving electroneutrality is to retain HCO_3^- along with Na^+, therefore expanding the extracellular fluid volume and increasing blood pressure. Obviously, to retain extra Na^+ (along with HCO_3^-), "permission" of the kidney is required.
- The mechanisms for renal retention of Na^+ and HCO_3^- include either (1) an enhanced reabsorption of filtered HCO_3^- or (2) a reduced glomerular filtration rate plus a source of HCO_3^- (e.g.: the ingestion of large amounts of milk and the absorbable antacid $CaCO_3$).
- Excessive mineralocorticoid activity is the main cause of metabolic alkalosis associated with hypokalemia and expanded circulating volume. The corresponding causes appear in Table 34.16.

Symptoms, Signs, Consequences

There are no specific diagnostic symptoms or signs of metabolic alkalosis [23, 31, 32, 36, 37]. Physical examination may reveal neuromuscolar irritability, such as tetany or hyperactive reflexes. These signs will be more pronounced if hypocalcemia is an accompanying feature, since the ionized Ca^{++} concentration decreases as pH rises. The symptoms and signs of accompanying hypokalemia have been discussed above.

It is recognized that in children with both normal (or contracted) and expanded circulating volume and metabolic alkalosis the assessment of the fluid volume status by physical examination and history may be quite inaccurate. This assumption is supported by the experience in infantile hypertrophic pyloric stenosis where the clinical assessment of the fluid volume status may be quite inaccurate, and the severity of metabolic alkalosis helps to define the amount of fluid replacement required.

Management

The most frequent causes of hypokalemic metabolic alkalosis associated with a normal or contracted "effective" circulating volume include intestinal (mostly gastric) or cutaneous fluid losses, and excessive diuretic therapy. These forms of metabolic alkalosis are termed "chloride responsive", because they are reversed by the oral or intravenous administration of Na^+Cl^-, K^+Cl^- and water. Many institutions hydrate infants with hypertrophic pyloric stenosis with a "near isotonic" parenteral solution containing glucose 5% (= 50 g/L), Na^+Cl^- 80–90 mmol/L and K^+Cl^- 20–30 mmol/L until correction of the acid-base and K^+ balance. The initial parenteral repair consists of a normal saline solution at least in chil-

dren with both hypokalemic alkalosis and rather severe hyponatremia (≤120 mmol/L).

Occasionally, severe metabolic alkalosis is additionally treated with (1) a carbonic anhydrase inhibitor like acetazolamide, which induces bicarbonaturia accompanied by Na^+- and K^+-losses, (2) with NH_4Cl, or (3) with HCl (through a central venous line). Finally, hemodialysis (or hemofiltration) with a low dialysate HCO_3^- in association with saline infusion has been advised for the treatment of severe metabolic alkalosis in advanced kidney disease.

In "chloride responsive" metabolic alkalosis the oral administration of K+ with any anion other than Cl^- (e.g.: citrate) prevents the correction of alkalosis.

Respiratory Acid-Base Disturbances

These acid-base disorders will not be discussed in this textbook of clinical nephrology with the exception of Table 34.17, which depicts the main causes.

Table 34.17 Causes of respiratory acidosis (hypoventilation) and alkalosis (hyperventilation)

• Respiratory acidosis (hypoventilation)
– Central nervous system (patient will not breathe!)
Cerebral
Posthypoxic brain damage
Cerebral trauma
Intracranial disease
Psychotropic drugs
Brain stem
Brain stem herniation
Encephalitis
Central sleep apnea
Severe metabolic alkalosis
Sedative or narcotic drugs
Upper airway reflexes
Bulbar palsy
Anterior horn cell lesion (including Guillan-Barré and poliomyelitis)
Disruption of airway
– Peripheral disorders (patient cannot breathe)
Respiratory muscle disease
Myasthenia, Guillain-Barré syndrome, myopathy, muscular dystrophy
Muscle fatigue or paralysis (including hypokalemic paralysis)
Airway and pulmonary disease
Interstitial lung disease (including lung fibrosis)
Obstructive disease (including upper airway obstruction, asthma, bronchiolitis, cystic fibrosis)
Obstructive sleep apnea
Obesity, kyphoscoliosis
• Respiratory alkalosis (hyperventilation)
– Hypoxia: intrinsic pulmonary disease, high altitude, congestive heart failure, cyanotic congenital heart disease
– Pulmonary receptor stimulation: pneumonia, asthma, interstitial lung disease, pulmonary edema, pulmonary thromboembolism
– Drugs: salicylates, nikethamide, catecholamines, theophylline, progesterone
– Central nervous disorders: subarachnoid hemorrhage, Cheyne-Stokes respiration, primary hyperventilation syndrome
– Miscellaneous: panic attacks with hyperventilation (rare before puberty), fever, sepsis, recovery from metabolic acidosis

Calcium

Balance

A 70 kg man contains one kg of Ca^{++} (= 25 mol), 99% of which resides in the skeleton in the form of hydroxyapatite and 1% of which is found in soft tissues and the extracellular space. Since Ca^{++} plays a crucial role in neuromuscular function, blood coagulation, and intracellular signaling, circulating Ca^{++} concentrations are maintained within a tight physiologic range. The Ca^{++} (and phosphate) homeostasis involves intestinal, bone, and renal function. Regulation of intestinal function is important because, in contrast to the complete absorption of dietary Na^{+}, K^{+} and Cl^{-}, that of Ca^{++} (like Mg^{++} and phosphate) is incomplete. This limitation is due both to the requirement for vitamin D and to the formation of insoluble salts in the intestinal lumen, such as calcium phosphate, calcium oxalate, and magnesium phosphate.

A normal adult ingests ≈1000 mg (= 25 mmol) of Ca^{++} per day, of which ≈40–50% may be absorbed. However, 300 mg (approximately 8 mmol) of Ca^{++} from digestive secretions is lost in the stool, resulting in the net absorption of no more than 10 to 20%. In the steady state, this amount of Ca^{++} is excreted in the urine. Within the blood Ca^{++}, ≈40% is bound to albumin, 15% is complexed with citrate, sulfate, or phosphate, and 45% exists as the physiologically important ionized form [38–40].

Considering that a large proportion of circulating Ca^{++} is bound to albumin, the determination of albumin (or the direct measurement of ionized Ca^{++}) is essential to the diagnosis of true hypocalcemia or hypercalcemia. The so-called Payne's formula [38–41] may be used for correction of total calcium to account for albumin binding:

$$\text{Adjusted } Ca^{2+}\left[\text{mmol / L}\right] = \text{measured } Ca^{2+}\left[\text{mmol / L}\right] + \frac{40 - \text{albumin}\left[\text{g / L}\right]}{40}$$

Although only a small fraction of the total body Ca^{++} is located in the plasma, it is the blood level of ionized Ca^{++} that is under control of calciotropic hormones:

(a) **vitamin D**,
(b) **parathyroid hormone** and
(c) the **Ca^{++}-sensing receptor**. This receptor, which is found on the cell surface of tissues such as the parathyroid gland, kidney, and bone, detects hypocalcemia and leads to enhanced secretion of parathyroid hormone. Summarizing the process briefly, a fall in circulating Ca^{++} in normal subjects leads to a compensatory increase in parathyroid hormone secretion, which returns the Ca^{++} level to normal by two major actions: increased Ca^{++} release from bone and stimulated production of 1,25-dihydroxyvitamin D, the active metabolite of vitamin D, resulting in an increase in intestinal Ca^{++} absorption [38–40].
(d) **Parathyroid hormone related peptide** is a further calciotropic hormone with the following identified actions: (1) During pregnancy, Ca^{++} is transferred from the maternal circulation to the fetus by a pump regulated by this hormone; (2) Parathyroid hormone related peptide levels are elevated during lactation and contribute substantially to the movement of Ca^{++} from the maternal skeleton to the mammary glands; (3) Finally, this peptide is involved in the pathogenesis of hypercalcemia of malignancies [38–40].

Hypocalcemia

Non-neonatal Hypocalcemia

Symptoms and Signs

Symptoms and signs of hypocalcemia, which is often asymptomatic, result from neuromuscular, ocular, ectodermal, dental, gastrointestinal, car-

diovascular, skeletal or endocrine dysfunctions, and are related to the severity and chronicity of the hypocalcemia (Table 34.18). Hypocalcemia manifests with a prolonged QT interval on standard electrocardiogram (Fig. 34.9). However, some signs and symptoms are unique to chronic

Table 34.18 Clinical signs and symptoms of hypocalcemia

• **Neuromuscular**
– **Tetany**
sensory dysfunction: circumoral and acral paresthesias
muscular dysfunction
Stiffness, myalgia, muscle spasms and cramps
Forced adduction of the thumb, flexion of the metacarpophalangeal joints and wrists, and extension of the fingers
Laryngismus stridulus (spasm of respiratory muscles and of glottis causing dyspnea)
Autonomic dysfunction: diaphoresis, bronchospasm, biliary colic
Trousseau sign: inflation of a sphygmomanometer above systolic blood pressure for 3–4 min induces a carpal spasm
Chvostek sign: ipsilateral tapping of the facial nerve just anterior to the ear followed by contraction of the facial muscles (the complete sign is contraction of corner of the mouth, the nose and the eye; contraction of the corner of the mouth alone often occurs in normal subjects)
– **Myopathy:** generalized muscle weakness and wasting with normal creatine kinase (myopathy represents more a feature of vitamin D deficiency than hypocalcemia per se; elevated parathyroid hormone level or hypophosphatemia may contribute to the myopathy)
– **Extrapyramidal disorders:** Bradykinetic movement disorders, sometimes dystonia, hemiballismus, choreoathetosis, oculogyric crises
– **Convulsions** (generalized or partial)
– **Mental retardation**, **psychosis**
• **Ocular**
– Cataract (rarely keratoconjunctivitis)
– Papilledema (often associated with benign intracranial hypertension; rarely optic neuritis is present)
• **Ectodermal** (especially in the context of severe, chronic hypocalcemia)
– Dry scaly skin
– Hyperpigmentation, dermatitis, eczema, and psoriasis
– Course, brittle, and sparse hair with patchy alopecia
– Brittle nails, with characteristic transverse grooves
– Candidiasis: usually as a component of **A**utoimmune **P**oly**E**ndocrinopathy-**C**andidiasis-**E**ctodermal **D**ystrophy (= APECED-association)
• **Dental** (dental hypoplasia, failure of tooth eruption, defective enamel and root formation, and abraded carious teeth)
• **Gastrointestinal**
– Loose stools (steatorrhea due to impaired pancreatic secretion)
– Gastric achlorhydria
• **Cardiovascular**
– Systemic hypotension, decreased myocardial function, congestive heart failure
– Prolonged QTc interval on standard electrocardiogram with tendency towards cardiac arrhythmias (clinically relevant if hypocalcemia is associated with hypokalemia and hypomagnesemia)
• **Skeletal**
– Rachitic findings
Delayed closure of the fontanelles
Parietal and frontal bossing
Craniotabes
Rachitic rosary: enlargement of the costochondral junction visible as beeding along the anterolateral aspects of the chest
Harrison sulcus caused by the muscular pull of the diaphragmatic attachments to the lower ribs

(continued)

Table 34.18 (continued)

Enlargement and bowing of the distal radius, ulna, tibia and fibula
Progressive lateral bowing of the femur and tibia
– Children with hypoparathyroidism: increased bone mineral density, osteosclerosis and thickening of the calvarium
– Children with pseudohypoparathyroidism: Albright's hereditary osteodystrophy, osteitis fibrosa cystica (due to normal skeletal responsiveness to parathyroid hormone)
• **Endocrine manifestations**
– Impaired insulin release
– Hypothyroidism, prolactin deficiency, and ovarian failure associated with polyglandular autoimmune syndromes

hypoparathyroidism and not hypocalcemia: these include candidiasis and dysmorphic changes in **A**utoimmune **P**oly **E**ndocrinopathy-**C**andidiasis-**E**ctodermal **D**ystrophy (= APECED-association). Among the symptoms of hypocalcemia, tetany, papilledema and seizures may occur in patients who develop hypocalcemia acutely. By comparison, ectodermal and dental changes, cataracts, basal ganglia calcification, and extrapyramidal disorders are features of chronic hypocalcemia and are common in hypoparathyroidism [38–40, 42].

Causes

Deficiency or impaired function of (a) parathyroid hormone, (b) vitamin D or (c) Ca^{++}-sensing receptor are major causes of reduced blood level of ionized Ca^{++}. Because bone Ca^{++} stores are so large, the major reason for hypocalcemia is decreased bone resorption. Sometimes acute events such as hyperphosphatemia, can produce hypocalcemia even though the regulatory systems are intact. The main causes of hypocalcaemia include vitamin D deficiency, Ca^{++} deficiency, impaired vitamin D metabolism, impaired parathyroid hormone action (secondary to end organ resistance), reduced production of parathyroid hormone, and abnormal Ca^{++}-sensing receptor or impaired renal function (Table 34.19).

Diagnostic Work Up

Hypocalcemia is a rather common clinical problem, the cause of which can very often be determined from the history (as with a breast-fed infant not receiving any supplementation of vitamin D presenting with non-febrile generalized convulsions, enlargement of the costochondral junction along the anterolateral aspects of the chest and enlargement of the wrist). In some cases, however, the underlying condition is not readily apparent. A detailed history documenting diet, lifestyle, family, and drug history, as well as development and hearing is important. The examination should include an assessment of skin, nails, teeth, and the skeleton, as well as the cardiovascular system. A comprehensive range of investigations should be performed at baseline, which have been divided into first and second line (Table 34.20). The objective of assessing urine Ca^{++} excretion is to establish whether the molar urine calcium/creatinine is inappropriately high in the presence of hypocalcemia. Reference values for urine calcium/creatinine ratio in young children are not well defined and will vary according to factors such as diet. The upper limits of normal urine Ca^{++} excretion in healthy children appear in the footnote of Table 34.20. Renal phosphate handling may be abnormal despite a blood phosphate within the quoted laboratory normal range, and should be assessed in more detail by determining the tubular maximum reabsorption threshold of phosphate (see phosphate).

Checking biochemistry of the parents and possibly siblings is crucial when inherited diseases such as hypocalcaemic hypercalciuria and hypophosphataemic rickets are suspected. It is also important to measure maternal Ca^{++} and vitamin D levels in the case of hypocalcaemia in infancy because of the link with maternal vitamin D deficiency and hyperparathyroidism. Maternal hyperparathyroidism is linked with adverse preg-

Table 34.19 Causes of hypocalcemia in infants and children

Parathyroid hormone level low
• **Abnormal production of parathyroid hormone**
– Magnesium deficiency[a]
– Following neck surgery
– Hypoparathyroidism (autosomal recessive, autosomal dominant, or X-linked)
– Di George anomaly (= 22q11 deletions), 10p13 deletion, Hall-Hittner or CHARGE-association (=Coloboma, Heart anomaly, Choanal Atresia, mental Retardation, Genital hypoplasia, and Ear anomalies), HDR-association (= Hypoparathyroidism, Deafness, Renal dysplasia)
– **A**utoimmune **P**oly**E**ndocrinopathy-**C**andidiasis-**E**ctodermal **D**ystrophy (= APECED-association)
– Infiltrative lesions such as Wilson's disease and thalassemia
– Mitochondrial diseases (e.g. Kearns Sayre syndrome)
• **Altered "set point"** (calcium sensing receptor activating mutations)
Intact parathyroid hormone level high
• **Hypovitaminosis D, calcium deficiency, impaired vitamin D metabolism**
– **Hypovitaminosis D**
Reduced vitamin D intake or production in the skin
Decreased intestinal absorption (e.g. celiac disease and cystic fibrosis)
– **Calcium deficiency**
– **Impaired vitamin D "metabolism"**
Severe liver disease
Drugs that "inactivate" vitamin D: anticonvulsants (phenobarbital, phenytoin, carbamazepine, oxcarbazepine), antimicrobials (isoniazid and rifampicin), antiretroviral drugs
Enzyme deficiency: defects of the 1-α-hydroxylase gene (= vitamin D dependent rickets type I)
End organ resistance to vitamin D (= vitamin D dependent rickets type II)
• **Signaling defects: pseudohypoparathyroidisms**
• **Renal failure, osteopetrosis, excessive fluoride intake**

[a] Severe chronic magnesium deficiency (≤0.45 mmol/L) causes hypocalcaemia by impairing parathyroid hormone secretion as well as parathyroid hormone action

Table 34.20 First and second line investigations in childhood hypocalcemia when the cause cannot be determined from the history and clinical examination

First line investigations	Second line investigations
• **Blood values**	
Phosphate[a], Magnesium	Autoantibody screen
Alkaline Phosphatase	Parental (and siblings)
Sodium, Potassium, Bicarbonate, Creatinine	Maternal Vitamin D_3-status
Intact Parathyroid Hormone	1,25-hydroxy vitamin D3
25-Hydroxyvitamin D_3 (= calcidiol)	Genetic studies (e.g. 22q11 deletion)
• **Urinary values**	
Urinalysis (for glucose, protein and pH)	
Calcium[a], Phosphate[b], Creatinine	
• **Imaging**	
Hand and wrist radiograph	Renal ultrasound
	Skull radiograph

[a] The upper limit of normal for urine calcium/creatinine in healthy children is 2.20 mol/mol (or 0.81 mg/mg) in infants aged 6–12 months, 1.50 mol/mol (or 0.56 mg/mg) in infants aged 13–24 months, 1.40 mol/mol (or 0.50 mg/mg) in infants aged 25–36 months, 1.10 mol/mol (or 0.41 mg/mg) in children aged 3–5 years, 0.80 mol/mol (or 0.30 mg/mg) in children aged 5–7 years and 0.70 mol/mol (or 0.25 mg/mg) in older children

[b] Calculate the maximal tubular reabsorption of phosphate as indicated in the section on phosphate

nancy outcome and causes transient hypocalcemia in the newborn because the fetal parathyroids are suppressed following exposure to high Ca^{++} levels in utero. An autoantibody screen including adrenal, parathyroid, smooth muscle and microsomal antibodies is useful in cases of isolated hypoparathyroidism and where APECED-association is suspected. Renal ultrasound scan looking for evidence of nephrocalcinosis or renal dysplasia is also often advised.

The biochemical picture of hypocalcemia can be categorized according to the presence of undetectable, normal or high levels of circulating parathyroid hormone, an approach that reflects the underlying pathophysiology [42].

- Undetectable or low levels of this hormone in the hypocalcemic child suggest hypoparathyroidism (Table 34.19). Aplasia or hypoplasia of the parathyroids is most commonly due to the DiGeorge syndrome associated with deletion of chromosome 22q11. In this syndrome, the characteristic clinical signs of the so called "CATCH-22" might be noticed: Cardiac malformations (especially conotruncal anomalies

like tetralogy of Fallot, truncus arteriosus communis or interrupted aortic arch), Abnormal facies, Thymic aplasia, Cleft palate and Hypocalcemia/Hypoparathyroidism). A similar phenotype including hypoparathyroidism has also been associated with deletions of chromosome 10p, while the HDR-association (**H**ypoparathyroidism, **D**eafness, and **R**enal dysplasia) is due to defects in the GATA3 gene. Defects in the parathyroid hormone gene are rare. Diseases such as APECED can present with hypoparathyroidism in the absence of the two other major manifestations, which are candidiasis and adrenal failure. There should be a high index of suspicion for this disease in all cases of hypoparathyroidism presenting in children older than 4 years. Children with APECED may have other "minor" features such as malabsorption, gallstones, hepatitis, dysplastic nails and teeth. Screening should be considered in the siblings of affected individuals. Mitochondrial disease is a rare cause of hypoparathyroidism but is not usually an isolated finding.

- Detectable parathyroid hormone values (low-normal or normal) in an asymptomatic individual raise the possibility of hypocalcemic hypercalciuria, an abnormality of the Ca^{++}-sensing receptor which can be assessed in more detail by determining urinary Ca^{++} excretion. This parameter is typically low in longstanding hypoparathyroidism, and a relatively high urine Ca^{++} excretion (molar urinary calcium/creatinine ratio $\geq$0.30) suggests hypocalcemic hypercalciuria. This abnormality is due to activating mutations of the Ca^{++}-sensing receptor with downshift of the setpoint for Ca^{++} responsive parathyroid hormone release. Mg^{++} levels are low in this disorder because the Ca^{++}-sensing receptor also detects this cation. Interestingly, the biochemical picture of hypocalcemic hypercalciuria sometimes resembles Bartter syndromes and includes hypokalemia and hyperbicarbonatemia.
- If blood creatinine is normal, thereby excluding renal insufficiency, then increased parathyroid hormone levels point towards a diagnosis of rickets[11] or pseudohypoparathyroidism. Vitamin D deficiency is still prevalent in the Western world. High-risk groups include families, where the maternal and child diet may be low in Ca^{++} and vitamin D and where exposure to sunlight can be limited. The diagnosis of Fanconi-De Toni-Debré syndrome should be considered in any hypocalcemic child with persistent glycosuria, phosphaturia, and acidosis. Pseudohypoparathyroidism is a heterogeneous disorder that results from signaling defects of the cell surface receptors. Patients may become hypocalcemic despite a compensatory increase in parathyroid hormone concentration, and may have other endocrine problems, such as primary hypothyroidism and hypogonadism that are also manifestations of an abnormal signaling mechanism. Some patients are overweight and mentally retarded.

Neonatal Hypocalcemia

Hypocalcemia is a common metabolic problem in newborns. During pregnancy, Ca^{++} is transferred from the maternal circulation to the fetus by a pump regulated by parathyroid hormone-related peptide. This process results in higher blood Ca^{++} in the fetus than in the mother and leads to fetal hypercalcemia, with total Ca^{++} level of ≈2.50–2.75 mmol/L in umbilical cord blood [38–40, 42].

The cessation of placental transfer of Ca^{++} at birth is followed by a fall in total blood Ca^{++} concentration to ≈2.00–2.25 mmol/L and ionized Ca^{++} to ≈1.10–1.35 mmol/L at 24 h. Ca^{++} subsequently rises, reaching levels seen in older children and adults by 2 weeks of age.

The definition of hypocalcemia depends upon birth weight: (a) in term infants or premature infants >1.50 kg birth weight, hypocalcemia is defined as a total Ca^{++} concentration <2.00 mmol/L or a ionized fraction <1.10 mmol/L; (b) premature infants with birth weight <1.50 kg are hypocalcemic if they have a total Ca^{++} concentration <1.75 mmol/L or a ionized fraction of <1.00 mmol/L [38–40, 42].

[11] In hypophosphataemic rickets, circulating parathyroid hormone and calcium are usually normal.

Symptoms and Signs

Neonatal hypocalcemia is usually asymptomatic. Among those who become symptomatic, the characteristic sign is increased neuromuscular irritability. Such infants are jittery and often have muscle jerking. Generalized or partial clonic seizures can occur. Rare presentations include inspiratory stridor caused by laryngospasm, wheezing caused by bronchospasm or vomiting possibly resulting from pylorospasm [38–40, 42].

Causes

The causes of neonatal hypocalcemia are classified by the timing of onset. Hypocalcemia is considered to be early when it occurs in the first 2–3 days after birth.

Early Neonatal Hypocalcemia

Early hypocalcemia is an exaggeration of the normal decline in Ca^{++} concentration after birth. It occurs commonly in premature infants, in infants of diabetic mothers, and after perinatal asphyxia or intrauterine growth restriction.

- Prematurity: One-third of premature infants and the majority of very-low-birth-weight infants develop hypocalcemia during the first 2 days after birth. Multiple factors contribute to the fall. They include hypoalbuminemia and factors that lower both total and ionized Ca^{++}, such as reduced intake of Ca^{++} because of low intake of milk, possible impaired response to parathyroid hormone, increased calcitonin and increased urinary Ca^{++} losses.
- Infants of diabetic mothers: Hypocalcemia occurs in 10–20% of infants of diabetic mothers. The lowest concentration typically occurs between 24 and 72 h after birth and often is associated with hyperphosphatemia. Hypocalcemia is caused by lower parathyroid hormone concentrations after birth in this condition compared to normal infants. Hypoparathyroidism is likely related to intrauterine hypercalcemia suppressing the fetal parathyroid glands. Concurrent hypomagnesemia is a further contributing factor.
- Birth asphyxia: Infants with birth asphyxia frequently have hypocalcemia and hyperphosphatemia. Possible mechanisms include increased phosphate load caused by tissue catabolism, decreased intake due to delayed initiation of feedings, renal insufficiency, acidosis, and increased serum calcitonin concentration.
- Intrauterine growth restriction: Hypocalcemia occurs with increased frequency in infants with intrauterine growth restriction. The mechanism is thought to involve decreased transfer of Ca^{++} across the placenta.

Late Neonatal Hypocalcemia

Late hypocalcemia develops after the second or third day after birth. It typically occurs at the end of the first week.

- Hypoparathyroidism: Hypoparathyroidism associated with excess phosphorus intake is the most common cause of late neonatal hypocalcemia. Hypoparathyroidism often occurs as part of a syndrome, including DiGeorge syndrome or, more rarely, mitochondrial cytopathies.
- Maternal hyperparathyroidism: Infants born to mothers with hyperparathyroidism frequently have hypocalcemia. The mechanism is related to increased transplacental Ca^{++} transport caused by maternal hypercalcemia, which results in excessive fetal hypercalcemia that inhibits fetal and neonatal parathyroid secretion. Affected infants typically develop increased neuromuscular irritability in the first 3 weeks after birth, but they can present later.
- Hypomagnesemia: Hypomagnesemia causes resistance to parathyroid hormone and impairs its secretion, both of which can result in hypocalcemia. The most common etiology in newborns is transient hypomagnesemia, although rare disorders of intestinal or renal tubular Mg^{++} transport can occur.
- Other causes: Critically ill or premature infants are exposed to many therapeutic interventions that may cause transient hypocalcemia including bicarbonate infusion resulting

in metabolic alkalosis, transfusion with citrated blood or infusion of lipids leading to formation of Ca^{++} complexes and decreased ionized Ca^{++}. Finally, mild hypocalcemia has been associated with phototherapy. Other rare causes include acute renal failure of any cause, usually associated with hyperphosphatemia, any disorder of vitamin D metabolism and rotavirus infections.

- High phosphate intake: Intake of excess phosphate is an historically important cause of late hypocalcemia that was seen in term infants fed bovine milk or a formula with a high phosphorus concentration. It has been postulated that the high phosphorus levels antagonize parathyroid hormone or may produce increased Ca^{++} and phosphorus deposition in bones. Symptomatic infants typically present with tetany or seizures at 5–10 days of age. Severe hyperphosphatemia and hypocalcemia also can be caused by phosphate enemas.

Hypercalcemia

Signs and Symptoms

Hypercalcemia is more difficult to diagnose than hypocalcemia because of the nonspecific nature of symptoms and signs (Table 34.21). Hypercalcemia manifests with a shortened QT interval on electrocardiogram (Fig. 34.9). Major symptoms include sekeletal pain, fatigue, anorexia, nausea and vomiting, and particularly important are polyuria and polydipsia. Changes in behavior and frank psychiatric disorders may also be a result of hypercalcemia. The extent of symptoms and signs is a function of both the degree of hypercalcemia and the rate of onset of the elevation in the blood concentration. Thus, a rather severe hypercalcemia of 3.50 mmol/L is asymptomatic when it develops chronically, while an acute rise to these values may cause marked changes in sensorium. It is worthy of mention, however, that symptoms and signs associated with hypercalcemia may be due to the elevation in the Ca^{++} concentration but also to the underlying disease [38–40, 43, 44].

Table 34.21 Symptoms and signs of hypercalcemia

• **General**
– Weakness
– Depression
– Anorexia
• **Central nervous system**
– Impaired concentration
– Increased sleep requirement
– Altered state of consciousness
– Mental retardation
– Polydypsia (and polyuria)
• **Muscular:** weakness
• **Ocular**
– Palpebral calcification
– Band keratopathy
– Conjunctival calcification
• **Dermal:** Pruritus and skin calcifications
• **Gastrointestinal**
– Constipation
– Anorexia, nausea, vomiting
– Pancreatitis
– Peptic ulcer
• **Cardiovascular**
– Shortened QTc interval on standard electrocardiogram[a]
– Arterial hypertension
• **Skeletal: joint pains (pseudogout)**
• **Renal dysfunction**
– Altered urinary concentration ability with polyuria and polydypsia
– Nephrolithiasis, nephrocalcinosis, renal failure
– Distal renal tubular acidosis

[a] without any major tendency towards cardiac arrhythmias

Causes

Hypercalcemia results when the entry of Ca^{++} into the circulation exceeds the excretion of Ca^{++} into the urine or deposition in bone. Since the major sources of Ca^{++} are the bone and the intestinal tract, hypercalcemia mostly results from increased bone resorption or from increased intestinal absorption. In some cases, however, multiple sites are involved in the development of hypercalcemia. The great majority of adult patients with elevated Ca^{++} level will be found to have either **primary hyperparathyroidism** or **malignancy** (this form of hypercalcemia is thought in many instances to be caused by secretion of parathyroid hormone related peptide), although the differential diagnosis is much longer. For these other causes of hypercalcemia,

which include **vitamin D (or A) intoxication**, **sarcoidosis**, **tuberculosis**, some **fungal infections**, **thyreotoxicosis**, **Addison's disease**, milk-alkali syndrome (= **calcium-alkali syndrome**) related to the prescription of Ca^{++}, absorbable alkali and vitamin D supplements, treatment with **thiazides** or **lithium** carbonate, **familial hypocalciuric hypercalcemia**, **prolonged immobilization** in subjects with high skeletal turnover (including adolescents) and the recovery phase of **rhabdomyolysis**, the use of the mnemonic VITAMINS TRAPS (Table 34.22) has been suggested. Children present with hypercalcemia less frequently than adults, but the

Table 34.22 Causes of hypercalcemia (please note that some causes of hypercalcemia are given twice)

• **Classical causes (Mnemonic VITAMINS TRAP)**
– **V**itamin D and vitamin A
– **I**mmobilization
– **T**hyrotoxicosis
– **A**ddison's disease
– **M**ilk-alkali syndrome (= calcium-alkali syndrome)
– **I**nflammatory disorders (granulomatous diseases with excessive production of calcitriol)
– **N**eoplastic-related disease[a]
– **S**arcoidosis
– **T**hiazides[b] and other drugs
– **R**habdomyolysis (recovery phase)
– **AIDS**
– **P**arathyroid disease[a] (including familial hypocalciuric hypercalcemia), **p**arenteral nutrition
• **Hypercalcemia associated with elevated calcitriol (1,25-dihydroxyvitamin D_3)**
– Sarcoidosis
– Acute granulomatous pneumonia, lipoid pneumonia
– Tuberculosis (and other mycobacterial infections)
– Wegener's granulomatosis
– Crohn's disease
– Hepatic granulomatosis
– Talc and silicone granulomatosis
– Cat scratch disease
– Neonatal subcutaneous fat necrosis
• **Hypercalcemia associated with elevated parathyroid hormone related peptide**
– Hypercalcemia of malignancy
– Some benign tumors (ovary, kidney, pheochromcytoma)
– Systemic lupus erythematosus
– HIV-associated lymphadenopathy
– Massive mammary hyperplasia
– During late pregnancy and lactation in hypoparathyroidism
• **Drugs associated with the development of hypercalcemia**
– **Common:** calcium, vitamin D, vitamin A, lithium, thiazides[b] (e.g.: hydrochlorothiazide, chlortalidone)
– **Less common:** omeprazole, theophyllin (toxic doses), recombinant growth hormone, foscarnet, hepatitis B vaccination, manganese toxicity
• **Rare causes of hypercalcemia with an unknown underlying mechanism**
– Infections: nocardiosis, brucellosis, cytomegaloviric infection (in AIDS), berylliosis
– Juvenile idiopathic arthritis
– Advanced chronic liver disease
• **Rare causes of hypercalcemia in infancy and young children**
– Reduced function of the calcium-sensing receptor
Deactivating mutations
Heterozygous: Familial hypocalciuric hypercalcemia

(continued)

Table 34.22 (continued)

Homozygous: Severe neonatal hyperparathyroidism
Autoantibodies directed at the calcium-sensing receptor
– Congenital hypoparathyroidism
– Idiopathic infantile hypercalcemia
– Jansens metaphyseal chondrodysplasia[c]
– Williams-Beuren syndrome
– Down syndrome
– Hypophosphatasia
– Congenital lactase deficiency
– Phosphate depletion in severe prematurity
– Renal tubular acidosis
– Primary hyperoxaluria
– Neonatal subcutaneous fat necrosis

[a] Malignancy and primary hyperparathyroidism account for 80–90% of cases of hypercalcemia in adulthood
[b] Although thiazides are frequently cited as a cause of hypercalcemia, it is more usual that they bring mild pre-existing hypercalcemia to light
[c] Consequence of a constitutive activation of the parathyroid hormone receptor

causes that are common in adults are also common in children. Young children and infants, however, present with hypercalcemia in association with some rather rare conditions seen almost exclusively in that population. **Idiopathic infantile hypercalcemia** is characterized by an increased sensitivity to vitamin D. It is the consequence of loss of function mutations in the gene that encodes the enzymatic system responsible for the inactivation of 25-hydroxyvitamin D, resulting in its decreased conversion into inactive metabolites [38–40, 43, 44]. Hypothermia treatment for neonatal asphyxia can sometimes lead to neonatal subcutaneous fat necrosis and consequentely hypercalcemia by elevated calcitriol (Table 34.22).

Diagnostic Work Up

The causes of hypercalcemia are often discerned clinically. Clinical history (calcium-alkali syndrome, which replaces the traditional term of milk-alkali syndrome, is currently a cause of hypercalcemia that results from the widespread use of over-the-counter Ca^{++} and vitamin D supplements), physical examination and rather simple laboratory data (circulating phosphate and creatinine; urinary Ca^{++}, phosphate and creatinine) and chest x-ray (looking for sarcoidosis) provide the correct diagnosis in many cases.

– **Step 1: Assess clinical and simple laboratory data**
– Clinical history and physical examination are useful in establishing the diagnosis of hypercalcemia induced by immobilization, medication or thyreotoxicosis, and the diagnosis of "syndromic" hypercalcemia, including Williams-Beuren syndrome, Down syndrome and Jansens metaphyseal chondrodysplasia. Measurement of the serum phosphate concentration and urinary Ca^{++} excretion also may be helpful in selected cases: hyperparathyroidism and the humoral hypercalcemia of malignancy induced by secretion of parathyroid hormone related peptide often present with hypophosphatemia resulting from inhibition of renal proximal tubular phosphate reabsorption. In comparison, the serum phosphate concentration is normal or elevated in granulomatous diseases, vitamin D intoxication, immobilization, thyrotoxicosis and metastatic bone disease. Calciuria is usually raised or high-normal in hyperparathyroidism and hypercalcemia of malignancy. Two conditions lead to relative hypocalciuria: thiazides, which directly enhance active reabsorption of Ca^{++} in the distal tubule, and familial hypocalciuric hypercalcemia, in which the fractional excretion of Ca^{++} is often <1.0% (further clues to

the possible presence of this disorder are a family history of hypercalcemia and few if any hypercalcemic symptoms).

– **Step 2: analyze parathyroid hormone level**
– An elevated parathyroid hormone concentration indicates the presence of primary hyperparathyroidism or a patient taking lithium. 10–20% of patients with primary hyperparathyroidism have a parathyroid hormone concentration in the upper end of the normal range: such a "normal" level, which indicates that the secretion is not suppressed, is virtually diagnostic of primary hyperparathyroidism, since it is still inappropriately high considering the presence of hypercalcemia. A low or low-normal parathyroid hormone level is consistent with all other non-parathyroid hormone-induced causes of hypercalcemia.
– **Step 3: Analyze vitamin D metabolites**
– The levels of vitamin D metabolites 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D are assessed if there is no obvious malignancy and parathyroid hormone levels are not elevated. An elevated 25-hydroxyvitamin D is indicative of either vitamin D intoxication or idiopathic infantile hypercalcemia. On the other hand, increased 1,25-dihydroxyvitamin D may be induced by direct intake of this metabolite or non-renal production in granulomatous diseases or lymphoma.

Management

The degree of hypercalcemia and the rate of rise of Ca^{++} level habitually determine symptoms and urgency of treatment [42, 43]:

– asymptomatic or mildly symptomatic hypercalcemia (total Ca^{++} <3.00 mmol/L) does not require immediate treatment. Similarly, Ca^{++} of 3.00–3.50 mmol/L is often well-tolerated chronically, and may not require urgent treatment (however, an acute rise to these concentrations may cause marked sensorium changes, which require more urgent measures).
– Total Ca^{++} concentration >3.50 mmol/L requires immediate treatment, regardless of symptoms.

The nonsurgical management of childhood hypercalcemia includes following points:

(a) Avoidance of the cause. For example, removal of exogenous vitamin D and Ca^{++} in children with vitamin D intoxication, calcium-alkali syndrome or idiopathic infantile hypercalcemia.
(b) Specific management. Steroids inhibit the effects of vitamin D and are particularly effective in hypercalcemia secondary to granulomatous diseases. The bisphosphonates, which inhibit skeletal Ca^{++} release, are effective in hypercalcemia that results from excessive bone resorption of any cause (including among others hypercalcemia of malignancy, hypercalcemia associated with neonatal subcutaneous fat necrosis and vitamin D intoxication). Pharmacologic doses of calcitonin reduce the Ca^{++} levels by decreasing bone resorption. The effect of calcitonin, which is limited to the first 48 h, is most beneficial in subjects with total Ca^{++} >3.50 mmol/L when combined with a bisphosphonate and administration of saline.
(c) Normal saline, administered at a rapid rate (initially 2800–3000 mL/m^2 body surface area daily), corrects possible volume depletion due to hypercalcemia-induced renal salt wasting and promotes renal Ca^{++} excretion. The loop diuretic furosemide is no longer recommended with the exception of cases with volume overload.

Magnesium

Balance

A 70 kg man contains ≈1 mole of Mg^{++}. About half of it is present in bone tissue, the other half in soft tissue, whereas no more than 1–2% of the total body Mg^{++} is present in extracellular fluids. Intracellular Mg^{++} serves as cofactor for many enzymes that produce and store energy via hydrolysis of adenosine triphosphate [40, 45, 46].

In healthy humans the total circulating Mg^{++} concentration is maintained within narrow limits

and ranges between 0.75 and 1.00 mmol/L.[12] Approximately 1/4 of circulating Mg^{++} is bound to albumin. For the remaining 3/4 of circulating Mg^{++} ≈10% is complexed to inorganic phosphate, citrate and other compounds, while 90% (≈2/3 of total circulating Mg^{++}) is in the form of free ion.

Mg^{++} balance, like that of other ions, is a function of intake and urinary excretion. In adults the daily Mg^{++} intake averages 0.23–0.28 mmol/kg (5.6–6.8 mg/kg) body weight. About 1/3 of this Mg^{++} is absorbed. In healthy adults there is no net gain or loss of Mg^{++} from bone so that balance is achieved by the urinary excretion of the absorbed 0.06–0.08 mmol/kg (1.5–1.9 mg/kg) body weight.

Only 15–25% of filtered Mg^{++} is reabsorbed in the proximal tubule and 5–10% in the distal tubule. The major site of Mg^{++} transport is the thick ascending limb of the loop of Henle where 60–70% of the filtered load is reabsorbed [40, 45, 46].

With negative Mg^{++} balance, the initial loss comes primarily from the extracellular fluid (equilibration with bone stores begins after several weeks). Thus, circulating Mg^{++} falls rapidly with negative Mg^{++} balance, leading to a conspicuous decrease in Mg^{++} excretion unless urinary Mg^{++} wasting is present. The fractional clearance of Mg^{++}, which is 3–5% in healthy subjects ingesting a normal diet, can fall to <0.5% with Mg^{++} depletion due to non-renal losses. This parameter is calculated from the following equation:

$$\frac{\text{Urinary}\,Mg^{++} \times \text{Circulating Creatinine}}{\text{Circulating}\,Mg^{++} \times \text{Urinary Creatinine}}$$

There is no protection against hypermagnesemia with loss of renal function. In this setting, high intake leads to extracellular Mg^{++} retention.

[12] Circulating magnesium levels can be reported in mmol/L, meq/L, mg/dL or mg/L. The valence of magnesium is 2 and its molecular mass 24.3 g/mol; therefore 0.50 mmol/L is equivalent to 1.00 meq/L, 1.22 mg/dL and 12.2 mg/L.

Hypomagnesemia

Hypomagnesemia, which is not rare, results either from intestinal (including dietary insufficiency) or renal losses (Table 34.23). In the presence of hypomagnesemia, the healthy kidney lowers Mg^{++} excretion to very low values. Hence, the diagnosis of hypomagnesemia caused by intestinal Mg^{++} losses (or low dietary Mg^{++} intake) is established by the demonstration of low urinary excretion of Mg^{++}. Conversely the diagnosis of hypomagnesemia caused by renal losses is established by the demonstration of inappropriately high (= "normal") urinary Mg^{++} excretion [40, 45, 46].

Table 34.23 Causes of hypomagnesemia

Decreased magnesium intake and intestinal losses
– Dietary deprivation
– Small bowel disorders, including acute or chronic diarrhea, malabsorption and steatorrhea, and small bowel bypass surgery
– Acute pancreatitis
– Paunier disease[a] (= hypomagnesemia with secondary hypocalcemia)
– Chronic management with proton-pump inhibitors
Renal losses
– Primary renal magnesium wasting diseases
– Drugs
Loop and thiazide-type diuretics
Drugs other than diuretics (aminoglycoside antibiotics, amphotericin B, cisplatin, pentamidine, cyclosporine, tacrolimus, foscarnet, cetuximab[b])
– Volume expansion
– Hypercalcemia
– Miscellaneous: recovery from acute tubular necrosis, following renal transplantation and during a postobstructive diuresis
Further causes
– Alcohol
– Refeeding syndrome
– Diabetes mellitus
– Following surgery
– "Hungry bone syndrome" following parathyroidectomy for hyperparathyroidism
Neonatal hypomagnesemia
– Maternal hypomagnesemia (including maternal diabetes mellitus)
– Intrauterine growth retardation

[a] Often combined with impaired renal magnesium conservation

[b] A monoclonal antibody against the epithelial growth factor receptor

Decreased Intake, Poor Intestinal Absorption or Intestinal Loss

Intestinal secretory losses, which contain some Mg^{++}, are continuous and not regulated. Although the obligatory losses are not large, marked dietary deprivation can lead to progressive Mg^{++} depletion. Mg^{++} loss will also occur when the intestinal secretions are incompletely reabsorbed as with most disorders of the small bowel, including acute or chronic diarrhea, malabsorption and steatorrhea, and small bowel bypass surgery. Prolonged use of proton pump inhibitors is an increasingly recognized cause of hypomagnesemia (these drugs interfere with the transport of this ion across the intestinal wall) [47]. Hypomagnesemia can also be seen in acute pancreatitis (saponification of Mg^{++} and Ca^{++} in necrotic fat is the underlying mechanism). Paunier disease or hypomagnesemia with secondary hypocalcemia is a very rare defect of intestinal Mg^{++} resorption (usually combined with impaired renal Mg^{++} conservation), which presents early in infancy with hypocalcemia responsive to Mg^{++} administration. The disease is caused by a loss of function mutation in an ion channel of the transient receptor potential gene family called TRPM6 [45, 46].

Renal Losses

Urinary Mg^{++} losses can be induced by different mechanisms.

Primary renal Mg^{++} wasting: these disorders are discussed in Chap. 37.

Drugs: Both loop and thiazide diuretics can inhibit net Mg^{++} reabsorption, while the K^+-sparing diuretics may lower excretion of Mg^{++}. The degree of hypomagnesemia induced by the loop and thiazide diuretics is generally mild, in part because the associated volume contraction will tend to increase proximal Na^+, water, and Mg^{++} reabsorption. Many further drugs can also produce urinary Mg^{++} wasting, as depicted in Table 34.23.

Volume expansion: Expansion of the extracellular fluid volume can decrease passive Mg^{++} transport. Mild hypomagnesemia may ensue if this is sustained.

Hypercalcemia: Ca^{++} and Mg^{++} seem to compete for transport in the thick ascending limb of the loop of Henle. The increased filtered Ca^{++} load in hypercalcemic states will deliver more Ca^{++} to the loop; the ensuing rise in Ca^{++} reabsorption will diminish that of Mg^{++}.

Miscellaneous: Mg^{++} wasting can be seen as part of the tubular dysfunction seen with recovery from acute tubular necrosis, following renal transplantation and during a postobstructive diuresis.

Alcohol: Excessive urinary excretion of Mg^{++} is common in alcoholic patients. Dietary deficiency, acute pancreatitis, diarrhea and refeeding also contribute to hypomagnesemia in these patients.

Further Causes

- Hypomagnesemia, together with hypophosphatemia, hypokalemia and increasing extracellular fluid volume, occurs in the context of refeeding syndrome (See: hypophosphatemia).
- Hypomagnesemia sometimes occurs in diabetes mellitus and is related in part to the degree of hyperglycemia.
- Hypomagnesemia can be seen following surgery, at least in part due to chelation by circulating free fatty acids.
- Hypomagnesemia can occur as part of the "hungry bone" syndrome in which there is increased Mg^{++} uptake by renewing bone following parathyroidectomy (for hyperparathyroidism).
- Hypomagnesemia is often encountered in cystic fibrosis patients with advanced disease, although sweat Mg^{++} concentration is normal in these patients. The causes are multiple, including aminoglycoside toxicity inducing renal magnesium-wasting and impaired intestinal magnesium balance. Interestingly, magnesium supplementation may lead to an improvement in respiratory muscle strength and mucolytic activity [48].

Neonatal Hypomagnesemia

Like in older children, in newborns hypomagnesemia may result from decreased Mg^{++} intake,

intestinal losses or renal losses. However, two peculiar causes of neonatal hypomagnesemia deserve consideration: (1) maternal hypomagnesemia and (2) intrauterine growth retardation.

- **Maternal hypomagnesemia:** Neonatal hypomagnesemia secondary to maternal hypomagnesemia is a recognized feature of maternal diabetes mellitus. However, maternal hypomagnesemia from any cause has been associated with neonatal hypomagnesemia.
- **Intrauterine growth retardation:** Hypomagnesemia sometimes occurs in infants whose birth weight is small in relation to their gestational age.

Symptoms, Signs, Consequences

Mg^{++} depletion is often associated with two biochemical abnormalities: (1) hypokalemia and (2) hypocalcemia. As a result, it is often difficult to ascribe specific manifestations solely to hypomagnesemia. The typical signs and symptoms of Mg^{++} depletion include tetany, positive Chvostek, Trousseau and Lust signs (the Lust sign, also called peroneal sign, is the dorsal extension and abduction of the foot, which is elicited by tapping the peroneal nerve on the lateral aspect of the fibula), or generalized convulsions. Generalized weakness and anorexia sometimes also occur. In addition, Mg^{++} depletion can induce ventricular arrhythmias, particularly during myocardial ischemia or cardiopulmonary bypass [40, 45, 46].

Hypokalemia

Hypokalemia, mostly accompanied by metabolic alkalosis, is common in hypomagnesemia. This association is in part due to underlying disorders that cause both Mg^{++} and K^+ loss, such as diuretic therapy and diarrhea. There is also evidence that concomitant Mg^{++} depletion aggravates hypokalemia and renders it refractory to treatment by potassium because Mg^{++} depletion increases distal K^+ secretion, as depicted in Fig. 34.14 [45, 46].

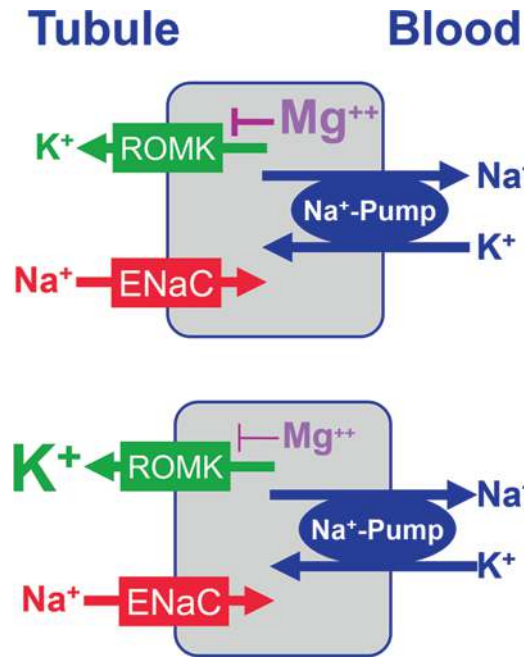

Fig. 34.14 Renal mechanism underlying hypokalemia in Mg^{++} depletion. In the distal nephron K^+ is taken up into cells across the basolateral membrane via Na^+ pump (blue oval) and secreted into luminal fluid via the apical ROMK K^+ channels (green rectangle). Na^+ is reabsorbed via epithelial Na^+ channels (ENaC, red rectangle). Intracellular Mg^{++} inhibits the ROMK K^+ channels and decreases K^+-secretion (upper panel). A decrease in intracellular Mg^{++} releases the Mg^{++}-mediated inhibition of ROMK K^+ channels, increases K^+-secretion (lower panel) and results in hypokalemia that is refractory to treatment by K^+. ROMK denotes renal outer medullary K^+ channel

Hypocalcemia

Hypocalcemia is the classical consequence of severe hypomagnesemia ($\leq$0.50 mmol/L). The following factors account for this tendency [40, 45, 46]:

- Inappropriately low circulating **parathyroid hormone** secretion.
- Inappropriately low **1,25-dihydroxyvitamin D**, the active metabolite of vitamin D.
- Bone resistance to **parathyroid hormone** (hypomagnesemia interferes with G protein activation in response to parathyroid hormone, thereby minimizing the stimulation of adenylate cyclase).

Repletion

Repletion of Mg^{++} is controversial in asymptomatic (mostly mild) hypomagnesemia. Oral repletion using lactate, oxide, pidolate or chloride salts is usually preferred. Because of the laxative effect of oral Mg^{++}, the amounts administered must be tailored to the individual patients (0.30 mmol/kg body weight of Mg^{++} per day in divided doses results in diarrhea in ≈10% of patients). The parenteral route is preferred in critically ill patients but the exact dosage is poorly understood. For true emergencies (e.g. generalized convulsions or ventricular arrhythmias) Mg^{++} is administered (either as sulphate or as chloride) intravenously over 1–2 min in a dosage of 0.15–0.20 mmol/kg body weight[13] (repeated if no response 5–10 min later). In subjects with moderate to severe but rather oligosymptomatic Mg^{++} deficiency, the mentioned dose is given over 4–6 h until circulating Mg^{++} returns to normal.

Inorganic Phosphate

Balance

In a 70 kg man, the body phosphate content amounts to ≈1% of the body weight, or 700 g, of which 85% is contained in the bone tissue and teeth, 14% in the soft tissues, and the remaining 1% in extracellular fluids [49].

In the blood, phosphate is found both as organic as well as inorganic salt but clinical laboratories measure the inorganic form. Of the circulating inorganic phosphate, ≈10% is bound to proteins, 5% is complexed with Ca^{++}, Mg^{++} or Na^{+} and 85% exists as ionized phosphate. The normal blood concentration of inorganic phosphate is highest during the neonatal period and early childhood and declines thereafter (Table 34.24) because infants and children retain phosphate avidly. There is a mean diurnal variation in concentration of phosphate of ≈0.20 mmol/L (≈0.6 mg/dL) with a nadir at 11.00 h, subsequently rising to a plateau at 16.00 h and peaking in the early night.

Table 34.24 Fasting values for circulating inorganic phosphate, fractional phosphate excretion and maximal tubular phosphate reabsorption in infancy and childhood

Age	Blood inorganic phosphate, mmol/L[a]	Fractional phosphate excretion, 10^{-2}	Maximal tubular reabsorption of phosphate, mmol/L[a]
0–3 months	1.62–2.40	11.9–38.7	1.02–2.00
4–6 months	1.78–2.21	3.50–34.9	1.27–1.88
6–12 months	1.38–2.15	10.3–20.0	1.13–1.86
1–2 years	1.32–1.93	5.50–23.3	1.05–1.74
3–4 years	1.02–1.92	≤18.4	0.90–1.78
5–6 years	1.13–1.73	0.60–15.0	1.02–1.62
7–8 years	1.06–1.80	≤16.8	0.98–1.64
9–10 years	1.13–1.70	1.80–14.1	1.00–1.58
11–12 years	1.04–1.79	1.80–12.1	0.97–1.65
13–15 years	0.97–1.80	≤12.6	0.91–1.68

[a] To obtain traditional units (mg/dL) multiply by 3.1

The average diet of a 70 kg man provides 800–1500 mg phosphate daily. As much as 2/3 of the dietary phosphate is absorbed in the gut but intestinal secretion, mainly in saliva and bile acids, adds 200 mg of phosphate into the intestinal lumen daily. Under steady-state conditions, the kidney is the most important modulator of the blood phosphate level, ensuring that urinary phosphate output is equivalent to the net phosphate absorption from the intestine. Phosphate is freely filtered across the glomerulus, and 80–90% of the phosphate is reabsorbed by the renal tubules (mostly in the proximal tubule) in subjects aged 6 months or more (Table 34.24). The renal tubular handling of phosphate is best expressed as fractional excretion of phosphate or, more precisely, as maximal tubular reabsorption of phosphate, which clarifies the relationship between circulating phosphate and urinary phosphate excretion. The fractional clearance of phosphate, the tubular phosphate reabsorption and the maximal tubular phosphate reabsorption are easily calculated, following an overnight fast, from plasma (P_{Ph}) and urinary (U_{Ph}) phosphate, and plasma (P_{Cr}) and urinary (U_{Cr}) creatinine, as follows [50]:

$$\text{Fractional excretion} = \frac{\mathbf{U_{Ph}} \times \mathbf{P_{Cr}}}{\mathbf{P_{Ph}} \times \mathbf{U_{Cr}}}$$

[13] Approximately 3.5–4.5 mg/kg body weight of elemental magnesium.

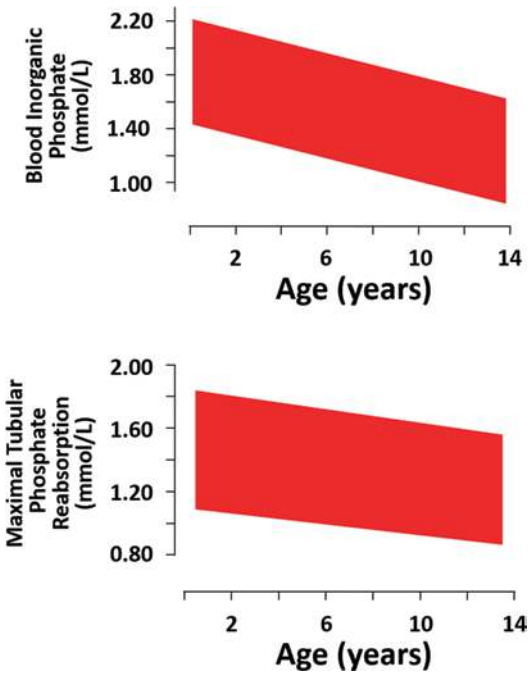

Fig. 34.15 Influence of age on fasting blood inorganic phosphate and maximal tubular reabsorption of phosphate. Blood inorganic phosphate and maximal tubular reabsorption of phosphate measured in infants, children and adolescents [50]

$$\text{Tubular phosphate reabsoption} = 1 - \frac{\mathbf{U_{Ph}} \times \mathbf{P_{Cr}}}{\mathbf{P_{Ph}} \times \mathbf{U_{Cr}}}$$

$$\text{Maximal reabsoption} = \mathbf{P_{Ph}} - \left(\frac{\mathbf{U_{Ph}} \times \mathbf{P_{Cr}}}{\mathbf{U_{Cr}}} \right)$$

The reference values [50] for the fractional excretion of phosphate and the maximal tubular reabsorption of phosphate are age dependent and appear in Table 34.24 and Fig. 34.15.

Three groups of hormonal factors regulate phosphate homeostasis [49]:

(a) **1,25-dihydroxyvitamin D** stimulates the intestinal phosphate absorption;
(b) **Parathyroid hormone** decreases the renal tubular reabsorption and causes phosphaturia;
(c) **"Phosphatonins"** are phosphaturic factors other than parathyroid hormone. **Fibroblast growth factor 23** (FGF-23) is currently considered the most important phosphatonin. FGF-23 is secreted by osteocytes and osteoblasts in response to phosphate loading or increased serum $1,25(OH)_2D_3$ levels and mainly targets the kidney in order to regulate the reabsorption of phosphate, the production and catabolism of 1,25-dihydroxyvitamin D and the expression of α-Klotho, an FGF-23 co-receptor and anti-ageing hormone.

Hypophosphatemia

Hypophosphatemia does not necessarily mean phosphate depletion since it can occur in the presence of a low, normal, or high total body phosphate. On the other hand, phosphate depletion may exist with normal, low, or elevated levels of blood phosphate [51, 52].

The normal phosphate level in adolescents and adults ranges between 0.97 and 1.80 mmol/L (2.9–5.4 mg/dL). In this age group, hypophosphatemia is arbitrarily divided into moderate cases (phosphate 0.32–0.65 mmol/L or 0.96–1.95 mg/dL) and severe cases (phosphate <0.32 mmol/L or <0.96 mg/dL). There are three major mechanisms by which hypophosphatemia can occur: (1) low dietary intake or poor intestinal absorption, (2) internal redistribution, and (3) increased urinary loss. In patients with hypophosphatemia caused by decreased intestinal absorption or internal redistribution the fractional excretion of phosphate and the maximal tubular reabsorption of phosphate are normal (Table 34.25). On the contrary, these parameters are low or inappropriately normal in patients with increased urinary losses [51–53].

Low Dietary Intake or Poor Intestinal Absorption

Given the fact that phosphate is ubiquitous in foods, the development of deficiency would be anticipated only in severe cases of malnutrition or in very-low-birth weight infants at the time of rapid postnatal growth. If phosphate restriction is severe and prolonged, or if intestinal absorption is reduced by the chronic use of phosphate binders, then the constantly reduced intestinal delivery may induce phosphate depletion.

Table 34.25 Causes of hypophosphatemia

• **With normal maximal tubular phosphate reabsorption and fractional excretion of phosphate**
– Low dietary intake or poor intestinal absorption
Low dietary intake: severe malnutrition, very-low-birthweight infants
Poor absorption: steatorrhea, chronic diarrhea, use of phosphate binders
– Internal redistribution
Refeeding syndrome in malnutrition (including diabetic ketoacidosis treated with insulin)
Respiratory alkalosis
Hungry bone syndrome after parathyroidectomy
• **With increased fractional excretion of phosphate**
– Hyperparathyroidism
– De Toni-Debré–Fanconi syndrome (= general impairment of the proximal tubule)[a]
– Gitelman syndrome and Bartter syndromes[b]
– Hypophosphatemic rickets[c]
– After kidney transplant (= posttransplant hypophosphatemia)

[a] Various drugs may cause an incomplete or, more rarely, a complete form of De Toni-Debré–Fanconi syndrome with hypophosphatemia including paracetamol poisoning, and treatment with ifosfamide, valproic acid, the iron chelator deferasirox or $ß_2$-adrenoreceptors (e.g.: albuterol)

[b] Hypophosphatemia is rather mild in these post-proximal tubular disorders

[c] At least in part explained by increased activity of fibroblast growth factor 23

Internal Redistribution

In the majority of cases, an acute shift in phosphate from the extracellular to the intracellular compartment is primarily responsible for lowering phosphatemia. The most frequent cause is refeeding syndrome, a recognized and potentially fatal condition that occurs when previously malnourished patients are fed. The fluid and electrolyte abnormalities noted in the refeeding syndrome and those noted in severe diabetic ketoacidosis following the administration of insulin therapy are similar.

Patients who are malnourished develop a total body depletion of phosphate, Mg^{++} and K^{+}. Nonetheless, their blood levels are maintained by redistribution from the intracellular space. The delivery of glucose as part of a feeding strategy causes a huge increase in the circulating insulin level that induces a rapid uptake of glucose, K^{+}, phosphate and Mg^{++} into cells. The blood concentration of these metabolites falls dramatically. In addition, the body begins to retain fluid, and the extracellular space expands. Although hypophosphatemia is the predominant feature of the syndrome, rapid falls in K^{+} and Mg^{++} levels, together with some tendency towards metabolic alkalosis, predispose to cardiac arrhythmias, while extracellular space expansion can precipitate acute heart failure in patients with cardiovascular disease. The most effective way to treat refeeding syndrome is to be aware of it. One should start feeding slowly and aggressively supplement and monitor phosphate, K^{+} and Mg^{++} for 4 days after feeding is started.

Another cause of hypophosphatemia in hospitalized patients is respiratory alkalosis. Severe hyperventilation can be seen in patients with anxiety, pain, sepsis, and in patients during mechanical ventilation. The fall in carbon dioxide will result in a similar change in the cells because carbon dioxide readily diffuses across cell membranes. The elevated pH stimulates the glycolysis, leading to an accelerated production of phosphorylated metabolites and a rapid shift of phosphate into the cells.

The hungry bone syndrome, characterized by massive deposition of Ca^{++} and phosphate in the bone, can occur after parathyroidectomy for long-standing hyperparathyroidism (both primary and secondary).

Urinary Loss

1. In hyperparathyroidism, both primary and secondary, there is an increased urinary loss of phosphate.
2. Fanconi-De Toni-Debré syndrome is characterized by a general impairment of the proximal tubule leading to urinary loss of compounds normally reabsorbed by the proximal tubule. It results in hypophosphatemia, glucosuria, hyperaminoaciduria, uricosuria, and hyperbicarbonaturia (causing renal tubular acidosis).
3. The urinary phosphate excretion is also increased in patients with hereditary hypophosphatemic rickets and tumor-induced osteomalacia and rickets, as discussed in Chap. 55.

4. Recent data demonstrate a mild tendency towards urinary loss of phosphate in Gitelman and Bartter syndromes [54].
5. Following kidney transplant, hypophosphatemia has been described in the absence of both hyperparathyroidism and other signs of proximal tubule dysfunction.

Symptoms, Signs, Consequences

Phosphate depletion can cause a variety of symptoms and signs [49, 51–53]. Two major mechanisms are responsible for these symptoms: (a) decrease in intracellular adenosine triphosphate, and (b) in diphosphoglycerate. In adults, hypophosphatemia is symptomatic when the phosphate level is <0.35 mmol/L. Hypophosphatemia may be asymptomatic under certain clinical situations: patients recovering from diabetic ketoacidosis and patients with prolonged hyperventilation are usually asymptomatic because often there is not real phosphate depletion. The clinical features of phosphate depletion appear in Table 34.26.

Management

Hypophosphatemia does not automatically mean that phosphate replacement is indicated. To determine whether treatment is indicated, it is necessary to establish the cause of the hypophosphatemia, in which the history and the clinical setting are important. The identification and treatment of the primary cause usually leads to normalization of the circulating phosphate level. As an example, the hypophosphatemia found in patients with diabetic ketoacidosis will usually correct spontaneously with normal dietary intake. However, replacement therapy is needed in patients with hypophosphatemia in combination with evidence of renal or gastrointestinal phosphate loss, the presence of underlying risk factors, and particularly if there are the clinical manifestations described above [53].

The safest mode of therapy is oral. Cow's milk is a good phosphate source: it contains 1 g (32 mmol) elemental phosphate per liter. Alternatively, oral preparations in the form of sodium phosphate or potassium phosphate can be used. The average adult patient requires 1–2 g (32–64 mmol) phosphate per day for 7–10 days to replenish body stores. An important side effect of oral supplementation is diarrhea.

Intravenous phosphate, usually 2.5–5.0 mg/kg (0.08–0.16 mmol/kg) over 6 h is given in symptomatic patients, who cannot take milk or tablets. More aggressive repletion with phosphate has been advocated but the magnitude of the response is unpredictable (close monitoring of phosphate level is crucial). Side effects of intravenous phosphate repletion are hypocalcemia, metastatic calcification, hyperkalemia associated with K^+-containing supplements, volume excess, hypernatremia, metabolic acidosis, and hyperphosphatemia.

Burosumab, a fully human monoclonal antibody against FGF-23, is used for the treatment of hereditary hypophosphatemic rickets. The drug has demonstrated superior efficacy in correcting hypophosphatemia and clinical symptoms compared to conventional therapy consisting of oral phosphate and active vitamin D (Chap. 55).

Table 34.26 Symptoms, signs and consequences of phosphate depletion

• Skeletal muscle and bone: proximal myopathy, rhabdomyolysis[a]
• Cardiovascular system: impaired myocardial contractility
• Respiratory system: respiratory failure (and failed weaning)
• Neurological system: paresthesias, tremors, seizures, features resembling Guillain-Barré syndrome or Wernicke encephalopathy
• Hematological system: hemolysis, impaired granulocyte chemotaxis and phagocytosis causing Gram-negative sepsis, altered platelet function and thrombocytopenia

[a] In patients with rhabdomyolysis, hypophosphatemia can be masked by the release of phosphate from the injured muscle

Hyperphosphatemia

Spurious or artefactual hyperphosphatemia has been observed if hemolysis occurs during the collection or processing of blood samples. Spurious hyperphosphatemia due to interference with analytical methods occurs in patients with

hyperglobulinemia, hyperlipidemia or hyperbilirubinemia and following contamination with heparinized saline from indwelling catheters or in subjects receiving liposomal amphotericin [55]. True hyperphosphatemia indicates either an increased phosphate load or a decreased renal phosphate excretion, as shown in Table 34.27. High dietary ingestion of phosphate alone rarely causes hyperphosphatemia with the exception of newborns and infants fed cow's milk, whose phosphate content is six times greater than human milk [49].

Table 34.27 Causes of hyperphosphatemia

• **Artifactual or spurious**
• **Increased phosphate load (with normal fractional excretion of phosphate and normal maximal tubular phosphate reabsorption)**
– High dietary intake or increased intestinal absorption
Newborns and infants fed cow's milk (rather than breast milk or adapted formula milk)
Parenteral administration of phosphate salts
Large amounts of phosphate-containing laxatives, phosphate enemas[a]
Vitamin D intoxication
– Internal redistribution
Tumor lysis syndrome (before treatment and after initiation of cytotoxic therapy)
Rhabdomyolysis
Lactic and ketoacidosis (or severe hyperglycemia alone)[b], including severe dehydration in the context of acute diarrhea
• **Decreased renal phosphate excretion**
– Reduced renal function (either acute or chronic)
– Increased renal tubular phosphate reabsorption (= decreased fractional phosphate excretion and increased maximal tubular phosphate reabsorption)
Hypoparathyroidism (and pseudohypoparathyroidism)
Acromegaly
Drugs: growth hormone, bisphosphonates, dipyridamole
Idiopathic childhood nephrotic syndrome
Familial hyperphosphatemic tumoral calcinosis

[a] The danger of hyperphosphatemia secondary to phosphate enema is especially high in children less than 2 years of age

[b] Metabolic acidosis blunts glycolysis and therefore cellular phosphate utilization. In addition, tissue hypoxia or insulin deficiency also play a crucial role

Acutely or chronically impaired renal function plays at least a partial role in most instances of hyperphosphatemia, including physiologically low glomerular filtration rate to explain the inability of the neonate to eliminate excess phosphate, mild renal insufficiency (due to volume contraction secondary to diarrhea) in subjects ingesting large amounts of phosphate-containing laxatives or mild to moderate tubulointerstitial injury secondary to intrarenal accumulation of uric acid in tumor lysis syndrome (see "hyperkalemia").

Familial hyperphosphatemic tumoral calcinosis is a recessive disorder characterized by hyperphosphatemia due to an increased maximal tubular phosphate reabsorption. Affected subjects present with extra-articular soft tissue deposition of calcium phosphate. This very rare disease (a kind of mirror image of some forms of hypophosphatemic rickets) results from inactivating mutations that lead to deficiency of circulating fibrolast growth factor 23 [49].

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35 Renal Fanconi Syndromes and Other Proximal Tubular Disorders

Detlef Bockenhauer and Robert Kleta

Introduction

Fanconi first described the concept that defective renal proximal tubule reabsorption of solutes might contribute to "non-nephrotic glycosuric dwarfing with hypophosphataemic rickets in early childhood" [1]. Rickets and albuminuria secondary to kidney disease was described some 50 years previously but attributed to a disorder of adolescence [2]. Fanconi's first case presented at 3 months with rickets and recurrent fevers. She had glycosuria and albuminuria and progressed to terminal renal failure by 5 years of age. At autopsy the renal tubule cells appeared filled with crystals, which were thought to be cystine. In subsequent reports, Debré, de Toni and Fanconi all described series of children with rickets, glycosuria and albuminuria [3–5]. In acknowledgment of this pioneering work, we now refer to this symptom constellation as Fanconi-Debre-de Toni syndrome, or just short as "renal Fanconi syndrome" (RFS). The presentation, course and outcome of the described children, however, varied markedly. This reflects that RFS is not a uniform entity, but a diagnosis of proximal tubular dysfunction, which can be due to a variety of different causes (see Tables 35.1 and 35.2). RFS can be isolated or in the context of multiorgan disorders, congenital or acquired, transient or permanent, associated with progression to end-stage kidney disease (ESKD) or with stable kidney function throughout. Moreover, it can differ in the extent and severity of tubular dysfunction. Severe and generalized proximal tubular dysfunction is seen in cystinosis whilst many children with e.g. Dent disease or Lowe syndrome may have no clinically significant disturbance of phosphate and bicarbonate transport [39]. Indeed, there is some debate at what point proximal tubular dysfunction can be called RFS [40].

Here, we use the term RFS to include disorders with dysfunction of multiple proximal tubular pathways, but recognize that not every transport system need be affected. This clinical and biochemical heterogeneity is likely to arise from the multiple mechanisms involved in proximal tubular transport, reflecting not only the bulk of solute and water reabsorption but also the reuptake of proteins, amino acids, vitamins, cytokines and many other substances. RFS do not therefore have a common and single pathogenetic basis but reflect the interplay of a number of different biochemical processes. Identification of the underlying etiology is therefore of utmost importance, as it informs management and prognosis.

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Table 35.1 Congenital causes of renal Fanconi Syndrome (RFS) by age of onset. (Adapted from [6])

Onset	Disorder	Associated features	Diagnostic test
Neonatal	Galactosemia	Liver dysfunction, jaundice, encephalopathy, sepsis	Red cell galactose 1-phosphate uridyl transferase
	Mitochondrial disorders	Usually multisystem dysfunction (brain, muscle, liver, heart)	Lactate/pyruvate (plasma lactate may be normal due to urinary losses), muscle enzymology
	Tyrosinemia	Poor growth, hepatic enlargement and dysfunction	Plasma amino acids, urine organic acids (succinylacetone)
Infancy	Fructosemia	Rapid onset after fructose ingestion, vomiting, hypoglycemia, hepatomegaly	Hepatic fructose-1-phosphate aldolase B
	Cystinosis	Poor growth, vomiting, rickets ± corneal cystine crystals	Leukocyte cystine concentration, molecular diagnosis (*CTNS*)
	Fanconi Bickel syndrome	Failure to thrive, hepatomegaly, hypoglycemia rickets, severe glycosuria, galactosuria	molecular diagnosis (*GLUT2*)
	Lowe's syndrome	Males (X-linked), cataracts, hypotonia, developmental delay	Clinical and molecular diagnosis (*OCRL*)
	RFS—GATM	Autosomal dominant, RFS recognisable in infancy, usually "mild" RFS, advanced CKD in adulthood	molecular diagnosis (*GATM*)
	RFS—EHHADH	Autosomal dominant, RFS recognisable in infancy, no progressive CKD	molecular diagnosis (*EHHADH*)
Childhood	Cystinosis	see above	
	Dent disease	Males (X-linked), hypercalciuria, nephrocalcinosis	Molecular diagnosis (*CLCN5, OCRL*)
	Wilson's disease	Hepatic & neurological disease, Kayser-Fleischer rings	Copper, coeruloplasmin, molecular diagnosis (*ATP7B*)

Table 35.2 Acquired causes of the renal Fanconi syndrome

Drugs and toxins
Anti-cancer drugs
Ifosfamide (see text)
Streptozocin [7, 8]
Antibiotics
Aminoglycoside (see text)
Expired tetracyclines [9, 10]
Anti-retrovirals
Adefovir/Cidofovir/Tenofovir [11–15]
ddI [16, 17]
Heavy metals
Lead poisoning [18]
Cadmium [19]
Sodium valproate [20]
Aristolochic acid (Chinese herb nephropathy) [21–23]
Toluene/Glue sniffing [24]
Fumaric acid [25]
Suramin [26]
Paraquat [27]
L-Lysine [28]
Renal disorders
Tubulointerstitial nephritis [29]
Membranous nephropathy with anti-tubular basement antibodies [30–38]

Clinical Features

The presenting clinical features of RFS in childhood are usually failure-to-thrive and rickets, although patients with RFS in the context of a systemic disorder may first be identified via the extra-renal manifestations, such as the cataracts in Lowe syndrome, or myopathy in mitochondrial cytopathies.

The failure-to-thrive is presumably due to the high-volume losses, with patients preoccupied with drinking, rather than caloric intake. Some patients exhibit features of a secondary nephrogenic diabetes insipidus, further compounding the water losses from impaired proximal reabsorption [41, 42].

Rickets is the consequence of renal phosphate losses (see below), as well as impaired proximal hydroxylation of vitamin D. The critical step in the formation of active Vitamin D (i.e. 1,25 OH-cholecalciferol) is mitochondrial 1α-hydroxylation in the proximal tubule. For this to occur, cholecalciferol, bound to its car-

rier vitamin D-binding protein (a low-molecular weight protein) needs to be reabsorbed from the tubular lumen, a process impaired in RFS [43].

Biochemical Abnormalities

Excessive urinary levels of a wide range of solutes and substances normally reabsorbed in the proximal tubule are the biochemical hallmarks of RFS.

Proteinuria

Proteinuria **in RFS** is made up of albumin, low molecular weight proteins and tubular enzymes, such as retinol binding protein (RBP), α-1 microglobulin, β-2 microglobulin, N-acetylglucoseaminidase, alanine aminopeptidase. The urinary level of these very sensitive markers of proximal tubular dysfunction is markedly elevated, especially if RBP is assayed [44, 45]. Albuminuria can be highly variable in RFS, but is typically below nephrotic range proteinuria [46]. In a family with autosomal dominant isolated RFS without apparent glomerular dysfunction the total amount was roughly 1g per day in affected adults [47]. This is consistent with some estimates of the amount of filtered albumin, which would usually undergo tubular reabsorption, at 0.4–1 g albumin per 1.73 m^2 per day [48, 49]. However, more recent estimates suggest that the amount of filtered albumin may actually be much higher, reaching several grams per day [50]. Indeed, there are multiple reports of patients with genetically defined defects in proximal tubular protein reabsorption, such as pathogenic variants in *OCRL*, *CLCN5* or *CUBN*, excreting several grams of protein in the urine per day [51–53]. Whether this amount of albuminuria is solely tubular or does reflect secondary glomerular damage remains controversial [54]. Interestingly, patients with proximal tubular disorders, even when they have nephrotic range proteinuria, typically have normal levels of plasma albumin and do not exhibit oedema [53]. Thus, the total amount of protein loss in RFS can be variable, reflecting the degree of impairment of reabsorption and potential concomitant glomerular dysfunction.

It is important to remember that urine dipsticks primarily detect larger proteins including albumin and thus can miss the mostly low-molecular weight proteinuria of RFS. Tubular proteinuria may be seen in some forms of nephrotic syndrome, likely reflecting associated tubulointerstitial damage [55, 56].

Aminoaciduria

The aminoaciduria seen in RFS is generalized and its pattern is influenced by plasma values, so that in rare situations of severe protein malnutrition, aminoaciduria as analyzed on thin-layer chromatography, may be recorded as “normal” or “mild” [44]. Quantitative analysis in urine and plasma by ion-exchange chromatography should be used to determine the degree and specific nature of aminoaciduria.

Organic Aciduria

Organic acids, including citrate and uric acid, are also exclusively reabsorbed in the proximal tubule and excretion is thus increased in RFS [57]. Consequently, patients with RFS typically have hyperuricosuria with hypouricemia, as well as hypercitraturia [58]. Moreover, transport of drugs, such as probenecid, furosemide or penicillin, can be affected, potentially altering pharmacokinetics [59]. The increased excretion of lactate can lead to normalization of plasma lactate levels in mitochondrial cytopathies, resulting in another potential diagnostic pitfall [60].

Glycosuria

Renal glycosuria in RFS reflects the impaired proximal tubular ability for glucose reabsorption, so that glycosuria occurs with normal blood glucose levels. As RFS is typically associated with marked polyuria, the urinary glucose concentration may be less than the 5 mmol/L and thus missed by dipstick measurement [39]. Formal

laboratory measurement, ideally of a 24-h urine collection, should thus be used to detect and quantify glycosuria. Normally, less than 1.5 mmol (300 mg)/day/1.73 m^2 are excreted [61], but this can increase to several grams daily with defects in proximal tubular reabsorption [62].

Renal Tubular Acidosis

Since the proximal tubule is the key site for bicarbonate reabsorption, bicarbonaturia is a typical feature of RFS with consequent metabolic acidosis (see also Chap. 35). The degree to which the threshold for reabsorption is reduced is variable, according to the underlying cause of RFS. In severe acidosis, filtered bicarbonate is reduced to a level below the threshold for proximal reabsorption and urine pH falls below 5.3 if distal acidification is intact. The hyperchloremic metabolic acidosis contributes to loss of skeletal calcium and consequent hypercalciuria. Unfortunately, treatment with alkali supplementation is complicated by the ongoing proximal losses, as soon as the plasma bicarbonate level rises above the threshold capacity for reabsorption. Frequent daily dosing of alkali supplementation can help to sustain bicarbonate levels in the normal range, or at least closer to it.

Phosphaturia

Renal phosphate wasting with secondary hypophosphatemia is another hallmark of proximal tubular dysfunction, leading to rickets or bone disease. Urine phosphate handling is usually assessed as the tubular reabsorption (TRP), the complement to the fractional excretion of phosphate (FEP). Thus, it is calculated as: TRP [%] = 100 − FEP [%]. Usually, a TRP >70% is considered normal, however this can be misleading. If the plasma phosphate level is decreased, the filtered load of phosphate may be close to or below the threshold of tubular phosphate reabsorption and TRP can therefore be misleadingly "normal". To account for the filtered load, urinary phosphate excretion can be assessed using the tubular threshold concentration for phosphate excretion, corrected for glomerular filtration rate (TmP/GFR) [63]. It is calculated as follows: TmP/GFR = (Phosphate plasma − Phosphate urine/Creatinine urine × Creatinine plasma). Normal values are age-dependent and listed in Table 35.3.

Table 35.3 Normal age-specific values for TmP/GFR (derived from [64–66])

Age	mmol/L
<1 month	1.48–3.43
1–3 months	1.48–3.30
4–6 months	1.15–2.60
7 months–2 years	1.10–2.70
2–4 years	1.04–2.79
4–6 years	1.05–2.60
6–8 years	1.26–2.35
8–10 years	1.10–2.31
10–12 years	1.15–2.58
12–15 years	1.18–2.09
>15 years	0.80–1.35

Hypercalciuria

Approximately 70% of filtered calcium is reabsorbed in the proximal tubule and, consequently hypercalciuria is another characteristic of RFS. It is further compounded by the acidosis-mediated calcium release from bone (see above). Nephrocalcinosis and stone formation can ensue, but interestingly is rather uncommon. Presumably, the polyuria inherent in RFS is protective against these complications, as may be the increased luminal concentration of citrate (see organic aciduria, above) [67].

Hypokalemia

Potassium, like almost all other electrolytes, is predominantly reabsorbed in the proximal tubule, making hypokalemia another typical feature. It can further be compounded by hyperaldosteronism if electrolyte and fluid losses result in volume contraction [68–70].

Other Substances

Carnitine is reabsorbed in the proximal tubule and is therefore lost in excess in RFS. Low plasma carnitine concentrations have been reported in children with cystinosis and tyrosinemia [71, 72] leading to plasma and muscle deficiencies of carnitine, which could contribute to the myopathy in these disorders. Moreover, losses of vitamins, carrier proteins and chemokines have all been described in RFS [46, 73].

Pathogenesis

The variation in etiology, manifestations and severity of RFS make it unlikely that there is a single common pathogenetic mechanism. Most studies of the pathogenesis have, of necessity, focused on one biochemical pathway. However, *in vivo*, it is more likely that a number of interlinked biochemical processes are disrupted in a variable manner.

Disruption of Energy Production

The high transport activity of the proximal tubule requires a large amount of energy. Thus, disruption of the energy supply is an obvious etiology of RFS. Consequently, it is not surprising that RFS is a frequent complication of mitochondrial cytopathies [60]. In fact, most inherited primary forms of RFS that have been genetically solved to date appear to be associated with mitochondrial dysfunction [74–76] (see below: Primary renal Fanconi syndromes).

Mitochondrial dysfunction has also been implicated in the development of RFS in cystinosis (see Chap. 30). Similar studies have been undertaken in models of tyrosinemia, which is associated with excessive accumulation of succinylacetone (SA). SA reduced sodium-dependent uptake of sugar and amino acids across rat brush border membranes [77] and intra-peritoneal injection of SA to rats led to development of RFS [78]. SA inhibits sodium-dependent phosphate transport by brush border membrane vesicles, decreases ATP production and inhibits mitochondrial respiration [79]. Administration of maleic acid, used to create an animal model of RFS, causes a reduction in ATP and phosphate concentrations, NaK ATPase activity and coenzyme A [80].

Glutathione Depletion

Historically, another line of investigation has looked into the role of glutathione (GSH) in the pathogenesis of RFS. GSH has a number of key cellular roles including post-translational protein modification, xenobiotic detoxification and it acts as a major antioxidant. Some such studies have mainly focused on cystinosis (Chap. 30), yet deficiency of GSH has also been implicated in other forms of the RFS. Ifosfamide toxicity, which leads to RFS, may be mediated by its interaction with γ-glutamyl transpeptidase, a precursor of GSH synthesis, and by hepatic metabolism to chloroacetaldehyde [81]. Incubation of chloroacetaldehyde with isolated human renal proximal tubules was associated with depletion of GSH, coenzyme A, acetyl-coenzyme A and ATP [82]. Wistar rats injected with ifosfamide develop RFS, associated with GSH depletion which is attenuated by treatment with melatonin [82]. Addition of ochratoxin A, the presumed toxin causing RFS in Balkan Endemic Nephropathy, to rat proximal tubular cells causes an elevation of reactive oxygen species and depletion of cellular GSH [83]. However, other data suggest that specific renal proximal tubular uptake and metabolism of ifosfamide is causative for the side effect of RFS [84].

Reduced Activity of Cotransporters

Increased solute excretion in RFS could result from reduced expression or activity of sodium-coupled cotransporters, which mediate proximal tubular reabsorption. In the animal model of maleic acid induced RFS, decreased *Slc34a1* mRNA expression and consequent reduced NaPi2a protein were observed [85].

Mice lacking hepatocyte nuclear factor 1 alpha (HNF1α), a transcription factor expressed in liver, pancreas, kidney and intestine, develop RFS, abnormal bile metabolism and diabetes [86]. HNF1α −/− mice had reduced expression of sodium-coupled transporters for glucose (SGLT2) and phosphate (NaPi1 and NaPi4) but normal levels of NaPi2a, the major phosphate transporter [87]. In addition, one primary form of proximal tubular dysfunction (see below: Primary renal Fanconi syndromes) is associated with a homozygous mutation in *SLC34A1* [88].

Disruption of the Endocytic Pathway (Megalin/Cubilin)

An important task of the proximal tubule is to reabsorb filtered proteins, including peptide hormones, as well as small carrier proteins binding fat-soluble vitamins and trace elements. Therefore, by reabsorbing filtered proteins the proximal tubule actively participates in the homeostasis of hormones, trace elements and vitamins. In addition, some lipoproteins, such as apolipoprotein A-I and A-IV are also reabsorbed in the proximal tubule [89]. Reabsorption of the vast majority of filtered proteins is mediated by two endocytic receptors: megalin and cubilin (reviewed in [90, 91]). These receptors contain several protein-binding domains and protrude from the microvilli, which make up the brush border of the proximal tubule, into the tubular lumen. Once a protein is attached, the receptor-ligand complex moves towards the base of the microvilli into clathrin-coated pits, which then bud off into the cytoplasm to form endosomes (see Fig. 35.1). Subsequently, the receptors are recycled back to the membrane to mediate further uptake. The fate of the protein ligands is different: most are degraded by acid hydrolysis after fusion of the endosome to a lysosome, while others, such as vitamins, are released back into the blood circulation across the basolateral membrane. In this fashion, the megalin/cubilin complex assumes a role in the regulation of several hormonal pathways, the importance of which becomes apparent, when considering, for instance, calcium-regulation: Megalin competes with the PTH receptor for the binding of filtered PTH and renders it non-functional by endocytosis and subsequent delivery to a lysosome for degradation [93]. In contrast, megalin/cubilin facilitates activation of Vitamin D by binding of filtered 25-OH vitamin D-Vitamin D binding protein and thus allowing uptake into the proximal tubule cell and activation by 1α-hydroxylase [43, 94].

Megalin is a large transmembrane protein belonging to the family of low-density lipoprotein receptors and was originally identified as the target of antibodies causing Heymann nephritis in rats [95]. It is expressed in epithelial cells of a variety of other tissues active in endocytosis [92]. Megalin-deficient mice mostly die in utero, but those that survive indeed show Fanconi-type low-molecular weight proteinuria, confirming the central role of megalin in endocytosis in the proximal tubule [96]. Interestingly, mutations in *LRP2*, the gene encoding megalin, were identified as the cause of Donnai-Barrow syndrome [97]. Donnai-Barrow syndrome is characterized by facial and ocular anomalies, sensorineural hearing loss and proteinuria. However, whilst these patients have, as expected, low-molecular weight proteinuria, there is no generalized proximal tubular dysfunction [98].

Cubilin is a peripheral membrane protein and is dependent on megalin to initiate endocytosis after ligand binding. These two proteins are co-expressed in proximal tubule and along the endocytic pathway and work in tandem to mediate protein uptake [90, 91]. Cubilin is otherwise known as the intrinsic factor-vitamin B12 receptor [99]. Loss-of-function mutations are associated with juvenile megaloblastic anemia or Imerslund-Graesbeck disease [100, 101]. The anemia is caused by deficient intestinal endocytosis of intrinsic factor-vitamin B12. In addition, some of these patients also have a selective low-molecular weight proteinuria that identifies those proteins requiring cubilin for endocytosis, such as albumin, transferrin, immunoglobulin light chains and α1- and β2-microglobulin [102]. Yet, as with Megalin, mutations in Cubilin are not associated with complete RFS.

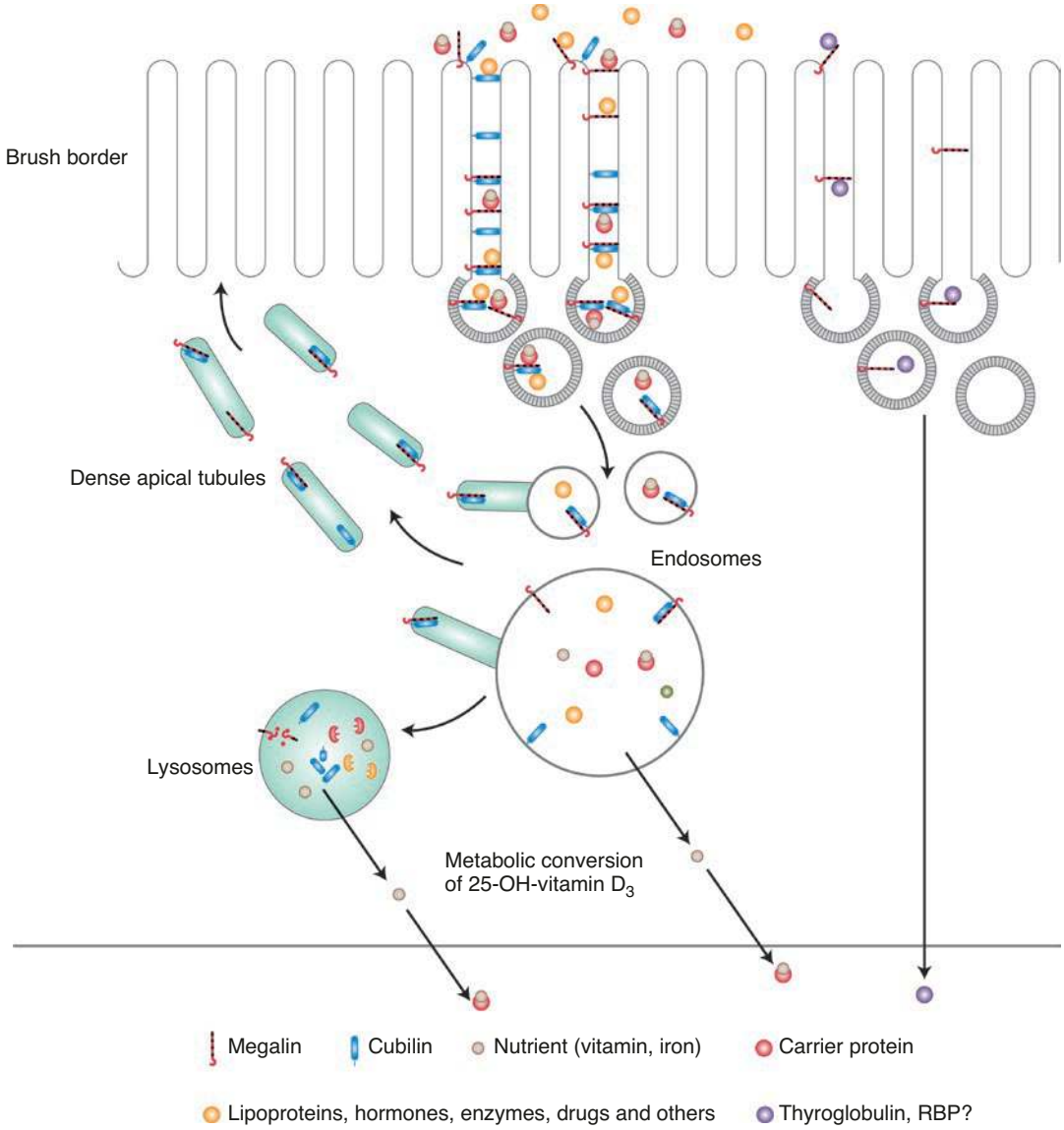

Fig. 35.1 Role of Megalin and Cubilin in proximal tubular transport. The receptors Megalin and Cubilin stick out from the brush border into the lumen of the proximal tubule. Once bound to a ligand (such as LMWP, nutrients etc.), the complex is endocytosed and ligand and receptor are separated by the low pH in the endosomes. The receptors are subsequently recycled to the membrane. (From [92])

Decreased expression of both megalin and cubilin at the brush border has been described in a mouse model of Dent disease [103]. Decreased levels of megalin have also been found in the urine of patients with Dent disease and Lowe syndrome, suggesting defective trafficking of the receptors as a mechanism of the low-molecular weight proteinuria seen in affected patients [104, 105]. The loss of vitamins, hormones and trace elements associated with endocytic dysfunction may explain some of the clinical heterogeneity seen in RFS.

Primary Renal Fanconi Syndromes

Sometimes, RFS appears in patients without an identified cause. The majority of these primary or idiopathic cases occur in adulthood, but some have also been reported in children [106]. A small number of cases occur in families and different modes of inheritance have been reported [47, 107–111]. Amongst the families with autosomal-dominant inheritance two distinct forms are recognized: (1) RFS with progressive chronic kidney disease (OMIM 134600), which is related to mutations in *GATM* [75] and (2) RFS with preserved GFR into advanced age (OMIM 615605) [88]. *GATM* encodes arginine-glycine amidinotransferase, an enzyme involved in creatine synthesis and recessive mutations are associated with a neurological disorder, cerebral creatine deficiency (OMIM 612718). However, the dominant mutations associated with RFS do not cause creatine deficiency and affected patients have no associated neurological manifestations. Instead, these dominant mutations cause linear aggregates of GATM in mitochondria, which are visible on electron microscopy in biopsy specimen. These aggregates appear to affect mitochondrial fission with consequent elongation of mitochondria, enhanced reactive oxygen species production and activation of the inflammasome, which in turn, is thought to contribute to tubulointerstitial fibrosis and progressive chronic kidney disease [75].

In RFS with preserved GFR so far only one specific dominant mutation in *EHHADH* has been identified (c.7G>A). *EHHADH* encodes a peroxisomal enzyme involved in fatty acid oxidation and, similar to *GATM*, this mutation does not lead to enzyme deficiency. Instead, the variant creates a novel mitochondrial targeting motif, leading to misrouting of the enzyme from the peroxisome to the mitochondria, where it interferes with fatty acid oxidation [74, 112]. While EHHADH is expressed in all organ systems, clinical manifestations appear restricted to the proximal tubule only, highlighting the dependence of the proximal tubule on mitochondrial fatty acid oxidation, rather than glucose metabolism [74, 112–114].

Another dominantly inherited form of RFS is associated with impaired insulin secretion (initially hyperinsulinism, later maturity onset diabetes in the young, OMIM 616026) and due to a specific missense mutation in *HNF4A* (p.Arg85Trp, also annotated as p.Arg63Trp or p.Arg76Trp, depending on reference sequence), encoding a transcription factor expressed in liver, pancreas and kidney [115, 116]. Interestingly, other dominant mutations in *HNF4A* are only associated with impaired insulin secretion, but not RFS. The exact mechanisms of how this particular mutations causes RFS are unclear, but impaired phosphorylation with subsequent changes in localization of HNF4A has been reported [76].

An autosomal recessive form of an incomplete RFS in two siblings has been attributed to a specific mutation in the phosphate transporter NaPi2a (*SLC34A1*), an in-frame 21bp duplication g.2061_2081dup; p.I154_V160dup [88]. It is yet to be clarified, how exactly this specific defect in proximal tubular phosphate transport causes a more generalized proximal tubular dysfunction, especially when considering that recessive mutations in *SLC34A1* have since been identified as the cause of a different disorder, infantile hypercalcaemia [117]. Interestingly, the specific mutation initially linked to proximal tubular dysfunction was subsequently linked also to infantile hypercalcaemia [118].

Treatment of these primary forms is symptomatic and in those with kidney failure, transplantation is an option.

Secondary Forms of Inherited Renal Fanconi Syndromes

Cystinosis

Nephropathic cystinosis, the most common cause of RFS in children, is covered in Chap. 30.

Tyrosinemia

The tyrosinemias are a group of disorders affecting the metabolism of tyrosine. The most severe one is tyrosinemia type 1, due to a defect in the enzyme fumarylacetoacetate hydrolase. Severity of clinical symptoms is variable but typically includes hepatic dysfunction with progression to cirrhosis and risk of hepatic cancer, as well as a porphyria-like neuropathy. In addition, patients can develop a severe RFS and chronic renal impairment may eventually ensue. The enzymatic defect in tyrosinemia leads to an accumulation of succinylacetone, which is thought to cause the symptoms. In experimental models succinylacetone inhibits transport in the proximal tubule, potentially by inhibition of mitochondrial function [77–79]. In addition, it inhibits porphobilinogen synthetase, which may explain the porphyria-like neuropathy [119]. Further evidence for the pathogenic role of succinylacetone comes from the discovery that blockade of tyrosine metabolism further upstream effectively remedies the symptoms of tyrosinemia: mice deleted for the gene encoding fumarylacetoacetate hydrolase die in the neonatal period, but are rescued by the additional deletion of the 4-OH-phenylpyruvate dioxygenase (HPD) gene (the basis for tyrosinemia type 3) which prevents the accumulation of succinylacetone [120]. Similarly, administration of nitisinone, a blocker of HPD effectively prevents and even reverses the symptoms of tyrosinemia in the vast majority of patients [121]. Consequently, nitisinone is now the first line therapy for tyrosinemia together with a tyrosine- and phenylalanine-restricted diet [122]. In the roughly 10% of patients where this fails, liver transplantation is an option. However, even though transplantation corrects the enzymatic defect in the liver, elevated levels of succinylacetone are still found in the urine of these patients and some of them have persisting tubular defects [123, 124].

Mitochondrial Cytopathies

The proximal tubule has a high energy requirement in order to reabsorb the bulk of filtered solutes. Cellular energy is provided in the form of ATP, produced by the respiratory chain in mitochondria. Therefore, proximal tubular cells are rich in mitochondria and it is not surprising that the proximal tubule is particularly susceptible to mitochondrial dysfunction. Indeed, RFS is the most common renal manifestation of mitochondrial cytopathies [60]. The clinical manifestations of mitochondrial disorders are highly variable, but those with RFS typically have severe multi-organ involvement and present during infancy [60]. Neuromuscular manifestations usually predominate and the prognosis is often poor. Mitochondrial DNA mutations are inherited through the maternal line, as mitochondria derive from the maternal egg. An egg contains several mitochondria, each carrying their own DNA. Mutations in mitochondrial DNA can therefore be present in some mitochondria, but not in others within the same cell, a state termed heteroplasmy. Depending on the number of mitochondria with mutations passed on during cell division the ratio of mutated to healthy mitochondria can be highly variable within different tissues and cells, which may explain some of the clinical variability [125]. However, the majority of genes encoding the respiratory chain enzymes are encoded in the nuclear genome and mutations in these genes are typically inherited in autosomal-recessive fashion and affect all mitochondria uniformly.

An initial investigation in suspected mitochondrial cytopathies is typically to determine the ratio of lactate to pyruvate in the serum. However, in patients with RFS, this ratio is often normal, due to the grossly increased loss of organic acids in the urine [60]. Therefore, measurement of activity of respiratory chain enzymes should be performed in those patients with high suspicion of a mitochondrial cytopathy. Renal

histology is typically non-specific, showing tubular damage, but may show giant mitochondria [126, 127]. Some forms of mitochondrial cytopathies can be improved by supplementation with certain vitamins, especially those with a deficiency in the coenzyme Q10 [128]. Otherwise, no definitive treatment exists and management is only supportive for the renal manifestations.

Fanconi-Bickel-Syndrome

Fanconi-Bickel-syndrome is a rare autosomal-recessive glycogen-storage disease, caused by mutations in the gene *SLC2A2* encoding the glucose transporter GLUT2 [62, 129]. Patients typically present in infancy with hepatomegaly, failure-to-thrive and renal Fanconi-syndrome with excessive glucosuria [130]. GLUT2 is expressed in liver, intestine, pancreatic β-cells and proximal tubule cells. In hepatocytes, the transporter facilitates glucose uptake, as well as release. The impaired release leads to hepatomegaly and hypoglycemia during fasting, while the defective uptake causes post-prandial hyperglycemia. In the pancreas, it leads to impaired glucose sensing and insulin release [130, 131]. In the kidney, GLUT2 localizes to the basolateral membrane of the proximal tubule, easily explaining the excessive glucosuria, which has been reported to exceed 300 g per day [132] The RFS is less well understood, but may be due to impaired mitochondrial function [133]. Interestingly, *GLUT2*-deleted mice reproduce the hepatic and pancreatic phenotype and also have glucosuria, but RFS has not been reported [131]. The mouse model is therefore not helpful in understanding the mechanisms responsible for the RFS.

Mutations in *SLC2A2* associated with Fanconi-Bickel are typically severe, expected to completely abrogate GLUT2 function, although mutations with a milder phenotype with only mild glycosuria and LMWP have recently been described [134]. Interestingly, some heterozygous carriers of *SLC2A2* mutations can have isolated renal glucosuria and some milder recessive mutations, associated only with glucosuria and LMWP have also been described [134, 135].

Treatment consists of frequent feedings of slowly absorbed carbohydrates, as well as replacement of renal losses of water and solutes [136].

Fructose Intolerance

Fructose intolerance is due to a deficiency in the enzyme fructose-1-phosphate aldolase B, also simply called aldolase. Affected infants typically become symptomatic at weaning, with the introduction of fructose-containing food, such as fruits and vegetables. Patients develop nausea, vomiting and diarrhea and can progress to hypoglycemia, convulsions and shock. Proximal tubule dysfunction develops and is most obvious in the form of a renal tubular acidosis, which is compounded by accumulation of lactic acid in the blood. The mechanism of cellular dysfunction is thought to be intracellular phosphate depletion due to phosphorylation of accumulating fructose. Phosphate is required for the generation of the cellular fuel ATP [137]. Moreover, a direct association between aldolase and the vacuolar proton pump V-H^+-ATPase has been shown [138]. This pump is involved in bicarbonate reabsorption in the proximal tubule, as well as acid secretion in the distal tubule and an inhibition by defective aldolase may explain the pronounced acidosis seen in patients. Treatment consists of a fructose-free diet, which completely reverses the renal symptoms.

Galactosemia

A reversible and incomplete form of proximal tubular dysfunction can be seen in infants with classical galactosemia, an autosomal-recessive disorder due to loss-of-function of the enzyme galactose-1-phosphate uridyl transferase [139]. This is a key enzyme for the conversion of galactose to glucose. Affected infants typically present with failure-to-thrive and develop vomiting, diar-

rhea and jaundice after ingestion of galactose-containing feeds. Untreated, hepatomegaly with progression to cirrhosis, cataracts and mental retardation develop. Renal manifestation are aminoaciduria, albuminuria, acidosis and galactosuria, the latter due to the elevated blood galactose levels [140]. The mechanism of tubular dysfunction is unclear, but may be related to intracellular depletion of free phosphate, due to phosphorylation of the accumulating galactose.

The diagnosis is made by increased blood galactose levels and confirmed by demonstration of enzyme deficiency. Importantly, the RFS is completely reversible with treatment, which is the elimination of galactose from the diet.

ARC-Syndrome

The combination of arthrogryposis, renal dysfunction and cholestasis constitutes a rare autosomal recessive disorder, due to mutations in genes encoding vacuolar sorting protein involved in intracellular transport, including *VPS33B and VIPAR* [141, 142]. Affected neonates are identified by their contractures, conjugated hyperbilirubinemia and severe failure to thrive. In addition, giant platelets with a bleeding diathesis are observed. Renal manifestations include severe proximal tubular dysfunction, but nephrocalcinosis, nephrogenic diabetes insipidus and dysplasia have also been described [143]. No specific treatment exists and the prognosis is poor with patients typically dying in their first year of life [141, 143–146]. However, milder forms have been described [147].

Membranous Nephropathy with Anti-proximal Tubule Basement Membrane Antibodies

Several reports exist about an association between membranous nephropathy and RFS [30–38, 148]. In most cases, antibodies have been found directed against the basement membrane of the proximal tubule. In some cases, pulmonary symptoms associated with anti-alveolar basement membrane antibodies are also present [34]. Most likely the antibodies are directed against an antigen expressed in the glomerulus, as well, explaining the combination of glomerular and proximal tubular dysfunction. In two families the syndrome has been linked to a region on the X-chromosome, but no gene has yet been identified [36].

Dent Disease

Clinical Features

Dent disease is an X-linked recessive proximal tubulopathy, characterized by low-molecular weight proteinuria (LMWP) and hypercalciuria with nephrocalcinosis and nephrolithiasis, as well as progressive renal failure [149]. Patients may also have aminoaciduria, glucosuria and phosphaturia, consistent with generalized proximal tubular dysfunction. It was first described as hypercalciuric rickets by Dent and Friedman [150]. Clinical manifestations can vary enormously and once an underlying gene, *CLCN5*, was identified in 1996, it was realized that mutations in the same gene also caused related tubulopathies, previously thought to be distinct, namely X-linked recessive nephrolithiasis and Japanese idiopathic low-molecular-weight proteinuria [151–157]. Patients typically manifest with complications of hypercalciuria, such as hematuria, nephrocalcinosis or stones. Progression to ESKD is rare in childhood. Women very rarely can be affected, probably due to skewed X-chromosome inactivation [158]. The diagnosis is made by the presence of hypercalciuria and low-molecular weight proteinuria (see above: proteinuria) and typically a family history on the maternal side. Renal histology is non-specific, showing features of interstitial nephritis and calcium deposits and is thus not useful in establishing the diagnosis.

Genetics

The first gene identified to underlie Dent disease was *CLCN5*, encoding a proton-chloride antiporter expressed in the proximal tubule and especially on late endosomes and lysosomes [151]. Mutations in this gene are identified in approximately 60% of patients with a clinical diagnosis of Dent disease. A second gene, *OCRL*, encoding a phosphatidylinositol 4,5-bisphosphate 5-phosphatase (PIP_2-5-phosphatase), was identified later and mutations in this gene are found in approximately 15% of patients [159]. These patients are often referred to as having Dent2 disease, to distinguish them from CLCN5-based Dent disease. In the remaining approximately 25% of patients, no mutation in either gene is found, indicating that other genes may be responsible.

The identification of *OCRL* underlying Dent disease was surprising, as this gene also underlies Lowe syndrome, which besides renal proximal tubular dysfunction includes cataracts and developmental delay (see below). A genotype-phenotype effect has been hypothesized, as frameshift, splice site and nonsense mutations in *OCRL* causing Dent disease all cluster in exons 1–7, whereas those associated with Lowe syndrome mostly localize to the exons further downstream [160]. Use of potential alternate start codons in exons 7 and 8, which maintain the *OCRL* frame and, presumably, some functionality, could explain the milder phenotype in Dent disease. However, this hypothesis cannot explain, why some missense mutations in OCRL are associated with multiorgan disease (the Lowe phenotype) in some patients and predominant kidney involvement (the Dent phenotype) in others [160].

Pathophysiology

Whilst the identification of underlying genes has provided great insights, the pathogenesis of Dent disease is still incompletely understood. *CLCN5* clearly plays an important part in endocytosis in the proximal tubule. It is highly expressed in endosomes and lysosomes, where it co-localizes with endocytosed proteins [103]. *CLCN5* is likely to provide an electric shunt in the lysosome that neutralizes the electrical gradient otherwise created by the H^+-ATPase, to allow its efficient operation. However, *CLCN5* is not a voltage-gated chloride channel, as initially described, but in fact a Cl^-/H^+ antiporter, which thus would remove protons from the lysosomal lumen [161, 162]. However, the net effect of CLCN5 function still appears to favor lysosomal acidification, as loss of function of *CLCN5* has been shown to impair this process in mice and also in cultured human proximal tubular cells *in vitro* [103, 163]. Initially, the LMWP was thought to be a consequence of the impaired lysosomal function, but there is evidence for a role of CLCN5 beyond the lysosome. CLCN5 is also expressed at the apical surface of proximal tubule cells, where it is important in the assembly of the endocytic complex containing megalin and cubilin (see above) [104, 164–166]. Indeed, megalin and cubilin expression at the brush border is dramatically reduced in *CLCN5*-deleted mice, as is the excretion of megalin in the urine in Dent patients and the mouse model [104, 105]. Therefore, the proteinuria seen in this disease likely reflects impaired receptor-mediated endocytosis [163]. This is consistent also with the involvement of OCRL in endocytosis (see Lowe syndrome, below). Indeed, the renal phenotype of Lowe syndrome strongly resembles that of Dent disease [39] and proteomic analysis of the urine of patients with Dent disease shows a similar pattern as in Lowe syndrome, suggesting that *CLCN5* and *OCRL* may participate in similar endocytic pathways [167]. Potentially, in patients with Dent2 disease, other PIP_2-5-phosphatases can compensate for the loss of *OCRL* except with respect to endocytosis and hypercalciuria. Redundancy in PIP_2-5-phosphatases is suggested by the fact that *OCRL*-deleted mice do not show any clinical phenotype [168]. Interestingly, children with Dent2 disease frequently have some extra-renal manifestations in the form of elevated plasma levels of LDH and CK and poorer growth [169]. In addition, mild developmental delay is noted

in some patients and it has been suggested that Dent2 disease is just a milder form of Lowe syndrome [170].

The hypercalciuria of Dent disease is poorly understood. One hypothesis is based on altered endocytosis of PTH and vitamin D-binding protein and a subsequently altered balance of calciotropic hormones [171]. Others propose a more direct role of CLCN5 in calcium handling by the kidney and bone [172, 173]. This discrepancy may in part be due to the fact that there are two different mouse strains with deleted *CLCN5* function, one of which has LMWP but no hypercalciuria [103].

Treatment

Treatment is mainly symptomatic and includes a large fluid intake to dilute urinary calcium and 1-α-OH vitamin D supplementation, to normalize PTH levels, so as to treat or prevent rickets. This needs to be monitored carefully, as excessive supplementation would worsen the hypercalciuria. Citrate supplementation has been helpful in a mouse model of the disease [174]. Thiazide diuretics have been shown to reduce calcium excretion at least in the short term and thus reduce the stone-forming risk in Dent disease [175] but are sometimes poorly tolerated. The reduction in urinary calcium excretion by thiazides in this disorder is surprising, as thiazides are thought to enhance calcium reabsorption in the proximal tubule, the segment affected in these patients [176].

Lowe Syndrome

Clinical Features

Lowe syndrome (oculo-cerebro-renal syndrome) was first described in 1952 as a clinical entity comprising "organic aciduria, decreased renal ammonia production, hydrophthalmus and mental retardation" [177]. Severity of symptoms varies, but in its complete form, patients are profoundly hypotonic with absent reflexes, have severe mental impairment, congenital cataracts, glaucoma and RFS [178, 179]. There is often a delay in establishing the diagnosis and the renal manifestations can be minimal in early years but gross LMWP is characteristic [44]. Most patients first present to the ophthalmologist due to congenital cataracts [180–182]. Interestingly, individual patients with pathogenic variants in *OCRL* and brain and kidney involvement, but without cataracts, have been described recently, further blurring the distinction between Dent2 disease and Lowe syndrome [160, 183, 184].

Motor development is typically delayed and most patients do not achieve independent walking before 3 years of age. Mental impairment can be very variable, but in one study most patients had IQ measured around 50, yet with 25% in the normal range (>70). Seizures have been reported in about a third of patients [185]. Brain imaging, if performed, may show white matter changes, cerebral atrophy or periventricular cysts [186–188]. Behavioral abnormalities in the form of temper tantrums, negativism and obsessive behavior are another typical feature that can be extremely difficult for the families [189, 190].

The renal phenotype, as discussed above, is predominated by LMWP and hypercalciuria, but can involve more generalized proximal dysfunction, such as a metabolic acidosis and phosphate wasting [39]. Hypercalciuria is presumably due to impaired proximal reabsorption, but may be compounded by increased intestinal absorption, which OCRL may be involved in regulating [191]. Associated with the hypercalciuria is nephrocalcinosis/lithiasis with is seen in about two thirds of Lowe patients.

Renal histology is non-specific, showing some distortion of proximal tubular architecture and later also glomerular changes [192]. Most patients exhibit a slow progression of renal insufficiency with ESKD typically reported during the fourth and fifth decade of life [193, 194]. Using the Schwartz-Haycock formula with usual k-values substantially overestimates the GFR in children with Lowe syndrome from serum creatinine when compared to formal GFR measurements, probably due to the low muscle mass of these patients [39].

In addition to the manifestations in eyes, brain and kidneys, other clinical symptoms can occur. These include:

- platelet dysfunction with increased bleeding risk [195]
- a debilitating arthropathy [196]
- growth failure [178], which is independent of GFR [170]
- skeletal abnormalities, such as kyphosis, scoliosis, joint hypermobility and hip dislocation [197]. Some of these features may be secondary to the neurological features, such as the muscular hypotonia
- dental abnormalities, such as eruption and dental cysts [198]
- dermal cysts [199]

Genetics

The gene underlying Lowe syndrome was cloned in 1992 and named OCRL [200].

Mutations are identified in approximately 80–90% of cases suspected of Lowe syndrome and in one study roughly one third of these occurred de novo [160]. About two thirds of mutations in patients diagnosed with Lowe syndrome are nonsense, frameshift and splice site changes, the remainder missense plus a few gross deletions. The presence of lens opacities has been suggested to identify female mutation carriers, although the reported sensitivity is variable [44, 181, 201–204].

Pathophysiology

Since identification of the underlying gene, much progress has been made towards understanding the pathophysiology. The OCRL protein contains several functional domains, including PIP_2-5-phosphatase located in the Golgi apparatus. Impairment of this phosphatase function results in elevated cellular levels of PIP_2. PIP_2 levels affect vesicle trafficking at the Golgi [205] and may also account for alterations in the actin cytoskeleton seen in fibroblasts from patients with Lowe's syndrome [206], resulting in altered endosomal membrane trafficking [207] (Fig. 35.2). Besides, the phosphatase domain, the

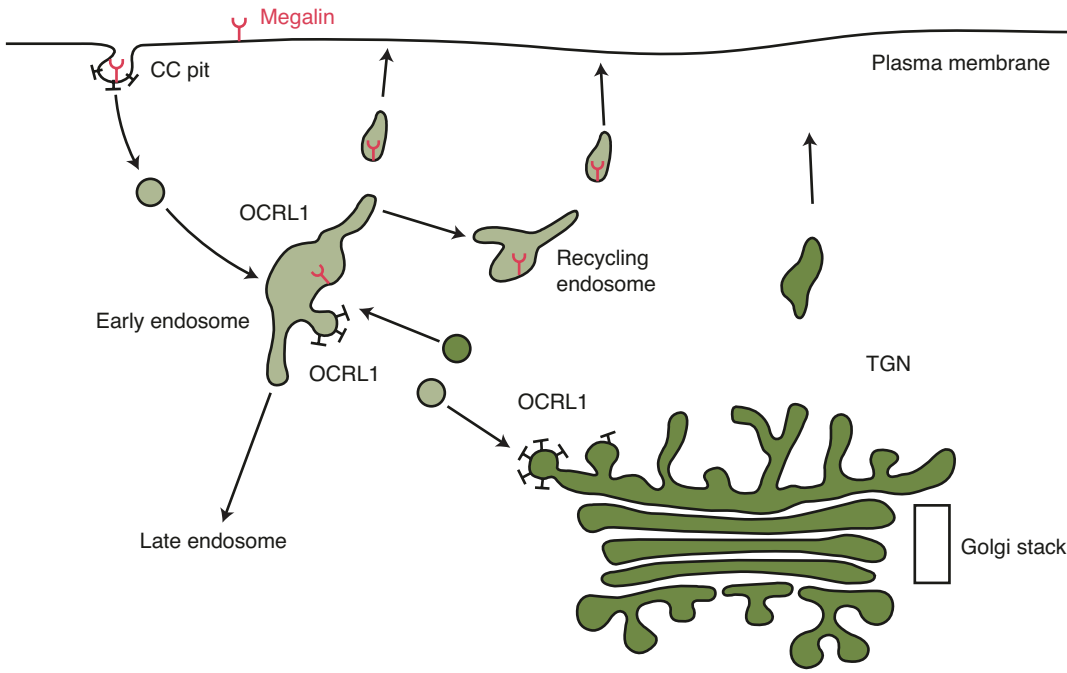

Fig. 35.2 Role of OCRL in regulation of traffic between apical membrane, endosomes and the trans-Golgi network. OCRL is present in clathrin-coated pits (CC) on the apical membrane of proximal tubular epithelial cells, early endosomes and the trans-Golgi network (TGN). (Adapted from [208])

OCRL protein also contains several other domains that are important for endosomal trafficking via protein-protein interactions, including an N-terminal PH (pleckstrin homology) domain, an ASH (ASPM-SPD2-Hydin) domain, and a C-terminal RhoGAP (Rho GTPase activating) domain [209]. Missense mutations within the RhoGAP domain were identified in some patients with Lowe syndrome, highlighting its importance for OCRL function [210].

Beyond the role in endocytosis, intracellular trafficking and regulation of the actin skeleton, OCRL has also been implicated in a wide range of cellular processes, including cell migration, cell polarity, cell-cell interaction, cytokinesis, mitochondrial function and cilia formation. Indeed, it has been suggested that Lowe syndrome could be considered a mitochondrial cytopathy [211] or, more recently, a ciliopathy [212]. It remains to be determined, to what degree these multiple mechanisms are clinically relevant and contribute to the variable phenotype associated with OCRL mutations.

Treatment

There is no specific treatment of Lowe syndrome and thus, management is symptomatic. Supplementation of electrolytes, alkali and vitamin D is based on biochemistries, and vitamin D is usually needed in 1-α- hydroxylated forms. Oversupplementation could worsen the hypercalciuria, so monitoring parathyroid hormone (PTH) and calcium levels is advised. Nutritional support with tube feeding may be helpful in the more severely affected patients.

The bleeding diathesis from the platelet dysfunction may be ameliorated with tranexamic acid and this should be considered prior to elective surgeries.

Cataract surgery is usually performed in the first year of life, but vision is nevertheless typically impaired and rarely better than 20/70 [213]. Anti-epileptic drugs can help in those patients suffering from recurrent seizures [187].

Acquired RFS

Many exogenous causes of RFS have been reported and are listed in Table 35.2. The mechanism of tubular damage is often unclear, although mitochondrial dysfunction has been implicated in some forms (see below).

Treatment, aside from supportive measures, is always the removal of the offending agent, which typically reverses the symptoms. Except for those compounds, where blood levels can be measured (such as lead or aminoglycosides), no specific diagnostic tests exist. Thus, the diagnosis is typically made through suspicion of an exogenous cause and its subsequent removal.

Chemotherapeutic Agents

Ifosfamide is the chemotherapeutic agent most commonly associated with RFS, which is seen in up to 10% of patients. Risk factors include total dose, reduced renal mass, young age or the combination with other nephrotoxic agents, such as cisplatin [214–218]. Symptoms typically reverse within weeks after cessation of the drug, but in some cases persist for several years and chronic impaired kidney function is possible [219, 220]. A mechanism has been proposed implicating specific ifosfamide uptake and metabolization in the proximal tubular cells [84].

Antibiotics and Antiretrovirals

Aminoglycosides are well known for their nephrotoxic side effects. In a small percentage of patients, they can also induce RFS. In fact, aminoaciduria has been proposed as a highly sensitive marker for aminoglycoside-induced renal injury, at least in the rat model [221]. The mechanism of damage is thought to be mitochondrial dysfunction: aminoglycosides target bacterial ribosomes where they induce faulty protein synthesis and since mitochondrial ribosomes bear structural resemblances to those of bacteria, they

are vulnerable to the toxicity [222]. The risk appears to be related to the dosage and length of treatment and symptoms typically reverse after cessation of the drug (reviewed in [223]).

With the advent of retroviral treatment, tenofovir has become an increasingly common cause of RFS, although rarely seen in children as risk factors for tenofovir toxicity include older age, pre-existing kidney disease and low body mass [11, 224]. The exact mechanism is unclear, but histology, if obtained, typically shows dysmorphic mitochondria [224].

Treatment

Specific therapy of RFSs depends on the underlying cause. The renal features of galactosemia and hereditary fructose intolerance are reversed by appropriate dietary therapy. Removal of the causative toxin or drug usually ameliorates the tubulopathy although some drugs (e.g. ifosfamide) can cause long-term dysfunction. Nitisinone (NTBC (2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione)), reverses the RFS in tyrosinemia (see above). Otherwise, treatment of RFS is mainly supportive. In severe cases, rehydration initially with 0.9% saline and careful electrolyte correction is necessary and can be hazardous if not monitored and managed by experts. Historically, fatalities occurred during rehydration with glucose-containing solutions, which exacerbated the profound hypokalemia. Rapid correction of acidosis can precipitate hypocalcemic seizures, exacerbated by the "hungry bone" phenomenon [225]. Once stabilized, large amounts of alkali (3–20 mmol/kg/day) are often needed to maintain acid-base homeostasis. As so often in tubular disorders, the more supplementation is given, the higher the filtered load and consequently, the more is lost in the urine. Smaller, but frequent dosing can help to maintain more consistent plasma levels and to reduce urinary losses from brief spikes in filtered load, associated with intermittent large doses, as well as side effects, such as diarrhoea. Alkali supplementation is typically prescribed in the form of sodium or potassium bicarbonate or citrate, or as a compound preparation. Supplements of sodium chloride may also be needed. For some children, provision of all the above supplements fails to correct the biochemical disturbances and growth failure persists. Indomethacin or an alternative non-steroidal anti-inflammatory agent may be helpful in such cases [226]. Carnitine supplements have been used to correct the plasma and muscle carnitine deficiencies that can occur due to renal losses. Hypophosphataemic rickets is treated with phosphate supplementation and 1-α-calcidol or calcitriol.

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Bartter-, Gitelman-, and Related Syndromes

36

Siegfried Waldegger, Karl Peter Schlingmann, and Martin Konrad

Basic Principles of Ion Transport in the TAL and the Early DCT

With respect to their role in sodium reabsorption, the TAL and early DCT form a functional unit that separates tubular sodium chloride from water. Compared to sodium reabsorption in the other nephron segments, which occurs via sodium hydrogen exchange or by sodium channels in the proximal nephron and in the ASDN, respectively, TAL and early DCT sodium transport is accomplished primarily by the active reabsorption of sodium together with chloride from the tubular fluid. These nephron segments are relatively water-tight and thus prevent osmotically driven absorptive flow of water. About 30% of the total sodium load provided by glomerular filtration is absorbed along the TAL and—via counter current multiplication—contribute to medullary interstitial hypertonicity. TAL sodium reabsorption thus not only accounts for the—in quantitative terms—most important mechanism of sodium retention (apart from the proximal nephron, which reabsorbs about 60% of the filtered sodium load), but also generates the osmotic driving force for water reabsorption along the CD. For this reason, disturbances in TAL salt reabsorption result in both salt-wasting and severely reduced urinary concentrating capacity (i.e. water loss). In contrast, DCT mediated salt reabsorption accounts for only about 5% of the filtered sodium load and does not contribute to the urinary concentrating mechanisms. Impaired DCT salt reabsorption therefore does not interfere with urinary concentrating capability, although the accompanying saluresis indirectly increases renal water excretion even with normal urine osmolalities.

Transepithelial sodium chloride reabsorption in the TAL and DCT is driven by secondary active transport processes that depend on a low intracellular sodium concentration maintained by active extrusion of sodium by the basolateral sodium-potassium-ATPase (sodium pump). By far the majority of TAL sodium reabsorption depends upon the operation of the furosemide-sensitive sodium-potassium-chloride cotransporter (NKCC2) with about half of the sodium taking the transcellular route and half taking a paracellular route by cation-selective intercellular pathways (Fig. 36.1a). Potassium that enters the TAL cell by sodium-potassium-chloride cotransport (1 potassium ion being transported with 1 sodium and 2 chloride ions) recycles back to the tubular urine through KCNJ1 (ROMK).

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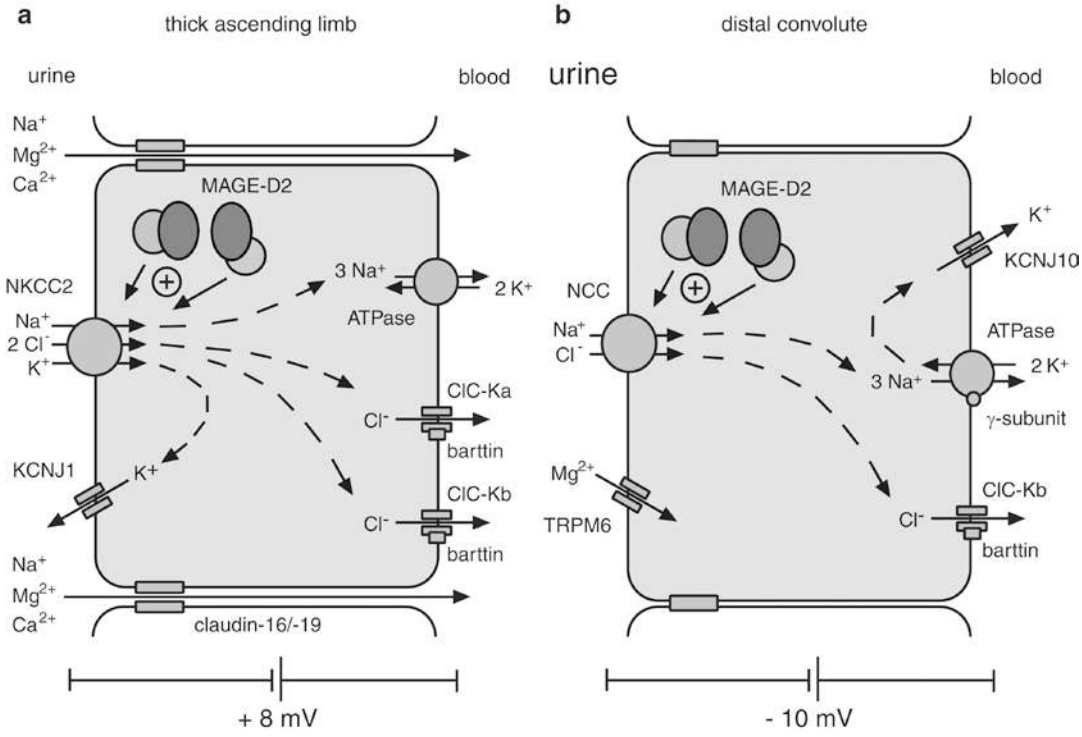

Fig. 36.1 Mechanisms of sodium reabsorption along the distal nephron. The key transport proteins and ion channels are shown for the thick ascending limb (**a**) and the distal convolute (**b**), for details please see text

This not only guarantees proper activity of NKCC2-mediated transport along the entire length of the TAL by replenishment of urinary potassium that otherwise would rapidly decrease along the TAL through reabsorption by NKCC2. Even more important, luminal potassium secretion in addition establishes a lumen-positive transepithelial voltage gradient that provides—in terms of energy recovery—a low-priced driving force for paracellular transport of cations like sodium, calcium, and magnesium. The essential functions of the TAL thus not only include the reabsorption of sodium chloride but also that of magnesium and calcium. Noteworthy, all of the TAL chloride reabsorption occurs by the transcellular route. Overall parity of sodium (with ~50% transcellular and ~50% paracellular) and chloride (100% transcellular) reabsorption is due to the stoichiometry of the apical NKCC2 cotransporter that transports two chloride ions for each sodium ion (Fig. 36.1a).

Taken together, the initial step of transcellular sodium chloride and paracellular sodium transport across the TAL epithelium critically depends on the proper activity of NKCC2 and KCNJ1.

In contrast to the TAL, sodium chloride reabsorption in the DCT occurs almost exclusively by the transcellular route (Fig. 36.1b). Luminal sodium chloride uptake is mediated by the electroneutral thiazide-sensitive sodium chloride cotransporter NCC that is structurally related to the NKCC2 protein, but transports 1 sodium ion together with 1 chloride ion without potassium. A relevant apical potassium conductance seems not to exist in early DCT cells, that instead express TRPM6 cation channels that permit apical magnesium entry. Inhibition of NCC transport by long term administration of thiazides or by genetic ablation in animal models has been shown to reduce the number of DCT cells, which might explain impaired renal magnesium reabsorption with consequent hypomagnesemia

observed in human diseases caused by impaired NCC mediated transport.

DCT and TAL cells differ with respect to the apical entry step for sodium chloride, however, as mentioned above, basolateral sodium release in both cell types is accounted for by the sodium pump. Moreover, epithelial cells in TAL and early DCT share similar pathways for basolateral chloride exit. In both cell types two highly homologous ClC-K type chloride channel proteins (ClC-Ka and ClC-Kb) associate with their beta subunit barttin to form a basolateral chloride conductance, which accounts for the release of the majority of reabsorbed chloride ions (Fig. 36.1a, b).

Taken together, NCC mediates early DCT cell sodium chloride uptake and ClC-K channels in association with barttin account for basolateral chloride release.

In the transition zone between the TAL and DCT a plaque of closely packed epithelial cells morphologically different from TAL- and DCT-cells forms the *macula densa*. Together with closely adjacent extraglomerular mesangial cells and granular cells of the afferent arterioles appendant to the same nephron, these specialized tubular cells assemble the juxtaglomerular apparatus. *Macula densa* cells serve an important function in coupling renal hemodynamics with tubular reabsorption in that they monitor the sodium chloride concentration of the tubular fluid. Via paracrine signalling molecules like prostaglandin E_2 (PGE_2), ATP, adenosin, and NO, the *macula densa* provides a feed-back mechanism, which adapts glomerular filtration to tubular reabsorption (tubuloglomerular feedback, TGF). In case of an increased sodium chloride concentration at the *macula densa* the TGF induces afferent arteriole vasoconstriction and decreases renin release, whereas a decreased *macula densa* sodium chloride concentration dilates the afferent arteriole and increases renin release. To sense the tubular sodium chloride concentration the *macula densa* takes advantage of essentially the same repertoire of transport proteins as found in salt-reabsorbing TAL cells. Via apical sodium chloride uptake (NKCC2 and KCNJ1) and basolateral chloride release (ClC-K and barttin) changes in luminal sodium chloride concentration are translated in alterations of basolateral transmembrane voltage. This again results from recycling of potassium into the tubular lumen, which guarantees an asymmetric - hence electrogenic - transcellular transport of sodium chloride, which results in basolateral membrane depolarization. This in turn regulates among other processes voltage-sensitive calcium entry, which triggers a series of intracellular signalling events eventually resulting in the release of the above mentioned paracrine signals. Owing to these combined functions in transepithelial transport and sensing of tubular sodium chloride, impaired activity of one of the participating proteins not only results in salt-wasting due to reduced TAL salt-reabsorbing capacity, but also abrogates the TGF as an important safety valve, which otherwise would reduce the filtered sodium chloride load by decreasing glomerular filtration. In fact, blinding of the *macula densa* for the tubular sodium chloride concentration with resultant disinhibition of glomerular filtration might constitute the single most important mechanism underlying the severe salt-wasting observed in impaired TAL salt transport.

Taken together, NKCC2, KCNJ1, ClC-K chloride channels, and barttin participate in the salt sensing-mechanism of the *macula densa*. Impaired function of one of these proteins affects the TGF and prevents adjustment of glomerular filtration with tubular salt-reabsorbing capacity, which further aggravates renal salt wasting [1].

Hypokalemic Salt-Wasting Tubular Disorders

With the exception of the medullary collecting duct that is primarily responsible for the reabsorption of water, reabsorption of sodium chloride from the glomerular filtrate at least in quantitative terms constitutes the key function of all nephron segments. Given the normal daily amount of 170 liters of glomerular filtrate produced by adult kidneys, at a normal plasma

sodium concentration of 140 mmol/L and plasma chloride concentration of 105 mmol/L the filtered load of sodium and chloride per 24 h amounts to 23.8 mols (about 550 g) and 17.9 mols (about 630 g), respectively. Healthy kidneys manage the reabsorption of more than 99% of the filtered load, with about 60% by the proximal tubule, 30% by the TAL, 5% by the early DCT, and the remainder by the aldosterone-sensitive distal nephron (ASDN). Impairment of sodium transport in any of these nephron segments causes a permanent reduction in extracellular fluid volume, which in turn causes compensatory activation of sodium conserving mechanisms, i.e. stimulation of renin secretion and aldosterone synthesis. Accordingly, with intact ASDN function, the primary symptoms of renal salt-wasting like hypovolemia with tendency for reduced arterial blood pressure mix with those of secondary hyperaldosteronism, which increases ASDN sodium retention at the expense of an increased potassium excretion eventually resulting in hypokalemia. In case of renal salt-wasting, hypokalemia thus indicates proper function of the ASDN and points to the involvement of nephron segments upstream to the ASDN.

As mentioned above, sodium reabsorption along the TAL and early DCT is coupled to the reabsorption of chloride. Sodium-wasting caused by defects in these nephron segments hence is accompanied by decreased reabsorption of chloride. Unlike sodium, which at least partially may be recovered by increased reabsorption along the ASDN, chloride irretrievably gets lost with the urine. Accordingly, the urinary chloride-loss exceeds that of sodium and for the sake of electroneutrality has to be balanced by other cations like ammonium or potassium. Loss of ammonium, the main carrier of protons in the urine, results in metabolic alkalosis, potassium loss in addition aggravates hypokalemia caused by secondary hyperaldosteronism. For this reason, hypochloremia with metabolic alkalosis, in addition to severe hypokalemia characterizes salt-wasting due to defects along the TAL and early DCT.

Finally, sodium reabsorption along the proximal tubule via the sodium proton exchanger and the carboanhydrase is indirectly coupled to the reabsorption of bicarbonate. Proximal tubular salt-wasting thus—in addition to hypokalemia—is accompanied by urinary loss of bicarbonate resulting in hyperchloremic metabolic acidosis.

Taken together, in the state of renal salt-wasting the determination of plasma potassium, chloride, and bicarbonate concentrations allows for the rapid assessment of the affected nephron segment. Of note, in this context the determination of the plasma sodium concentration is not very helpful, since changes in plasma sodium—the more or less exclusive extracellular cation accounting for plasma osmolality—reflects disturbances in the osmoregulation (i.e. water balance) rather than in the regulation of sodium balance.

Apart from more general disturbances of proximal tubular function which among other transport processes affect proximal tubular sodium reabsorption (as seen in Fanconi syndrome), no hereditary defects specifically affecting the proximal tubular sodium proton exchanger have been described in humans. By contrast, several genetic defects affect sodium chloride transport along the TAL and DCT and will be the focus of the following section.

Renal Salt-Wasting with Hypokalemia and Hypochloremic Metabolic Alkalosis

Historical Overview and Nomenclature

In 1957, two pediatricians described an infant with congenital hypokalemic alkalosis, failure to thrive, dehydration, and hyposthenuria, who finally died at the age of 7.5 months [2]. Some years later, two patients with normotensive hyperaldosteronism, hyperplasia of the juxtaglomerular apparatus, metabolic alkalosis and severe renal potassium wasting were characterized by the endocrinologist Frederic Bartter [3]. Other features of this syndrome were increased activity of the renin-angiotensin system and a relative

vascular resistance to the pressor effect of exogeneously applied angiotensin II. Following these original reports, hundreds of such Bartter syndrome (BS) cases have been described. While all shared the findings of hypokalemia and hypochloremic alkalosis, patients differed with respect to age of onset, severity of symptoms, degree of growth retardation, urinary concentration capacity, magnitude of urinary excretion of potassium and prostaglandins, presence of hypomagnesemia, and extents of urinary calcium excretion.

Gitelman and colleagues pointed to the susceptibility to carpopedal spasms and tetany in three BS cases [4]. Tetany was attributed to low plasma magnesium levels secondary to impaired renal conservation of magnesium. Further examination of these patients in addition revealed low urinary calcium excretion [5]. Consequently, the association of hypocalciuria with renal magnesium-wasting was regarded as a hallmark to separate the then defined Gitelman syndrome (GS) from other forms of BS [6]. Interestingly, both patients in Bartter's original report displayed positive Chvostek's sign and carpopedal spasms. Indeed, in a review of the original observations described by Bartter et al., one of the coauthors conceded that the majority of patients seen by both endocrinologists perfectly matched the later description of Gitelman [7].

Phenotypic homogeneity of BS was challenged even more seriously when the pediatricians Fanconi and McCredie described high urinary calcium excretion and medullary nephrocalcinosis in preterm infants initially suspected of having BS [8, 9]. Descriptions of this variant in the literature became more frequent in the 1980s, most likely because advances in neonatal medicine resulted in higher survival rates of extremely preterm born babies. The neonatologist Ohlsson finally described the antenatal history with maternal polyhydramnios, which likely predisposed to premature birth [10]. Immediately after birth, profound polyuria puts such patients at great risk for life-threatening dehydration. Contraction of the extracellular fluid (ECF) volume is accompanied by markedly elevated renal and extrarenal prostaglandin E_2 (PGE_2) production. Treatment with prostaglandin synthesis inhibitors effectively reduced polyuria, ameliorated hypokalemia, and improved growth [11, 12]. Another variant of this severe, prenatal-onset salt-wasting disorder was first described in a Bedouin family. It differs from the above mentioned antenatal variant of BS by the presence of sensorineural deafness, absence of medullary nephrocalcinosis, and slowly deteriorating renal function [13]. The last variant that has been described is X-linked transient antenatal BS. This disorder is of peculiar interest because at onset the clinical course is very severe and still many patients die from complications. Interestingly, the disease phenotype is self-limited and clinical symptoms disappear within a few weeks to months of life [14].

Taken together, renal salt-wasting syndromes associated with hypokalemia and hypochloremic metabolic alkalosis (frequently subsumed as "Bartter syndrome" in a broader sense) present with marked clinical variability. Severe, early onset forms (**antenatal Bartter syndrome, aBS**) with symptoms directly arising from profound salt-wasting with extracellular volume depletion contrast with milder late onset forms primarily characterized by the features of secondary hyperaldosteronism (**Gitelman syndrome, GS**). In between these two extremes, the Bartter syndrome *sensu stricto* (**classic Bartter syndrome, cBS**) presents as a disorder of intermediate severity. Variable extents of extracellular volume depletion and secondary electrolyte disturbances contribute to a rather variable disease phenotype, which in its extremes may mimic aBS or GS.

This classification based on clinical criteria was enriched by clarification of the underlying *genetic* defects, of which all but one follow an autosomal recessive mode of inheritance. As disclosed by molecular genetic analyses, aBS results from disturbed salt reabsorption along the TAL due to defects either in NKCC2 [15], KCNJ1 (ROMK) [16], Barttin [17], or both ClC-Ka and ClC-Kb [18]. X-linked transient aBS is caused by mutations in MAGE-D2, a chaperone-like molecule affecting the expression of NKCC2 and NCC during fetal life [14]. The cBS is caused by dysfunction of ClC-Kb [19], which impairs salt transport to some extent along the TAL and in

Table 36.1 Molecular genetics of renal salt wasting disorders

	BS1	BS2	BS3	BS4a	BS4b	BS5	GS	EAST/SeSAME
OMIM	601678	241200	607364	602522	613090	300971	263800	612780
Gene	*SLC12A1*	*KCNJ1*	*CLCNKB*	*BSND*	*CLCNKA* + *CLCNKB*	*MAGED2*	*SLC12A3*	*KCNJ10*
Protein	NKCC2	KCNJ1 (ROMK)	ClC-Kb	Barttin	ClC-Ka + ClC-Kb	MAGE-D2	NCC	KCNJ10
Inheritance	AR	AR	AR	AR	AR	XLR	AR	AR

OMIM Online Mendelian Inheritance in Man, *AR* autosomal recessive, *XLR* X-linked recessive

particular along the DCT. A pure defect of salt reabsorption in the DCT due to dysfunction of NCC finally results in GS [20].

Given the significant clinical overlap within the different subtypes of BS and also with GS, it is more and more accepted to describe the subtypes by the underlying genetic abnormality (Table 36.1). According to this molecular genetic classification, Bartter syndrome type 1 **(BS1)** refers to a defect in NKCC2 (gene name *SLC12A1*), **BS2** in KCNJ1 (*KCNJ1*), **BS3** in ClC-Kb (*CLCNKB*), **BS4a** in Barttin (*BSND*), **BS4b** to a digenic defect in ClC-Ka and ClC-Kb, and **BS5** in MAGE-D2. Previously patients with a Bartter-like phenotype with gain of function mutations in the calcium sensing receptor (CaSR) have been described [21, 22] and sometimes referred to as BS5, but this designation has been abandoned in accordance with the current OMIM nomenclature. **GS**, owing to disturbed NCC (*SLC12A3*) function, was not included in this classification despite its apparent relatedness to this group of disorders.

Another facet of the clinical and genetic heterogeneity of inherited renal salt wasting disorders unsurfaced in 2009, when a new autosomal recessive clinical syndrome characterized by epilepsy, ataxia, sensorineural deafness and renal salt wasting with or without mental retardation was described under the acronyms EAST or SeSAME syndrome [23, 24]. **EAST/SeSAME** syndrome is caused by loss-of-function mutations in the *KCNJ10* gene encoding the inwardly-rectifying potassium channel KCNJ10 (Kir4.1). The renal tubular defect disturbs the reabsorption of sodium chloride in the DCT and thus symptoms closely resemble GS with hypokalemic alkalosis, hypomagnesemia and hypocalciuria.

Taken together, renal salt-wasting with hypokalemia and hypochloremic metabolic alkalosis becomes manifest in three clinically defined syndromes: BS, GS, and EAST syndrome.

Genetic Disorders with Hypokalemic Salt-Wasting

Bartter Syndrome Type 1 (BS1)

Disruption of sodium chloride reabsorption in the TAL due to inactivating mutations of the *SLC12A1* gene which encodes NKCC2 causes BS1, a severe disorder with onset *in utero*. Within the second trimester, fetal polyuria leads to progressive maternal polyhydramnios. Untreated, premature delivery occurs around 32 weeks of gestation. The most striking abnormality of the newborns is profound polyuria. With adequate fluid replacement, daily urinary outputs can easily exceed half of the body weight of the newborn (>20 mL/kg/h). Despite ECF volume contraction and presence of high AVP levels, urine osmolality hardly approaches that of plasma, indicating a severe renal concentrating defect. Salt reabsorption along the TAL segment is also critical for urine dilution, which explains that urine osmolality on the other hand typically does not decrease below 160 mosmol/kg. Some preserved ability to dilute urine might be explained by an adaptive increase of salt reabsorption in the DCT which functions as the most distal portion of the diluting segment. This moderate hyposthenuria clearly separates NKCC2-deficient patients from polyuric patients with nephrogenic diabetes insipidus, who typically display urine osmolalities below 100 mosmol/kg.

Within the first months of life, nearly all patients develop medullary nephrocalcinosis in parallel with persistently high urinary calcium excretion. Remarkably, conservation of magnesium is not affected to a similar extent and

NKCC2-deficient patients usually do not develop hypomagnesemia. This is even more surprising given that mutations in either *CLDN16* or *CLDN19* which both encode tight junction proteins that mediate paracellular transport of divalent cations along the TAL, invariably cause both hypercalciuria and hypermagnesiuria with subsequent hypomagnesemia [25, 26]. With respect to magnesium transport, the difference between both disorders might be explained by an upregulation of magnesium reabsorption parallel to a compensatory increase of sodium chloride reabsorption in DCT cells in case of a NKCC2 defect [27].

Bartter Syndrome Type 2 (BS2)

Patients with mutations in the *KCNJ1* gene encoding the ATP-sensitive inwardly rectifying potassium channel KCNJ1 (ROMK) similarly show a history of maternal polyhydramnios, prematurity with a median age of gestation of 33 weeks, vasopressin-insensitive polyuria, isosthenuria, and hypercalciuria with secondary nephrocalcinosis. As in the case of NKCC2 dysfunction, the severity of the symptoms argues for a complete defect of sodium chloride reabsorption along the TAL. The mechanism of RAAS activation is virtually identical to that proposed for NKCC2-deficient patients. However, despite the presence of high plasma aldosterone levels, KCNJ1-deficient patients exhibit transient hyperkalemia in the first days of life [28]. The simultaneous appearance of hyperkalemia and hyponatremia resembles the clinical picture of mineralocorticoid-deficiency (which however shows low aldosterone levels) or that of pseudohypoaldosteronism type I (PHA-I; high aldosterone levels). Indeed, several published cases of PHA-I turned out to be misdiagnosed and subsequent genetic analysis revealed KCNJ1 mutations as the underlying defect [29]. The severity of initial hyperkalemia decreases with gestational age [30]. Hyperkalemia may be attributed to the additional role of KCNJ1 in the cortical collecting duct (CCD) where it participates in the process of potassium secretion. Although less pronounced as compared to NKCC2-deficiency, the majority of KCNJ1-deficient patients develop hypokalemia in the later course of the disease. The transient nature of hyperkalemia may be explained by the upregulation of alternative pathways for potassium secretion in the CCD.

Bartter Syndrome Type 3 (BS3)

BS3, previously often designated as "classic Bartter syndrome", is caused by mutations in *CLCNKB* (encoding the basolateral chloride channel ClC-Kb). This subtype of BS is characterized by a very high clinical variability which might be partly explained by an alternative chloride extrusion pathway in the basolateral membrane of TAL cells, namely ClC-Ka. Several studies have indicated that the clinical variability is not related to a certain type of mutation [31, 32]. Even the most deleterious mutation, which implies the absence of the complete *CLCNKB* gene and which affects nearly 50% of patients, can cause varying degrees of disease severity. Features of tubular dysfunction distal from the TAL predominate, suggesting a major role of ClC-Kb along the DCT. Although TAL salt transport can be impaired to a variable extent, its function is never completely perturbed.

With respect to renal function, the neonatal period in ClC-Kb-deficient patients usually passes without major problems. Maternal polyhydramnios is observed in only one fourth of the patients and usually is mild. Accordingly, duration of pregnancy is not substantially shortened. More than half of the patients are diagnosed within the first year of life. Symptoms at initial presentation include failure to thrive, dehydration, muscular hypotonia, and lethargy. Laboratory examination typically reveals low plasma chloride concentrations (down to 60 mmol/L), decreased plasma sodium concentration, and severe hypokalemic alkalosis. At first presentation, electrolyte derangement is usually more pronounced as compared to the other variants of BS. However, because renal salt wasting progresses slowly and polyuria may be absent, medical consultation may be delayed. Plasma renin activity is greatly increased, whereas plasma aldosterone concentration is only slightly elevated. This discrepancy might be attributed to negative feed-back regulation of aldosterone

incretion by hypokalemia and alkalosis. Therefore, normal or slightly elevated aldosterone levels under conditions of profound hypokalemic alkalosis are in fact inappropriately low.

Urinary concentrating ability is preserved at least to a certain extent and a number of patients with BS3 achieve urinary osmolalities above 700 mosmol/kg in morning urine samples. Because renal medullary interstitial hypertonicity is critically dependent on sodium chloride reabsorption in the TAL, the ability to concentrate urine above 700 mosmol/kg indicates nearly intact TAL function despite of ClC-Kb deficiency. Moreover, the integrity of TAL function is also reflected by the finding that hypercalciuria is not a typical feature of ClC-Kb dysfunction and—if present—occurs only temporarily. The majority of patients exhibit normal or even low urinary calcium excretion. Accordingly, medullary nephrocalcinosis—a hallmark of pure TAL dysfunction—is rare. The plasma magnesium concentration gradually decreases over time owing to impaired renal magnesium conservation, as is observed in other forms of abnormal DCT function. Accordingly, several ClC-Kb deficient patients exhibit both hypomagnesemia and hypocalciuria, a constellation which usually is thought to be highly indicative for an NCC-defect. ClC-Kb deficiency thus may mimic GS.

Bartter Syndrome Type 4a (BS4a)

In 2001, a new player in the process of salt reabsorption along the TAL and DCT was identified—the ClC-K channel beta-subunit barttin—following the discovery of molecular defects in *BSND* in patients with a very rare variant of BS with sensorineural deafness [17]. Because Barttin had no homology to any known protein, its physiologic function remained unclear until its role as an essential beta-subunit of the ClC-K channels was demonstrated [33, 34].

Two ClC-K isoforms of the ClC family of chloride channels are highly expressed along the distal nephron, with ClC-Ka being primarily expressed in the thin ascending limb and decreasing expression levels along the adjacent distal nephron. Its close homologue, ClC-Kb, is predominantly expressed in the DCT. Along the TAL, both channel isoforms are equally expressed. Barttin, which is found in all ClC-K expressing nephron segments, is essential for proper ClC-K channel function in that it facilitates the transport of ClC-K channels to the cell surface and modulates biophysical properties of the assembled channel complex.

In affected individuals, the Barttin defect seems to completely disrupt chloride exit across the basolateral membrane in TAL as well as DCT cells. Accordingly, patients display the severest salt-wasting kidney disorder described so far. As with defects of NKCC2 and KCNJ1, the first symptom of a Barttin defect is maternal polyhydramnios due to fetal polyuria beginning at approximately 22 weeks of gestation. Again, polyhydramnios accounts for preterm labor and extreme prematurity. Postnatally, patients are at high risk of volume depletion. Plasma chloride levels fall to approximately 80 mmol/l, a further decrease usually can be avoided by close laboratory monitoring and rapid intervention. Polyuria again is resistant to vasopressin and urine osmolalities range between 200 and 400 mOsmol/kg.

Unlike patients with loss-of-function mutations in KCNJ1 and NKCC2, Barttin-deficient patients exhibit only transitory hypercalciuria [35]. Medullary nephrocalcinosis is absent, yet progressive renal failure is common with histologic signs of pronounced tissue damage like glomerular sclerosis, tubular atrophy, and mononuclear infiltration. The mechanisms underlying the deterioration of renal function are not yet understood. The lack of hypercalciuria, however, may be explained by disturbed sodium chloride reabsorption along the DCT. Isolated DCT dysfunction as seen in GS (see below) or after long-term inhibition of NCC-mediated transport by thiazides is known to induce hypocalciuria. This effect might counter-balance the hypercalciuric effect of TAL-dysfunction in case of a combined impairment of salt reabsorption along the TAL and DCT. In contrast to calcium, the renal conservation of magnesium is impaired, leading to hypomagnesemia. This might be explained by the disruption of both magnesium reabsorption pathways, the paracellular one in the TAL and the transcellular one in the DCT, respectively.

Barttin defects are invariably associated with sensorineurinal deafness. Elucidation of the

pathogenesis of this rare disorder has provided a deeper insight into the mechanisms of potassium rich endolymph secretion in the inner ear: Marginal cells of the *stria vascularis* contribute to the endolymph formation by apical potassium secretion. Transcellular potassium transport is mediated by the furosemide-sensitive Na-K-2Cl-cotransporter type 1 (NKCC1) ensuring basolateral potassium entry into the marginal cells. Voltage-dependent potassium channels mediate apical potassium secretion into the endolymph. Proper function of NKCC1 requires basolateral recycling of chloride. Deafness associated with Barttin defects suggests that this recycling is enabled by the ClC-K/barttin channel complex.

A Digenic Disorder: Bartter Syndrome Type 4b (BS4b)

The concept of the physiologic role of Barttin as a common beta-subunit of ClC-K channels was substantiated by the description of patients harbouring inactivating mutations in both the ClC-Ka and ClC-Kb chloride channels, respectively [18]. The clinical symptoms resulting from this digenic disease are indistinguishable from those of Barttin-deficient patients. This observation not only proves the concept of the functional interaction of Barttin with both ClC-K isoforms but also excludes important other functions of Barttin not related to ClC-K channel interaction.

X-Linked Transient Bartter Syndrome (BS5)

Transient forms of antenatal BS have been first described in the 1990s [36, 37]. The clinical course is characterized by early development of severe polyhydramnios, high prenatal mortality and excessive neonatal salt wasting in the surviving infants. This disease condition is mainly observed in male infants and surprisingly the disease manifestations spontaneously resolve within a few weeks to several months of life. Mutations in *MAGED2* encoding the melanoma-associated antigen-D2 have been identified to underly the disorder [14]. As expected for an X-linked disease, transient BS (BS5) is almost exclusively observed in boys. Only two female infants have been described to date [38]. Functional data indicate that MAGED2 regulates the expression of NKCC2 and NCC, especially during fetal life. Because of the transient character of the disease, it is of crucial importance to establish the exact diagnosis in order to avoid therapies such as NSAIDs which are no longer necessary by nature of the disease.

Gitelman Syndrome (GS)

DCT epithelia contain two cell types: early DCT cells (DCT1) which express the thiazide-sensitive sodium chloride cotransporter (NCC) as its predominant apical sodium entry pathway, and further distal residing late DCT cells (DCT2), which express the epithelial sodium channel (ENaC) as the main pathway for apical sodium reabsorption. Both sodium entry pathways are inducible by aldosterone. Early DCT and late DCT cells probably also differ with respect to their function in divalent cation transport.

Genetic defects in *SLC12A3* encoding NCC result in only mild renal salt wasting. Initial presentation frequently occurs at school age or later with the characteristic symptoms being muscular weakness, cramps, and fatigue. Whereas a history of salt craving is common, urinary concentrating ability typically is preserved. Laboratory examination shows a typical constellation of metabolic alkalosis, low normal chloride levels, hypokalemia, and hypomagnesemia, urine analysis shows hypocalciuria. Family studies revealed that electrolyte imbalances are present from infancy, although most of the affected infants displayed no obvious clinical signs. Of note, the combination of hypokalemia and hypomagnesemia exerts an exceptionally unfavorable effect on cardiac excitability, which puts these patients at high risk for cardiac arrhythmia.

The pathognomonic feature of GS is the dissociation of renal calcium and magnesium handling, with low urinary calcium and high urinary magnesium levels. Subsequent hypomagnesemia causes neuromuscular irritability and tetany. Decreased renal calcium elimination together with magnesium deficiency favors deposition of mineral calcium as demonstrated by increased bone density as well as chondrocalcinosis. Although the combination of hypomagnesemia and hypocalciuria is typical for GS, it is neither a specific nor universal finding. Clinical observa-

tions in GS patients disclosed intra- and inter-individual variations in urinary calcium concentrations which can be attributed to gender, age-related conditions of bone metabolism, intake of magnesium supplements, changes in diuresis and urinary osmolality, respectively. Likewise, hypomagnesemia might not be present from the beginning. Because less than one percent of total body magnesium is circulating in the blood, renal magnesium loss can be balanced temporarily by magnesium release from bone and muscle stores as well as by an increase of intestinal magnesium reabsorption. Accordingly, the strict definition of hypomagnesemia with coincident hypocalciuria in order to separate GS from BS3 appears arbitrary.

The mechanisms compromising distal magnesium reabsorption and favoring reabsorption of calcium are not yet completely understood. The occasional co-existence of hypomagnesemia and hypocalciuria in ClC-Kb deficient patients indicates that this phenomenon is not restricted to NCC defects but is rather a consequence of impaired transcellular sodium chloride reabsorption along the early DCT. It is tempting to speculate, that with a functional defect of early DCT cells, which in addition to sodium chloride normally reabsorb magnesium by apical TRPM6 magnesium channels, these cells are replaced by late DCT cells, which reabsorb sodium via ENaC channels and calcium via epithelial calcium channels (TRPV5). Accordingly, reabsorption of magnesium would decrease and that of calcium increase. Moreover, other phenomena like for example the redistribution of renal tubular sodium chloride reabsorption to more proximal nephron segments (proximal tubule and TAL) might contribute to alterations in renal calcium and magnesium handling.

EAST/SeSAME Syndrome

In 2009, a newly described autosomal recessive clinical syndrome characterized by epilepsy, ataxia, sensorineural deafness and renal salt wasting with/without mental retardation was described under the acronyms EAST or SeSAME syndrome [23, 24]. EAST/SeSAME syndrome is caused by loss of function mutations in the *KCNJ10* gene encoding the inwardly-rectifying potassium channel KCNJ10 (Kir4.1). The expression pattern of KCNJ10 fits the disease phenotype with high expression levels in brain, the stria vascularis of the inner ear, and in the distal nephron, especially in the DCT. Here, KCNJ10 is localized at the basolateral membrane of DCT cells where it is thought to function in collaboration with the Na^+K^+-ATPase as it might allow for a recycling of potassium ions entering the tubular cells in countermove for the extruded sodium [24]. Loss of KCNJ10 function most likely leads to a depolarization of the basolateral membrane and thereby to a reduction of the driving force for basolateral anion channels as well as sodium-coupled exchangers. By this mechanism, KCNJ10 defects could also affect the putative Na^+/Mg^{2+} exchanger and possibly explain the magnesium wasting observed in EAST/SeSAME syndrome. Moreover, it could be demonstrated that lack of KCNJ10 decreases basolateral chloride conductance and results in a diminished expression of NCC in the apical membrane [39]. These results could explain the salt loss observed in EAST/SeSAME patients. Interestingly, the renal phenotype of KCNJ10 knockout mice had not been thoroughly studied until the description of the human disease [40]. However, the reevaluation of KCNJ10 knockout mice clearly demonstrated renal salt wasting leading to significant growth retardation [23].

Patients usually present early in infancy with generalized tonic-clonic seizures, speech and motor delay, as well as severe ataxia leading to an inability to walk, intention tremor, and dysdiadochokinesis. In addition they exhibit a severe hearing impairment. Renal salt wasting may develop or be recognized only later during the course of the disease [41]. Closely resembling GS, the renal phenotype includes the combination of hypokalemic alkalosis, hypomagnesemia and hypocalciuria.

A summary of the most important clinical features and the ordinary age of disease manifestation is given in Table 36.2.

Table 36.2 Main clinical and biochemical characteristics of different renal salt wasting disorders

	BS1	BS2	BS3	BS4a BS4b	BS5	GS	EAST/SeSAME
Age at onset	Prenatally	Prenatally	0–5 years	Prenatally	Prenatally	>5 years	Infancy
Polyhydramnios	Severe	Severe	Absent-mild	Severe	Very severe	Absent	Absent
Leading symptoms	Polyuria Hypochloremia Alkalosis Hypokalemia	Polyuria Hypochloremia Alkalosis Transient neonatal hyperkalemia	Hypokalemia Hypochloremia Alkalosis Failure to thrive	Polyuria Hypochloremia Alkalosis Hypokalemia	Polyuria Hypochloremia Alkalosis Hypokalemia	Hypokalemia Alkalosis Hypomagnesemia	Hypokalemia Alkalosis Hypomagnesemia
Calcium excretion	High	High	Variable	Variable	High	Low	Low
Nephrocalcinosis	Very frequent	Very frequent	Rare, mild	Rare, mild	Rare, mild	Rare, mild	Rare, mild
Other findings			Mild Hypomagnesemia	Deafness Risk for CKD Risk of ESKD	Large for gestational age Transient disease	Growth retardation Chondrocalcinosis	Ataxia Deafness Seizures Mental retardation

CKD chronic kidney disease, *ESKD* end stage kidney disease

Treatment

As with other hereditary diseases the desirable correction of the primary genetic defects is not yet feasible. In the case of salt-wasting kidney disorders, however, the correction of secondary phenomena like increased renal prostaglandin synthesis or disturbed electrolyte homeostasis have been part of treatment virtually from the first description of the diseases. To the present, the cornerstones in the treatment of renal salt-wasting are non-steroidal anti-inflammatory drugs (NSAID) and long-term salt and electrolyte substitution [1].

In all subtypes of BS inhibition of renal and systemic prostaglandin synthesis leads to reduced urinary prostaglandin E_2 (PGE_2) excretion, dramatically decreases polyuria, converts hyposthenuria to isosthenuria, reduces hypercalciuria, and stimulates catch up growth [11, 12, 30]. Maintenance of euvolemia in the immediate postnatal period by meticulous replacement of renal fluid and salt loss is of central importance before starting NSAID therapy, which might precipitate acute renal failure if extracellular volume is depleted. There is long standing experience with the unselective cyclooxygenase (COX) inhibitor indomethacin which is started at 0.05 mg/kg per day and may be gradually increased to 1.5 mg/kg per day according to its effects on urinary output, renal PGE_2-synthesis and blood aldosterone levels. However, the potential benefit of indomethacin in preterm infants and neonates should be weighed against potential risks of severe gastrointestinal complications, i.e. ulcers, perforation and necrotizing enterocolitis [42, 43]. In particular, indomethacin therapy of newborns with KCNJ1 defects (BS2) may be complicated by oliguric renal failure and severe hyperkalemia. At any age, KCNJ1-deficient patients are particularly sensitive to indomethacin, with doses well below 1mg/kg/day being sufficient to maintain normal plasma potassium levels.

Gastrointestinal side effects (gastritis and peptic ulcers) are also the main drawbacks of longterm indomethacin therapy. These might be reduced by the use of COX-2 specific inhibitors (e.g. celecoxib), which show a comparable effect on renal salt wasting but adversely affect blood pressure [44]. A convincing explanation for these unsurpassed effects of NSAIDs is still missing although a reduction of glomerular filtration and blockage of an aberrant tubulo-glomerular feedback certainly are important contributors. Despite these beneficial effects of NSAIDs, lifelong substitution of potassium chloride usually is required to prevent life-threatening episodes of hypokalemia. Additional potassium supplementation is more often required for NKCC2-deficient (BS1) than KCNJ1-deficient (BS2) patients [1, 30]. In single patients, treatment with a potassium-sparing diuretic (i.e. spironolactone) has been shown to effectively increase serum potassium levels. Also ACE inhibitors have been used in a few patients with success but should be used with caution because ACE inhibitors could impair the compensatory mechanisms for sodium reabsorption in the more distal nephron. Thiazides should not be used to reduce hypercalciuria, since they interfere with compensatory mechanisms in the DCT and further aggravate salt and fluid losses.

Patients with BSND (BS4) are managed primarily with intravenous fluids in neonatal intensive care units. In contrast to other forms of BS, and despite high levels of urinary PGE_2, the effect of indomethacin on growth and correction of electrolyte disorders is rather poor [35, 45]. Hypokalemic metabolic alkalosis persists despite high doses of sodium chloride and potassium chloride supplementation [35]. In a single patient, combined therapy with indomethacin and captopril was needed to discontinue intravenous fluids and improve weight gain [46]. A pre-emptive nephrectomy for refractory electrolyte and fluid losses and persistent failure to thrive, followed by peritoneal dialysis and successful renal transplantation has been reported in a 1-year-old child with BS4 [47].

Patients with cBS (BS3) are typically treated with NSAIDs. Indomethacin is the most frequently used drug, usually started within the first years of life at doses ranging from 1 to 2.5 mg/kg/day. Potassium supplementation (usually KCl, 1–3 mmol/kg/day) is mandatory in BS3, as hypo-

kalemia is often severe at presentation and is not fully corrected by indomethacin. If potassium chloride alone fails to correct hypokalemia, then addition of spironolactone (1–1.5 mg/kg/day) may be considered. ACE-inhibitors should be given with caution because of the risk of hypotension. Magnesium supplementation should be added when hypomagnesemia is present, but the correction is typically difficult [30].

In patients with GS, unrestricted salt intake as well as magnesium and potassium supplementations are the main therapeutic measures. Magnesium supplementation should be considered first, since magnesium repletion will facilitate potassium repletion and reduce the risk of tetany and other complications related to hypomagnesemia [48, 49]. All types of magnesium salts are effective, but their bioavailability is variable. Magnesium chloride, magnesium lactate and magnesium aspartate show higher bioavailability [48]. Magnesium chloride is recommended since it will also correct the urinary loss of chloride. The dose of magnesium must be adjusted individually in 3–4 daily administrations, with diarrhea being the limiting factor. In addition to magnesium, high doses of oral potassium chloride supplements are necessary in the majority of patients with GS [50]. Importantly, magnesium and potassium supplementation results in catchup growth [51, 52]. Spironolactone or amiloride can be useful, both to increase serum potassium levels in patients resistant to potassium chloride supplements and to treat magnesium depletion that is worsened by elevated aldosterone levels [53]. Both drugs should be started cautiously to avoid hypotension. Patients should be encouraged not to deny their usual salt craving, particularly if they practice regular physical activity. Following the pathophysiology with salt loss distal to the *macula densa* and thus not involving disturbances of the tubuloglomerular feeback, prostaglandin inhibitors are less frequently used in GS, since urinary PGE_2 levels are usually normal. However, in a recent controlled, randomized crossover study, Blanchard et al. demonstrated that indomethacin in GS effectively increases potassium levels. In this study, it was even more effective than amiloride or eplerenone [54]. Considering the occurrence of prolonged QT interval in up to 50% GS patients [55, 56], QT-prolonging medications should be used with caution.

Although GS adversely affects the quality of life [57], information about the long-term outcome of these patients are lacking. Renal function and growth appear to be normal, provided lifelong supplementation. Progression to renal failure is extremely rare in GS: only 2 patients with GS who developed end-stage renal disease have been reported [58, 59].

For further details, please see two recent expert consensus statements summarizing the current knowledge on diagnosis and management of BS and GS [60, 61].

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Disorders of Calcium and Magnesium Metabolism

37

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Calcium Physiology

Approximately one kilogram of the adult human body consists of elemental Ca^{2+}, of which 99% are bound in bone. The extracellular fluid contains only ~1000 mg of Ca^{2+}. Following national boards of nutrition, a daily uptake of 1000–1200 mg of elemental Ca^{2+} with a normal diet is recommended [1]. The intestine reabsorbs approximately 25–33% of the nutritional Ca^{2+} content [2]. In the kidney, ~800 mg of Ca^{2+} are filtered per day in the glomeruli of which 99% are reabsorbed along the renal tubule. Only ~10 mg (~0.1 mg/kg/day) are excreted with the urine. In plasma, ~50% of Ca^{2+} is present in the free ionized form, ~35% is protein-bound, and ~15% is complexed to bicarbonate, citrate or phosphate. The physiological range for blood Ca^{2+} equals 1.1–1.35 mmol/L for free, ionized Ca^{2+} and 2.2–2.6 mmol/L for total Ca^{2+} (in the presence of physiological whole protein levels) [3]. Only the free, ionized fraction is responsible for the biological Ca^{2+} effects.

The blood Ca^{2+} level is kept within a narrow physiological range by the concerted action of endocrine systems controlling intestinal Ca^{2+} uptake, renal Ca^{2+} excretion, and Ca^{2+} transport in bone and soft tissues. These comprise the parathyroid gland, active 1,25-$(OH)_2$-vitamin D_3, and the bone-derived phosphaturic hormone FGF-23 [4]. All three systems are tightly linked and influence each other's activity. Parathyroid hormone (PTH) increases intestinal Ca^{2+} absorption and Ca^{2+} release from bone, it promotes renal Ca^{2+} reabsorption while stimulating renal phosphate excretion. In contrast, active 1,25-$(OH)_2$-vitamin D_3 promotes intestinal Ca^{2+} and phosphate absorption as well as renal Ca^{2+} and phosphate conservation thereby ensuring sufficient Ca^{2+} and phosphate supply for bone mineralization. Finally, FGF-23 together with its co-factor klotho increases renal phosphate excretion by inhibiting proximal tubular phosphate reabsorption, but also negatively regulates active 1,25-$(OH)_2$-vitamin D_3 by inhibiting its activation and promoting its degradation [5]. The common aim of this endocrine interplay is to keep extracellular Ca^{2+} levels constant while supplying sufficient amounts of Ca^{2+} for soft tissues and bone mineralization.

At the glomerulus, the ionized fraction of serum Ca^{2+} is freely filtered. The majority of filtered Ca^{2+} (60–70%) is reabsorbed in the proximal tubule (Fig. 37.1) [6]. Classically, Ca^{2+} transport in the proximal tubule is considered to occur via the paracellular pathway driven by active transcellular Na^+ reabsorption and paracellular water flow [7]. Apical uptake of Na^+ into the

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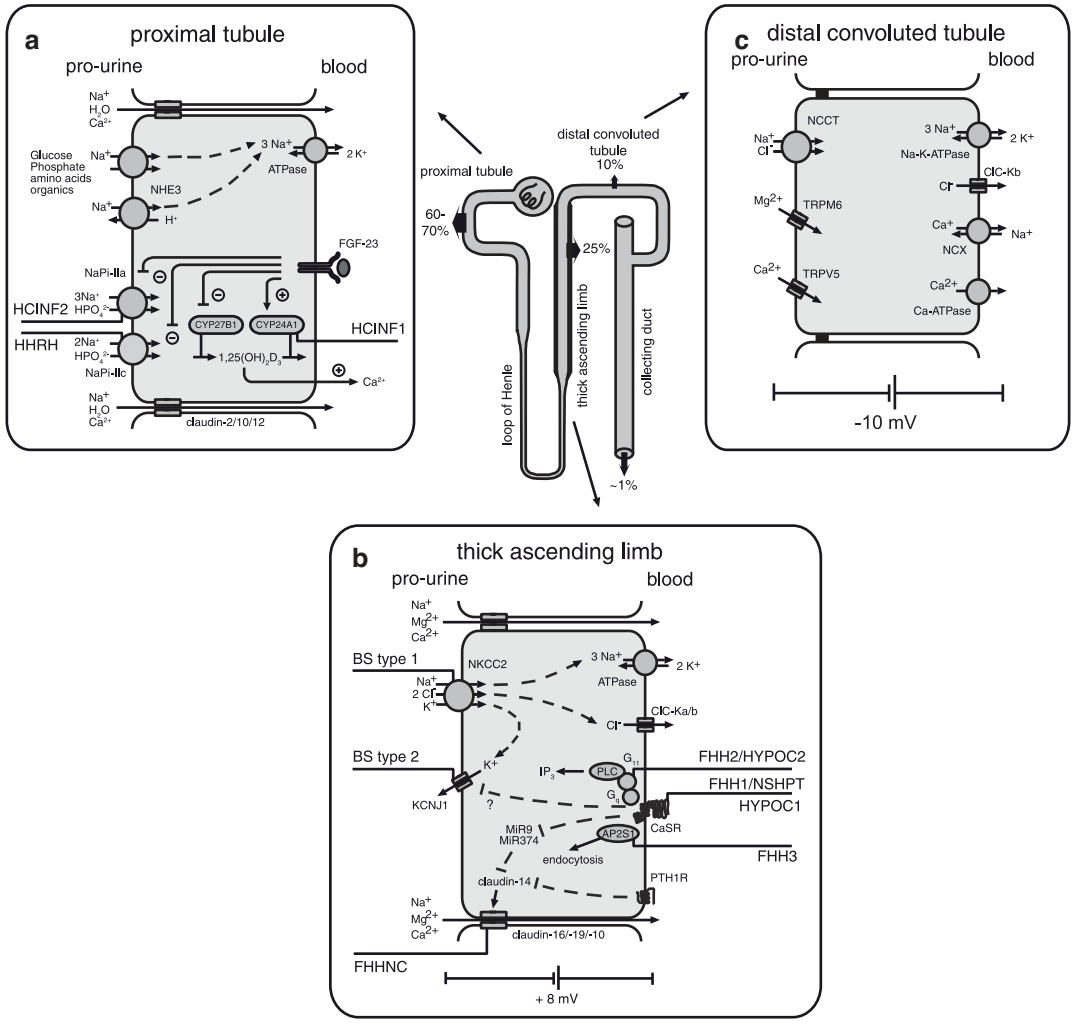

Fig. 37.1 Ca^{2+} reabsorption along the kidney tubule. (**a**) In the proximal tubule (PT), Ca^{2+} is reabsorbed via a paracellular pathway through tight junctions containing claudin-2. Also, the enzymes responsible for vitamin D activation, 1a-hydroxylase (CYP27B1), and vitamin D inactivation, 24-hydroxylase (*CYP24A1*), are expressed in PT cells and functionally linked to PO_4^{3-} reabsorption via NaPi-IIa (*SLC34A1*) and NaPi-IIc (*SLC34A3*). (**b**) In the thick ascending limb (TAL), Ca^{2+} is reabsorbed together with Mg^{2+} through paracellular tight junctions composed by claudin-16 and -19. This process is negatively regulated by basolateral Ca^{2+}-sensing receptor (CaSR) and PTH. Mutations in the CaSR and its associated proteins lead to mirror-like changes in calcium metabolism. (**c**) In the distal convoluted tubule (DCT), Ca^{2+} is actively reabsorbed via the transcellular pathway involving an apical entry through TRPV5 and a basolateral exit via Ca^{2+}-ATPase and Na^+/Ca^{2+}-exchanger (NCX). Hereditary defects affecting tubular Ca^{2+} reabsorption are indicated

proximal tubular cell is achieved via the Na^+/H^+-exchanger NHE3 (*SLC9A3*) followed by basolateral extrusion via Na^+/K^+-ATPase [8]. The paracellular space in the proximal tubule has a high permeability for ions and water that is conferred by tight junctions composed of claudin-2, claudin-10, and claudin-12 [9, 10]. Interestingly, recent data also indicate transcellular Ca^{2+} transport in the proximal tubule involving apically expressed Ca^{2+}-sensing receptor (CaSR) and the Ca^{2+}-permeable ion channel TRPC3 (transient receptor potential family C member 3) [11].

Around 25% of filtered Ca^{2+} is reabsorbed in the thick ascending limb (TAL) of the loop of

Henle. Here, Ca^{2+} transport is passive and paracellular in nature and occurs together with magnesium (Fig. 37.1). It is driven by a lumen-positive transepithelial voltage generated by active transcellular salt reabsorption. Next to changes in transcellular NaCl reabsorption, the permeability and cation selectivity of the paracellular pathway play a critical role in determining Ca^{2+} and Mg^{2+} reabsorption. Paracellular tight junctions in the TAL are composed of a complex set of claudin proteins conferring different properties: Claudin-16 and claudin-19 facilitate paracellular Ca^{2+} and Mg^{2+} transport, but also influence Na^+ permeability while sealing the paracellular space for Cl^- [12]. Furthermore, paracellular movement of Ca^{2+} and Mg^{2+} is modified by claudin-10 which is thought to form a paracellular Na^+ pore [13]. Paracellular Ca^{2+} and Mg^{2+} transport in the TAL is regulated by a complex network involving claudin-14 as well as the CaSR and PTH receptor, both expressed at the basolateral membrane [14–16]. Claudin-14 interacts with claudin-16 and diminishes paracellular cation permeability in vitro. Under physiologic conditions, the expression of claudin-14 is suppressed via a microRNA pathway [17]. By activation of the CaSR, extracellular Ca^{2+} is able to relieve this suppression and to induce claudin-14 expression, thus inhibiting paracellular divalent cation reabsorption [15]. The CaSR is a G-protein-coupled receptor that stimulates phospholipase C through G-proteins G_q and G_{11} resulting in the production of inositol 1,4,5-trisphosphate. This in turn leads to a reduction in PTH secretion from the parathyroid gland as well as increased urinary Ca^{2+} and Mg^{2+} excretion rates [18]. Finally, claudin-14 expression and therefore paracellular cation transport are also directly regulated by PTH via basolaterally expressed PTH-receptor PTH1R [16].

The distal convoluted tubule (DCT) only reabsorbs a small proportion of filtered Ca^{2+} (10–15%), however, as for Mg^{2+}, the DCT determines the final urinary excretion as there is no Ca^{2+} reabsorption beyond this segment. Transcellular Ca^{2+} reabsorption in the DCT is a sequential process of three steps: apical entry into the epithelial cell through Ca^{2+}-permeable ion channels (TRPV5), binding to calbindin-D_{28k} for diffusion through the cytoplasm, and basolateral exit through either a Ca^{2+}-ATPase (PMCA1b, *ATP2B1*), or a Na^+/Ca^{2+}-exchanger (NCX1, *SLC8A1*) [19]. Transcellular Ca^{2+} reabsorption in the DCT is critically influenced by PTH and active 1,25-OH_2-vit D_3 that regulate the expression levels of all the mentioned transport proteins [20]. In addition, Ca^{2+} (as well as Mg^{2+}) handling in the DCT appears to depend on dietary K^+ and the rate of active transcellular salt reabsorption [21, 22].

Disturbances of Calcium Homeostasis

Disturbances in Ca^{2+} metabolism comprise states of Ca^{2+} deficiency as well as Ca^{2+} excess usually detected in the form of hypo- or hypercalcemia. Whereas hypocalcemia represents a more common finding and is usually apparent by typical clinical signs and symptoms, hypercalcemia in infancy and childhood is a rare event and may remain unrecognized as clinical symptoms are rather unspecific. An overview on symptoms, causes, and the diagnostic work-up of pediatric hypocalcemia and hypercalcemia is provided in Chap. 31. Here, we focus on hereditary disorders of Ca^{2+} metabolism. These typically manifest with characteristic changes in serum and urine Ca^{2+} levels. As for acquired disorders of Ca^{2+} homeostasis, the diagnostic work-up, next to the parallel measurement of serum and urine electrolytes and creatinine, comprises the determination of parathyroid hormone (PTH) and vitamin D metabolites.

Hypercalciuria

The assessment of urinary Ca^{2+} excretion rates provides important information on alterations of renal Ca^{2+} conservation. Reference values for urinary Ca^{2+} excretion rates in infants and children are provided in Table 37.1 [23]. An increased urinary Ca^{2+} excretion may either reflect a primary disturbance in renal calcium handling or may reflect changes in renal Ca^{2+} handling in order to

Table 37.1 Reference ranges for urinary calcium excretion in children

	Urinary Ca/Crea mg/mg (mol/mol)	
Age (years)	5th percentile	95th percentile
0–1	0.03 (0.09)	0.81 (2.2)
1–2	0.03 (0.07)	0.56 (1.5)
2–3	0.02 (0.06)	0.50 (1.4)
3–5	0.02 (0.05)	0.41 (1.1)
5–7	0.01 (0.04)	0.30 (0.8)
7–10	0.01 (0.04)	0.25 (0.7)
10–14	0.01 (0.04)	0.24 (0.7)
14–17	0.01 (0.04)	0.24 (0.7)

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compensate for disturbances in body Ca^{2+} homeostasis or its hormonal regulation.

Hypercalciuria is the most common risk factor for nephrocalcinosis and stone formation in children [24]. The etiology is assumed to be multifactorial with a complex interaction of environmental and genetic factors. Still, despite an increased knowledge in the underlying genetic causes, the majority of patients are classified as having idiopathic hypercalciuria. A detailed overview on hypercalciuria and its role in the etiology of nephrocalcinosis and kidney stone disease is also provided in Chap. 44 "Renal Calculi".

In the past two decades, genetic studies have identified variants in an increasing number of genes involved in tubular Ca^{2+} reabsorption in patients with hypercalciuria, nephrocalcinosis and kidney stone disease. Classic examples of disturbed renal tubular calcium (and magnesium) reabsorption are hereditary defects in the tight junction proteins claudin-16 and -19 in patients with FHHNC (HOMG3, HOMG5), as well as activating and inactivating mutations in the CaSR producing mirror images of disturbed calcium homeostasis (ADH1, FHH1, NSHPT) (see below).

Disorders affecting proximal tubular Ca^{2+} reabsorption comprise Dent's disease (DENT1, DENT2) and Lowe syndrome (see Chaps. 32 and 44). Both disorders affect endosomal function in proximal tubular epithelial cells resulting in a complex dysfunction characterized by tubular proteinuria, hypercalciuria, renal Fanconi syndrome, and progressive renal failure [25, 26].

Next to different metabolic disorders affecting proximal tubular function leading to renal Fanconi syndrome (see Table 37.2), Fanconi-Bickel syndrome due to mutations in *SLC2A2* [27] is another generalized defect of proximal tubular function with hypercalciuria. Moreover, an isolated renal phenotype without hepatic symptoms and disturbance in glucose metabolism has been described in families with bi-allelic *SLC2A2* mutations [28].

Detailed descriptions of differential diagnoses for hereditary renal Fanconi syndrome and proximal tubulopathies are also provided in Chap. 32.

Hereditary defects of proximal tubular phosphate reabsorption and vitamin D degradation are discussed below (section "Hypercalcemia").

A recent study directly implicated defective paracellular Ca^{2+} reabsorption in the proximal tubule in the pathogenesis of hypercalciuria and renal calcifications since deletion of claudin-2 in mice resulted in hypercalciuria and renal calcifications [29]. The same study also identified a potentially pathogenic heterozygous variant in *CLDN2* in a family with multiple members affected by kidney stones and associated common genetic variants near the *CLDN2* locus with kidney stone risk [29]. No primary defects in transcellular Ca^{2+} reabsorption in the DCT have been linked to monogenic human disease to date. Deletion of the epithelial Ca^{2+} channel TRPV5 in mice resulted in severe hypercalciuria, compensatory intestinal hyperabsorption of Ca^{2+}, and reduced bone thickness [30]. A TRPV5 missense mutation was identified in a mouse model with dominant hypercalciuria [31]. In contrast, homozygous knock-out of basolateral NCX1 (SLC8A1) and PMCA1b (ATP2B1) in mice is embryonically lethal [32, 33].

Finally, hypercalciuria is observed in other renal tubular disorders including Bartter syndrome (see Chap. 33) and renal tubular acidosis (see Chap. 36).

Table 37.2 Monogenic disorders associated with hypercalciuria

Proximal tubule	**OMIM #**	**Inheritance**	**Gene**	**Protein**
Dent's disease type 1	300009	XLR	*CLCN5*	ClC-5 chloride channel
Dent disease type 2/Lowe syndrome	309000	XLR	*OCRL*	Inositol-polyphosphate-5-phosphatase
Nephropathic cystinosis	219800	AR	*CTNS*	Cystinosin cystine transporter
Tyrosinemia 1	276700	AR	*FAH*	Fumarylacetoacetate hydrolase
Fanconi renotubular syndrome 3	615605	AD	*EHHADH*	Enoyl-CoA-hydratase
Fanconi renotubular syndrome with MODY	600281	AD	*HNF4A*	Hepatocyte nuclear factor 4α
Fanconi-Bickel syndrome	227810	AR	*SLC2A2*	GLUT2 glucose transporter
Infantile hypercalcemia 1 (HCINF1), nephrocalcinosis, calcium nephrolithiasis	143880	AR, AD	*CYP24A1*	25-OH-vitamin D_3-24-hydroxylase
Infantile hypercalcemia 2 (HCINF2), nephrocalcinosis, calcium nephrolithiasis	616963	AR, AD	*SLC34A1*	Na-Po_4 co-transporter NaPi-IIa
Hypophosphatemic rickets with hypercalciuria (HHRH), nephrocalcinosis, calcium nephrolithiasis	241530	AR, AD	*SLC34A3*	Na-Po_4 co-transporter NaPi-IIc
Thick ascending limb	**OMIM #**	**Inheritance**	**Gene**	**Protein**
Bartter syndrome type 1	601678	AR	*SLC12A1*	NKCC2 co-transporter
Bartter syndrome type 2	241200	AR	*KCNJ1*	ROMK potassium channel
Bartter syndrome type 3	607364	AR	*CLCNKB*	ClC-Kb chloride channel
Bartter syndrome type 4a	602522	AR	*BSND*	Barttin subunit
Bartter syndrome type 4b	613090	DR	*CLCNKA/B*	ClC-Ka/ClC-Kb chloride channels
Bartter syndrome type 5	300971	XLR	*MAGED2*	MAGE-D2
Familial hypomagnesemia with hypercalciuria/nephrocalcinosis (FHHNC)(HOMG3)	248250	AR	*CLDN16*	Claudin-16 (paracellin-1), tight junction protein
Familial hypomagnesemia with hypercalciuria/nephrocalcinosis (FHHNC) and severe ocular involvement (HOMG5)	248190	AR	*CLDN19*	Claudin-19, tight junction protein
Autosomal dominant hypoparathyroidism 1 (ADH1)	601198	AD	*CASR*	CaSR, Ca^{2+}/Mg^{2+} sensing receptor
Autosomal dominant hypoparathyroidism 2 (ADH2)	615361	AD	*GNA11*	G-protein α-11
Collecting duct (CD)	**OMIM #**	**Inheritance**	**Gene**	**Protein**
dRTA 1 dRTA 4	179800 611590	AD AR	*SLC4A1*	AE1 anion echanger
dRTA 2 with progressive sensorineural deafness	267300	AR	*ATP6V1B1*	H^+-ATPase subunit B1
dRTA with/without sensorineural deafness	602722	AR	*ATP6V0A4*	H^+-ATPase subunit A4
dRTA with early onset sensorineural deafness	600791	AR	*FOXI1*	Forkhead box I1 transcription factor

Hypocalcemia

A detailed description of clinical symptoms and signs of hypocalcemia is provided in Chap. 31 (see Table 30.18). Following the approach outlined there (Table 30.19), the etiology of Ca^{2+} deficiency and hypocalcemia can be categorized according to the serum level of PTH [34]. Low levels of PTH indicate primary dysfunction of the parathyroid gland with impaired or absent PTH

production or release. Hereditary disorders involving abnormal parathyroid gland function include **primary hypoparathyroidism** due to mutations in the *PTH* gene (**isolated familial hypoparathyroidism 1**, OMIM #146200), due to **Di George syndrome** caused by microdeletions on chromosome 22q11 (OMIM #188400), or due to **HDR syndrome** (hypoparathyroidism, sensorineural deafness, renal dysplasia, OMIM #146255) caused by mutations in the transcription factor GATA3.

However, inappropriately low or normal levels of PTH may also point to an activating mutation in the *CASR* gene encoding the Ca^{2+} sensing receptor (CaSR) or in the *GNA11* gene encoding the accessory G-protein G-alpha-11 causing **autosomal-dominant hypocalcemia type 1 and 2**, respectively (ADH1, ADH2). These entities will be discussed in more detail below. ADH1 might be differentiated from the above mentioned disorders by determination of urinary Ca^{2+} excretion: In contrast to low urinary Ca^{2+} excretion found in primary forms of parathyroid dysfunction, urinary Ca^{2+} excretion is inappropriately high in face of hypocalcemia in patients with ADH1.

Elevated levels of PTH in the presence of normal renal function are also found in **acquired Ca^{2+}- and vitamin D-deficient rickets**. Whereas hypocalcemia might also be an associated finding in **renal Fanconi syndrome**, it is rather uncommon in other disorders of the proximal tubule such as hypophosphatemic rickets, Dent's disease or Lowe syndrome. These disorders are discussed in detail in Chaps. 32 and 44.

Pseudohypoparathyroidism represents a heterogeneous group of rare hereditary disorders with the common feature of PTH end-organ resistance and hypocalcemia despite elevated serum PTH levels (PHP1A OMIM #103580; PHP1B, OMIM #612462; PHP1C, OMIM #203330). Different (epi)genetic defects have been discovered as underlying causes, including loss of function mutations in the *GNAS* gene encoding the Gs-alpha isoform or methylation defects of the *GNAS* gene locus [35].

Autosomal Dominant Hypocalcemia

The extracellular **Ca^{2+}-sensing receptor (CaSR)** plays an essential role in Ca^{2+} and Mg^{2+} homeostasis by modulating PTH secretion and by directly regulating the rate of Ca^{2+} and Mg^{2+} reabsorption in the kidney [36]. The CaSR, a dimeric cell-surface protein of the G-protein receptor family, has a large extracellular domain that can bind extracellular Ca^{2+} at multiple sites [37]. Activation of the CaSR induces G_q and $G_{alpha\text{-}11}$ dependent phospholipase C signaling [18, 38], resulting in an inhibition of PTH release from the parathyroid gland and of renal tubular Ca^{2+} absorption. The function of the CaSR is inhibited by non-competitive binding of PO_4^{3-} to anion binding sites at the extracellular domain, leading to increased PTH secretion from the parathyroid gland [39]. Ca^{2+} sensing is also influenced by the level of CaSR surface expression. The CaSR is internalized from the plasma membrane by clathrin-mediated endocytosis. A central component of these clathrin-coated vesicles (CCVs) is the adaptor protein 2 (AP2) that forms a heterotetrameric complexes of α, β, μ, and σ (*AP2S1*) subunits [40]. Several diseases associated with both activating and inactivating mutations in the *CASR* gene, *GNA11*, and *AP2S1* have been described.

In the kidney, the CaSR is expression and basolateral localized in TAL, DCT, and intercalated cells of the cortical collecting duct (CCD) [41]. In addition, the CaSR is expressed in the apical membrane of the collecting duct as a putative sensor of urine Ca^{2+} levels [42]. Here, by adjusting aquaporin-2 expression, the CaSR is thought to adjust water diuresis to Ca^{2+} load, minimizing the risk of stone formation in the face of an increased urine Ca^{2+} excretion [43].

Autosomal dominant hypocalcemia 1 (ADH1) is caused by activating mutations in the *CASR* gene. Affected individuals typically manifest during childhood with seizures or carpopedal spasms. Laboratory evaluation reveals the typical combination of hypocalcemia and low PTH levels. Serum Ca^{2+} levels are usually in a range of 6–7 mg/

dL. In addition, many patients also exhibit moderate hypomagnesemia [44, 45]. Patients are often given the incorrect diagnosis of primary hypoparathyroidism on the basis of inappropriately low PTH levels. As indicated above, the differential diagnosis can be established by determination of urinary Ca^{2+} excretion which is low in primary hypoparathyroidism but usually increased in ADH1 patients. The differentiation of ADH from primary hypoparathyroidism is of particular importance because treatment with active vitamin D in ADH may result in a dramatic increase in hypercalciuria with subsequent nephrocalcinosis and impairment of renal function. Therefore, therapy with active vitamin D or Ca^{2+} supplementation should be reserved for symptomatic patients with the aim to maintain serum Ca^{2+} levels just sufficient for the relief of symptoms [45].

Activating *CASR* mutations lead to a lower setpoint of the receptor or an increased affinity for extracellular Ca^{2+} and Mg^{2+}. This inadequate activation by physiological extracellular Ca^{2+} and Mg^{2+} levels results in a diminished PTH secretion in the parathyroid gland as well as a decreased reabsorption of both divalent cations in the TAL. A severe degree of hypocalcemia and hypomagnesemia is observed in patients with complete activation of the CaSR at physiologic serum Ca^{2+} and Mg^{2+} concentrations who may also exhibit a Bartter-like phenotype [46]. In these patients, CaSR activation inhibits TAL-mediated salt and divalent cation reabsorption to an extent that cannot be compensated in more distal nephron segments [46].

Genetic heterogeneity in autosomal-dominant hypocalcemia (**ADH2**) was demonstrated by the discovery of heterozygous missense mutations in *GNA11* encoding the accessory G-protein $G_{alpha\text{-}11}$ of the CaSR [47, 48].

Hypercalcemia

Clinical Findings

In contrast to hypocalcemia, hypercalcemia represents a rather uncommon finding in infancy and childhood but requires prompt diagnostic work-up and targeted therapy [49, 50]. Hypercalcemia is defined as ionized Ca^{2+} above 1.35 mmol/L, usually with concomitant elevation of total serum Ca^{2+} levels above 2.7 mmol/L. While most children with a mild degree of hypercalcemia remain asymptomatic, more severe hypercalcemia can lead to a serious clinical disease pattern that may even be life-threatening, especially in infants. Symptoms of hypercalcemia include failure to thrive, weight loss, dehydration, fever, muscular hypotonia, constipation, irritability, lethargy, and disturbed consciousness. Cardiovascular findings may comprise bradycardia, shortened QT interval, and arterial hypertension (Table 37.3).

An impairment of renal function by volume contraction can further limit renal Ca^{2+} elimination and therefore aggravate hypercalcemia. On the other hand, the increase of renal Ca^{2+} excretion in hypercalcemic conditions is a risk factor for the development of nephrocalcinosis and kidney stone formation.

Table 37.3 Symptoms of acute hypercalcemia

Neurology
Muscle weakness
Irritability/confusion
Somnolence, stupor, coma
Abnormal behaviour
Headache
Gastrointestinal tract
Loss of appetite
Nausea/vomiting
Constipation
Abdominal cramping
Pancreatitis
Kidneys and urinary tract
Polyuria/polydipsia
Dehydratation
Nephrocalcinosis
Nephro-/urolithiasis
Musculoskeletal system
Bone pain
Ectopic calcifications
Heart/cardiovascular system
Shortened QT interval
Cardiac arrhythmia
Arterial hypertension

Diagnostic Workup

The assessment of past medical history should comprise medications including over-the-counter vitamin preparations and family history with a focus on disturbances in Ca^{2+} metabolism, renal disease and urolithiasis.

The etiology of hypercalcemia in children and especially infants significantly differs from that in adulthood where primary hyperparathyroidism and malignancy represent the most common underlying causes [51]. Especially in infants, rare hereditary disorders should be considered in the differential diagnosis [49].

The diagnostic work-up requires comprehensive laboratory testing including molecular genetics. Next to the parallel measurement of serum and urine electrolytes, the determination of PTH and vitamin D metabolites represents the most important element. Potential diagnostic parameters are summarized in Table 37.4.

Inappropriately normal or elevated levels of PTH point to a primary defect in the parathyroid gland (Fig. 37.2). Next to primary hyperparathyroidism, which is extremely rare in infancy and childhood, hypercalcemia with elevated PTH might be caused by a hereditary disorder of the CaSR or associated proteins. In contrast to dominant activating CaSR mutations as present in ADH1 (see above), inactivating CaSR mutations may be present in either heterozygous or homozygous/compound-heterozygous state leading to Familial Hypocalciuric Hypercalcemia 1 (FHH1) and Neonatal Severe Hyperparathyroidism (NSHPT), respectively (see below). Genetic heterogeneity in FHH was demonstrated in 2013 by discovery of heterozygous mutations in *GNA11* (FHH2) and *AP2S1* (FHH3) [40, 47].

Table 37.4 Evaluation of hypercalcemia in children

Laboratory tests	
Blood	Ionized and total calcium
	Sodium, potassium, chloride, magnesium, phosphate
	Renal function tests
	Alkaline phosphatase
	Blood gases
	Intact parathyroid hormone (iPTH)
	Vitamin D metabolites (25-OH-D_3, 1,25-$(OH)_2$-D_3)
	Optional: PTH-related peptide (PTHrP)
	Vitamin A
	Angiotensin converting enzyme (ACE)
	FISH (Williams-Beuren-Syndrom)
Urine	Calcium
	Sodium, potassium, chloride, magnesium, phosphate
	Creatinine
	->Ca^{2+}/Crea ratio and tubular phosphate reabsorption (TRP)
Imaging studies	
	Ultrasound examination of kidneys and urinary tract
	Optional:
	X-ray studies (long bones, thorax)
	Ultrasound of parathyroid glands

In face of an appropriate suppression of PTH, vitamin D metabolites should be evaluated. The determination of 25-OH-vitamin D_3 primarily helps to exclude overt vitamin D intoxication. While cut-off values vary in the literature, levels above 200 ng/mL are usually considered toxic [52]. Next, serum levels of active 1,25-$(OH)_2$-vitamin D_3 are of critical importance for further diagnostic considerations (Fig. 37.2). Elevated levels are not only observed in vitamin D poisoning, but also in disorders with extra-renal expression of 1α-hydroxylase including granulomatous disease or subcutaneous fat necrosis. In these disorders, 1α-hydroxylase (CYP27B1) is expressed by monocytes and, in contrast to renal tissue, not regulated by serum Ca^{2+}, PO_4^{3-}, PTH, and 1,25-$(OH)_2$-vitamin D_3. Finally, in hypercalcemic individuals with high levels of active 1,25-$(OH)_2$-vitamin D_3, hereditary disorders of vitamin D metabolism or renal phosphate handling should be considered (see below).

Treatment

Acute therapeutic measures in hypercalcemic patients primarily aim at the lowering of serum Ca^{2+} levels into the reference range resulting in a rapid relief of clinical symptoms. For this purpose, different pharmacologic approaches have been considered (Table 37.5).

Even before clarification of the underlying etiology, vitamin D supplements need to be

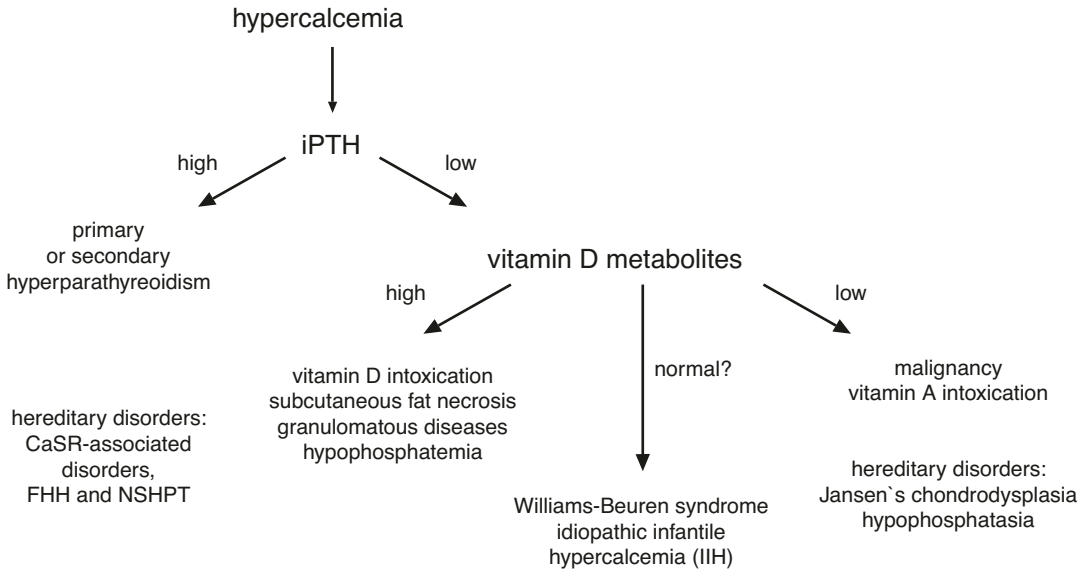

Fig. 37.2 Diagnostic approach in hypercalcemia in childhood. Central diagnostic steps are the determination of parathyroid hormone (PTH) and vitamin D-metabolites. Following this diagnostic approach, three major entities can be discerned: PTH-dependent hypercalcemia as present in primary hyperparathyroidism and in disorders due to inactivating mutation of the CaSR, vitamin D-induced hypercalcemia including idiopathic infantile hypercalcemia, and hypercalcemia due to third causes including also hypophosphatasia

Table 37.5 Therapy of hypercalcemia in children

General measures
Stop of vitamin D supplementation
Ca^{2+} restriction in enteral and parenteral nutrition
Specific measures
Promotion of renal Ca^{2+} excretion
– Furosemide 0.5–1 mg per kg body weight q6h
Inhibition of enteral Ca^{2+} absorption/inhibition of vitamin D-conversion
– Glucocorticoids, i.e. methylprednisolone ~1 mg/kg q.6h
– Na^{+}-cellulose phosphate
Inhibition of Ca^{2+} release from bone
– Bisphosphonates, i.e. pamidronate 0.5–1 mg/kg over 4–6 h
– Calcitonin 4–8 IU/kg body weight
Inhibition of vitamin D-activation by 1α-hydroxylase
– Imidazole derivates, i.e. ketoconazole 3–9 mg per kg body weight per day
Hemodialysis/hemofiltration

stopped and, if appropriate, a low-Ca^{2+} diet should be implemented. Vigorous rehydration, usually performed via the intravenous route, is a key therapeutic strategy in hypercalcemia independent of etiology. Quantities up to twice the daily fluid requirements have been described. Pharmacological treatment includes measures to decrease Ca^{2+} absorption from the intestine, to promote renal Ca^{2+} excretion, and to inhibit Ca^{2+} release from bone. To increase renal Ca^{2+} elimination loop diuretics that inhibit paracellular Ca^{2+} reabsorption in the TAL (such as furosemide) are widely used. A fast and effective approach to inhibit enteral Ca^{2+} absorption is the administration of glucocorticoids, i.e. prednisolone. Next to this intestinal effect, glucocorticoids also inhibit the conversion of 25-OH-vitamin D_3 into active 1,25-$(OH)_2$-vitamin D_3, an effect that is of special importance in vitamin D-mediated hypercalcemia. Sodium cellulose phosphate (SCP) is a non-absorbable cation exchange resin used for the removal of excess Ca^{2+} from the body [53]. It was initially used in patients with so-called absorptive hypercalciuria in order to decrease renal Ca^{2+} excretion and prevent stone formation [54]. In the acute phase of hypercalcemia calcitonin may be used because of its prompt and pronounced effect on serum Ca^{2+} levels, however, its therapeutic usefulness is limited by its short duration of action due to the develop-

ment of end organ resistance. In the presence of intermediate to severe symptomatic hypercalcemia, bisphosphonates such as pamidronate may be used [55]. If bisphosphonate therapy is considered, it is important to have the diagnostic work-up completed in advance to avoid misinterpretation of diagnostic tests for PTH and vitamin D metabolites. A class of drugs that are specifically used in vitamin D-mediated hypercalcemia are imidazole derivates, such as ketoconazole [56]. Next to their antifungal effect these compounds also inhibit mammalian cytochrome P450 enzymes. Via inhibition of 1α-hydroxylase (CYP27B1) they are able to effectively lower serum levels of active 1,25-$(OH)_2$-vitamin D_3 and consecutively normalize serum Ca^{2+} levels. In individual patients with extremely high serum Ca^{2+} levels hemofiltration and hemodialysis have been applied sucessfully [57].

Hypercalcemia with Inappropriately High PTH

Familial Hypocalciuric Hypercalcemia/ Neonatal Severe Hyperparathyroidism

Familial hypocalciuric hypercalcemia 1 (FHH1) and **neonatal severe hyperparathyroidism** (NSHPT) result from inactivating mutations of the CaSR present in either mono-allelic or bi-allelic state, respectively [58]. Genetic heterogeneity has been described in FHH with heterozygous variants in *GNA11* (**FHH2**) encoding accessory G-protein $G_{\alpha 11}$ of the CaSR and in *AP2S1* (**FHH3**) encoding adaptor protein σ1 involved in CaSR recycling from the plasma membrane [40, 47]. Therefore, all three subtypes of FHH share the pathophysiology of a decreased CaSR-mediated Ca^{2+} sensing. FHH patients typically present with mild to moderate hypercalcemia, accompanied by few if any symptoms, and often do not require treatment. However, the occurrence of pancreatitis and chondrocalcinosis has been described [59]. Urinary Ca^{2+} and Mg^{2+} excretion rates are markedly reduced and serum PTH levels are inappropriately high. In addition, affected individuals also show mild hypermagnesemia [60].

In contrast to FHH, patients with **NSHPT** with two mutant CaSR alleles usually present in early infancy with polyuria and dehydration due to severe symptomatic hypercalcemia (Table 37.9). Unrecognized and untreated, hyperparathyroidism and hypercalcemia result in skeletal deformities, extraosseous calcifications, and also severe neurodevelopmental deficit. Early treatment with partial to total parathyroidectomy therefore seems to be essential for outcome [61]. Serum PO_4^{3-} levels are typically low due to increased iPTH levels. Data on serum Mg^{2+} in NSHPT is sparse and contradictory [62]. However, elevations to levels around 50% above the reference range have been reported.

Hypercalcemia with Suppressed PTH and Inappropriately High 1,25-$(OH)_2$-Vitamin D_3

Idiopathic Infantile Hypercalcemia

Idiopathic Infantile Hypercalcemia (IIH) was first decribed in the 1950s after an endemic occurrence in the United Kingdom (UK) [63, 64]. Affected infants present with typical symptoms of severe hypercalcemia. Concomitant hypercalciuria typically leads to the development of early nephrocalcinosis (Table 37.9). The laboratory analysis reveals serum Ca^{2+} levels up to 5 mmol/L. Intact PTH is suppressed while levels of active 1,25-$(OH)_2$-vitamin D_3 are usually elevated or in the upper normal range. A role of exogenously administered vitamin D was suspected early on [65–67]. Some children exhibited a complex phenotype that became later known as the Williams Beuren syndrome [68, 69]. However, most hypercalcemic infants did not have syndromic features and were considered to be affected by a milder variant of the syndrome that was termed idiopathic infantile hypercalcemia (IIH) [63, 64].

The pathophysiology of IIH remained elusive until the discovery of loss-of-function mutations in the vitamin D catabolizing enzyme

25-OH-vitamin D_3-24-hydroxylase (CYP24A1) [70]. CYP24A1 is responsible for several sequential degradation steps that convert active 1,25-$(OH)_2$-vitamin D_3 into water soluble calcitroic acid [71, 72]. Loss-of-function mutations of *CYP24A1* lead to an accumulation and increased action of active 1,25-$(OH)_2$-vitamin D_3. Functional studies in vitro demonstrated a complete loss of enzyme function for most of the identified *CYP24A1* mutations [70]. In addition to the genetic analysis of the *CYP24A1* gene, the determination of 24-hydroxylated vitamin D metabolites by liquid chromatography/mass spectrometry (LC/MS) represents a quick and reliable test in the diagnosis of the disease [73].

The critical role of the cumulative dose of exogenous vitamin D is underscored by the following observations: Under the most commonly used dose of 500 IU vitamin D_3 per day, symptoms usually develop after several months. Higher doses of supplemental vitamin D most likely lead to an increased incidence of the disease in infancy and an earlier manifestation. Regimens using oral bolus doses of up to 600.000 IU provoke symptoms of acute vitamin D toxicity in infants with CYP24A1 deficiency while being tolerated well by healthy individuals [74]. Finally, omitting vitamin D supplementation in a genetically affected infant due to symptomatic disease in the older sibling prevented hypercalcemic episodes and the development of nephrocalcinosis [70].

After diagnosis of symptomatic hypercalcemia with inappropriately high levels of 1,25-$(OH)_2$-vitamin D_3, vitamin D prophylaxis is usually stopped and a low-Ca^{2+} diet might be instituted. The restriction of dietary Ca^{2+} has to be carefully monitored as it might lead to a defective mineralization of bone as well as an increased intestinal absorption of oxalate with subsequent risk of stone formation. Next to these immediate therapeutic measures, vigorous intravenous rehydration and a repertoire of strategies to reduce serum Ca^{2+} levels as described above are used (Table 37.5).

Currently, it remains an unanswered question, why many of the affected individuals after acute treatment in infancy do not show recurrence of symptomatic hypercalcemia during later life. Potentially, compensatory mechanisms, i.e. a down-regulation of 1α-hydroxylase (CYP27B1) are able to prevent an excessive activation of vitamin D. Laboratory parameters, i.e. suppressed PTH and inappropriately high values of 1,25-$(OH)_2$-vitamin D_3, are detectable for a long time during follow-up. However, nephrocalcinosis persists and together with recurrent nephrolithiasis might result in an impairment of renal function. Interestingly, since the initial description of *CYP24A1* mutations, several groups identified *CYP24A1* mutations also in adult patients with mild hypercalcemia, nephrocalcinosis, and recurrent kidney stone disease [75–77].

Genetic heterogeneity in IH was demonstrated by discovery of recessive mutations in *SLC34A1* encoding proximal-tubular sodium-phosphate co-transporter NaPi-IIa [78]. Patients with NaPi-IIa defects share phenotypic and biochemical features of patients with *CYP24A1* mutations but hypercalcemia is usually milder. In addition, they exhibit phosphate depletion and hypophosphatemia. The pathophysiology involves primary renal phosphate wasting, phosphate depletion, and suppression of the phosphaturic hormone FGF-23. FGF-23, next to its role in proximal-tubular phosphate reabsorption, negatively regulates vitamin D metabolism by inhibiting 1α-hydroxylase (*CYP27B1*) and promoting the degradation of active 1,25-$(OH)_2$-vitamin D_3 by 24-hydroxylase (*CYP24A1*) [78].

In addition to acute therapeutic measures also applied in patients with *CYP24A1* defects, hypercalcemic patients potentially benefit from PO_4^{3-} supplementation in order to control Ca^{2+} metabolism.

A significant subset of patients with NaPi-IIa defects may also be diagnosed with isolated early nephrocalcinosis or present later in childhood or adolescence with nephrolithiasis [79]. Of note, prenatally diagnosed renal calcifications due to *SLC34A1* mutations have even been described as a differential diagnosis for fetal hyperechogenic kidneys [80].

Very similar biochemical changes as in NaPi-IIa deficiency have also been reported in patients with *SLC34A3* defects encoding the

closely related proximal-tubular sodium-phosphate co-transporter NaPi-IIc [81]. Mutations in *SLC34A3* have initially been described in patients with **hypophosphatemic rickets with hypercalciuria** (HHRH) [82, 83]. Whereas a clinical manifestation with hypercalcemia in infancy has not been reported yet in a patient with *SLC34A3* defect, patients with bi-allelicas well as mono-allelic *SLC34A3* mutations appear to commonly present with nephrocalcinosis and/or kidney stones [81]. As individuals with CYP24A1 defects, patients with NaPi-IIa and NaPI-IIc defects are at risk to develop chronic renal failure [84].

Magnesium Physiology

Mg^{2+} is the second most abundant intracellular cation in the body. As a cofactor for many enzymes, it is involved in energy metabolism and protein and nucleic acid synthesis. It also plays a critical role in the modulation of membrane transporters and in signal transduction. Under physiologic conditions, serum Mg^{2+} levels are maintained at almost constant values. Homeostasis depends on the balance between intestinal absorption and renal excretion. Mg^{2+} deficiency can result from reduced dietary intake, intestinal malabsorption or renal loss. The control of body Mg^{2+} homeostasis is primarily accomplished in the kidney tubules.

The daily dietary intake of Mg^{2+} varies substantially. Within physiologic ranges, diminished Mg^{2+} intake is balanced by enhanced Mg^{2+} absorption in the intestine and reduced renal excretion. These transport processes are regulated by metabolic and hormonal influences [85, 86]. The principal site of Mg^{2+} absorption is the small intestine, with smaller amounts being absorbed in the colon. Intestinal Mg^{2+} absorption occurs via two different pathways: a saturable active transcellular transport and a nonsaturable paracellular passive transport (Fig. 37.3a) [86, 87]. Saturation kinetics of the transcellular transport system are explained by the limited transport capacity of active transport. At low intraluminal concentrations Mg^{2+} is absorbed primarily via the active transcellular route and with rising concentrations via the paracellular pathway, yielding a curvilinear function for total absorption (Fig. 37.3b).

In the kidney, approximately 80% of total serum Mg^{2+} is filtered in the glomeruli, of which more than 95% is reabsorbed along the nephron. Mg^{2+} reabsorption differs in quantity and kinetics depending on the different nephron segments. 15–20% are reabsorbed in the proximal tubule of the adult kidney. Interestingly, the premature kidney of the newborn is able to reabsorb up to 70% of the filtered Mg^{2+} in this nephron segment [88].

From early childhood onward, the majority of Mg^{2+} (around 70%) is reabsorbed in the loop of Henle, especially in the cortical TAL. Transport in this segment is passive and paracellular, driven by the lumen-positive transepithelial voltage (Fig. 37.4a). Although only 5–10% of the filtered Mg^{2+} is reabsorbed in the distal convoluted tubule (DCT), this is the part of the nephron where the fine adjustment of renal excretion is accomplished. The reabsorption rate in the DCT defines the final urinary Mg^{2+} excretion as there is no significant uptake of Mg^{2+} in the collecting duct. Mg^{2+} transport in this part of the nephron is an active transcellular process (Fig. 37.4b). The apical entry into DCT cells is mediated by a specific and regulated Mg^{2+} channel driven by a favorable transmembrane voltage [89]. The mechanism of basolateral transport into the interstitium is unknown. Here, Mg^{2+} has to be extruded against an unfavourable electrochemical gradient. Most physiologic studies favor a Na^{+}-dependent exchange mechanism [90]. Mg^{2+} entry into DCT cells appears to be the rate-limiting step and the site of regulation. Finally, 3–5% of the filtered Mg^{2+} is excreted in the urine.

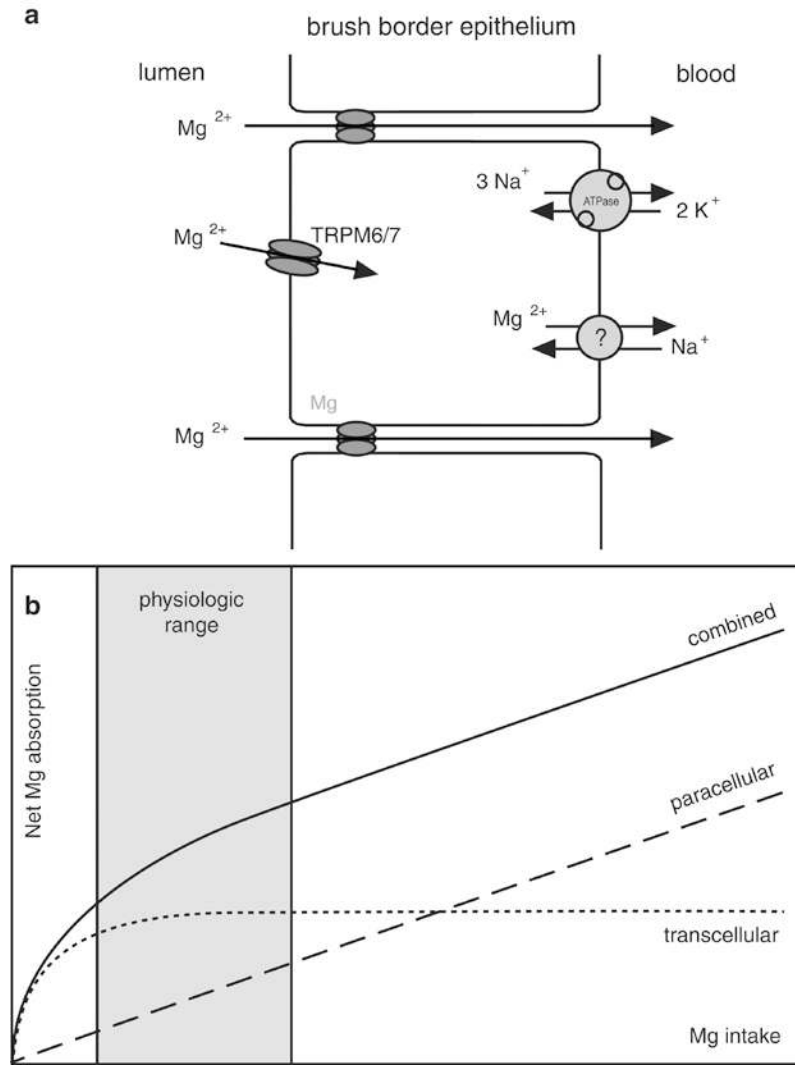

Fig. 37.3 Intestinal Mg^{2+} reabsorption. (**a**) Model of intestinal Mg^{2+} absorption via two independent pathways: passive absorption via the paracellular pathway and active, transcellular transport consisting of an apical entry through a putative Mg^{2+} channel and a basolateral exit mediated by a putative Na^+–coupled exchange. (**b**) Kinetics of intestinal Mg^{2+} absorption in humans. Paracellular transport linearly rising with intraluminal concentrations (dotted line) and saturable active transcellular transport (dashed line) together yield a curvilinear function for net Mg^{2+} absorption (solid line)

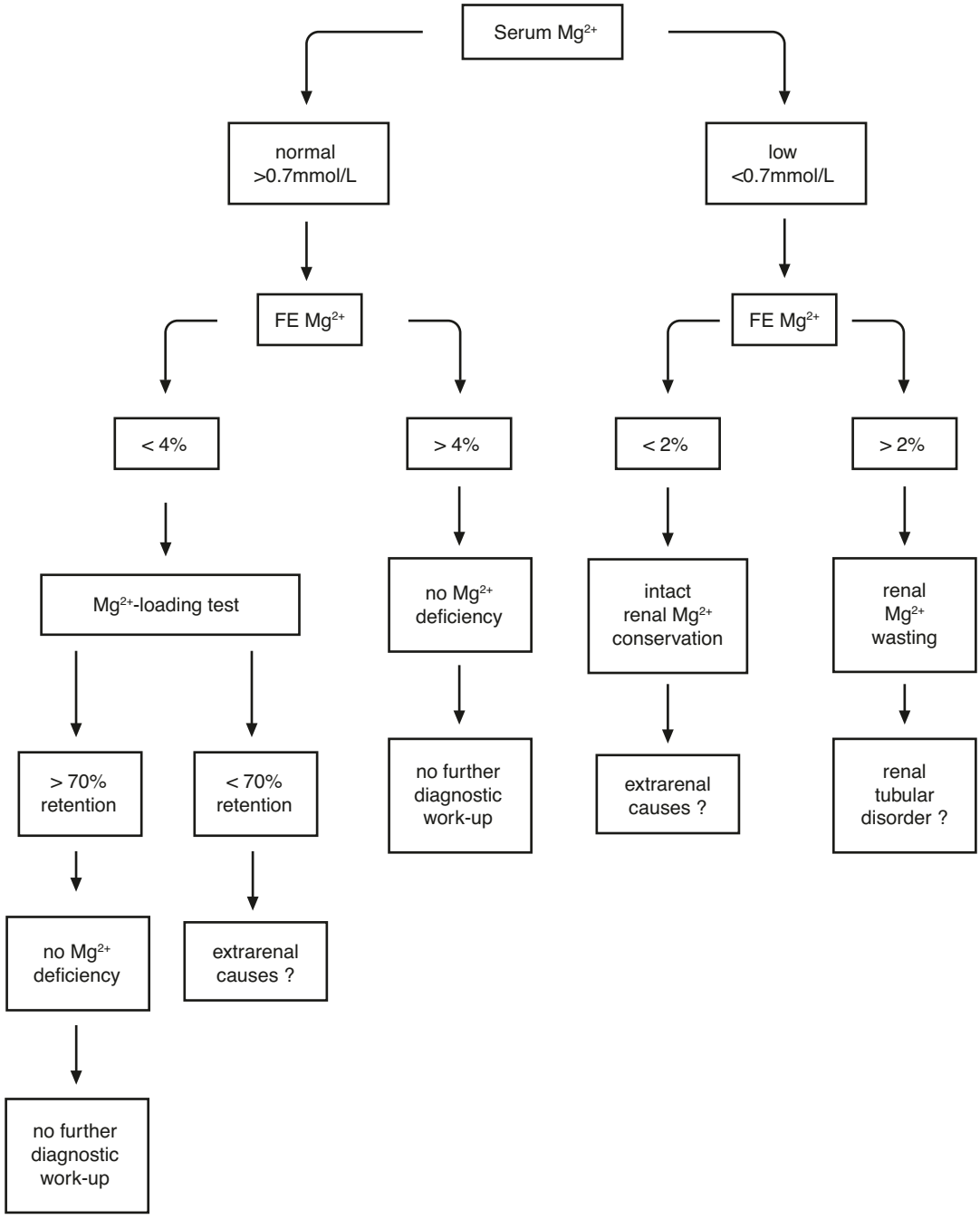

Fig. 37.4 Diagnostic workup in patients with suspected magnesium deficiency. In face of hypomagnesemia, the determination of urinary Mg^{2+} excretion allows for a distinction between renal Mg^{2+} wasting and extrarenal losses. In normomagnesemic individualswith low urinary magnesium excretions, an increased retention of Mg^{2+} in the Mg^{2+}-loading test might indicate Mg^{2+} deficiency. This test, however, requires an intact renal Mg^{2+} conservation process

Magnesium Depletion

Mg^{2+} depletion usually occurs secondarily to another disease process or to a therapeutic agent. Some disorders that can be associated with Mg^{2+} depletion are summarized in Table 37.6 [91]. Mg^{2+} may be lost via the gastrointestinal tract, either by excessive loss of secreted fluids or impaired absorption of both dietary and endogenous Mg^{2+}. Mg^{2+} depletion is common in patients with acute or chronic diarrhea. Malabsorption syndromes such as celiac disease may also result in Mg^{2+} deficiency. Also, acute severe pancreatitis may be associated with hypomagnesemia.

Excessive excretion of Mg^{2+} into the urine is another cause of Mg^{2+} depletion. Renal Mg^{2+} excretion is proportional to tubular fluid flow as well as to Na^+ and Ca^{2+} excretion. Therefore, both chronic intravenous fluid therapy with Na^+-containing fluids and disorders in which there is extracellular volume expansion may result in Mg^{2+} depletion. Hypercalcemia and hypercalciuria have been shown to decrease renal Mg^{2+} reabsorption and are probably the cause of excessive renal Mg^{2+} excretion and hypomagnesemia observed in many hypercalcemic states. A large variety of pharmacological agents also cause renal Mg^{2+} wasting and Mg^{2+} depletion (see below). Various renal diseases, e.g. chronic pyelonephritis or post-obstructive nephropathy may also be accompanied by Mg^{2+} losses.

During infancy and childhood, a substantial proportion of patients receiving medical attention for signs of hypomagnesemia are affected by inherited renal disorders associated with Mg^{2+} wasting. In these disorders, hypomagnesemia may either be the leading symptom or may be part of a complex phenotype resulting from tubular dysfunction. Finally, Mg^{2+} wasting may be caused by endocrine disorders, e.g. by hyperparathyroidism because of the hypercalcemia or within the context of a SIADH state. In SIADH, Mg^{2+} losses are explained by the volume expansion.

Table 37.6 Main causes of magnesium deficiency

Gastrointestinal loss
Prolonged nasogastric suction/vomiting
Acute and chronic diarrhea
Malabsorption syndromes (e.g., celiac disease)
Extensive bowel resection
Intestinal and biliary fistulas
Acute hemorrhagic pancreatitis
Renal loss
Chronic parenteral fluid therapy
Osmotic diuresis (e.g. due to presence of glucose in diabetes mellitus)
Hypercalcemia
Drugs (e.g. diuretics, aminoglycosides, calcineurin inhibitors)
Alcohol
Metabolic acidosis
Renal diseases
– Chronic pyelonephritis, interstitial nephritis, and glomerulonephritis
– Polyuria after acute renal failure
– Post-obstructive nephropathy
– Renal tubular acidosis
– After kidney transplantation
Inherited tubular diseases
Endocrine disorders
– Hyperparathyroidism
– Hyperthyroidism
– Hyperaldosteronism
Syndrome of inappropriate secretion of antidiuretic hormone (SIADH)

Manifestations of Hypomagnesemia

Mg^{2+} deficiency and hypomagnesemia often remain asymptomatic. Clinical symptoms are mostly not very specific and Mg^{2+} deficiency is frequently associated with other electrolyte abnormalities. The biochemical and physiologic manifestations of severe Mg^{2+} depletion are summarized in Table 37.7.

Hypokalemia

A common accompanying feature of Mg^{2+} depletion is hypokalemia [91]. During Mg^{2+} depletion, there is loss of K^+ from the cell with intracellular K^+ depletion, which is enhanced due to the inability of the kidney to conserve K^+. Attempts to replete the K^+ deficit with K^+ therapy alone may not be successful without simultaneous Mg^{2+}

Table 37.7 Major manifestations of magnesium depletion

Biochemical
Hypokalemia
Excessive renal potassium excretion
Decreased intracellular potassium
Hypocalcemia
Impaired parathyroid hormone (PTH) secretion
Renal and skeletal resistance to PTH
Resistance to vitamin D
Neuromuscular
Positive Chvostek's and Trousseau's sign
Spontaneous carpal-pedal spasm
Seizures
Vertigo, ataxia, nystagmus, athetoid and chorioform movements
Muscular weakness, tremor, fasciculation and wasting
Psychiatric: depression, psychosis
Cardiovascular
Electrocardiographic abnormalities
Prolonged PR- and QT-intervals
U-waves
Cardiac arrhythmia
Atrial tachycardia, fibrillations
"Torsades de pointes"
Gastrointestinal
Nausea, vomiting
Anorexia

supplementation. K^+ depletion may contribute to the electrocardiographic findings and cardiac arrhythmias observed in $Mg^{2}+$ deficiency. Secondary K^+ depletion in the presence of Mg^{2+} deficiency must be differentiated from renal tubular disorders mainly affecting the distal convoluted tubule (DCT) that lead to combined losses of both cations, such as Gitelman syndrome (see below).

Hypocalcemia

Hypocalcemia is a common finding in moderate to severe Mg^{2+} depletion and may be a major contributing factor to the increased neuromuscular excitability often present in Mg^{2+}-depleted patients. The pathogenesis of hypocalcemia is multifactorial. Impaired parathyroid hormone (PTH) secretion appears to be a major factor in hypomagnesemia-induced hypocalcemia. Serum PTH concentrations are usually low in these patients, and Mg^{2+} administration will immediately stimulate PTH secretion. Patients with hypocalcemia due to Mg^{2+} depletion also exhibit both renal and skeletal resistance to exogenously administered PTH, as manifested by subnormal urinary cyclic AMP (cAMP) and phosphate excretion and a diminished calcemic response. All these effects are reversed following several days of Mg^{2+} therapy. The paradoxical inhibition of PTH secretion in patients with severe hypomagnesemia was already described in the 1970s [92]. Later, the failure of the parathyroid gland to synthesize and secrete PTH was attributed to a defect in G-protein signaling within parathyroid cells [93]. As a functional consequence, the intracellular signaling pathways responsible for Ca^{2+}-sensing Receptor (CaSR)-mediated inhibition of PTH secretion are enhanced including the generation of inositol phosphates and the inhibition of cAMP. Because cAMP is also an important second messenger mediating PTH effects in kidney and bone, it was also postulated that there may be a defect in adenylate cyclase function as Mg^{2+} is both an essential part of the substrate (Mg-ATP) as well as an important co-factor for catalytic activity [94].

Vitamin D metabolism and action may also be abnormal in hypocalcemic Mg^{2+}-deficient patients. Resistance to vitamin D therapy has been reported in such cases. This resistance may be due to impaired metabolism of vitamin D because plasma concentrations of 1,25-dihydroxyvitamin D_3 are low. Because PTH is a major stimulator of 1,25-dihydroxyvitamin D_3 synthesis, the decrease in PTH secretion observed in hypomagnesemia and hypocalcemia may also be a cause of the impaired metabolism of vitamin D.

Neuromuscular Manifestations

Neuromuscular hyperexcitability may be the prominent complaint of patients with Mg^{2+} deficiency. Tetany and muscle cramps may be present. Generalized seizures may also occur. Other neuromuscular signs may include dizziness, dis-

equilibrium, muscular tremor, wasting, and weakness [91]. Although hypocalcemia often contributes to the neurologic signs, hypomagnesemia without hypocalcemia has also been reported to result in neuromuscular hyperexcitability.

Cardiovascular Manifestations

Mg^{2+} depletion may also result in electrocardiographic abnormalities as well as in cardiac arrhythmias [95], which may be manifested by tachycardia, premature beats, or a totally irregular cardiac rhythm (fibrillation). Cardiac arrhythmia is also known to occur during K^+ depletion; therefore, the effect of Mg^{2+} deficiency on K^+ loss may be a contributing factor (see section on Gitelman syndrome below) [91].

Clinical Assessment of Magnesium Deficiency

Although Mg^{2+} is an abundant cation in the body, more than 99% of it are located either intracellularly or in bone. The less than 1% of total Mg^{2+} present in the body fluids is the most easily accessible compartment for clinical testing, and the total serum Mg^{2+} concentration is the most widely used measure of Mg^{2+} status, although its limitations in reflecting Mg^{2+} deficiency are well recognized [96]. The reference range for normal total serum Mg^{2+} concentration is a subject of ongoing debate, but concentrations of 0.7–1.1 mmol/L are widely accepted. Because the measurement of serum Mg^{2+} concentration does not necessarily reflect the true total body Mg^{2+} content, it has been suggested that measurement of ionized serum Mg^{2+} or intracellular Mg^{2+} concentrations might provide more precise information on Mg^{2+} status. However, the relevance of such measurements to body Mg^{2+} stores has been questioned because the ionized serum Mg^{2+} and intracellular Mg^{2+} did not correlate with tissue Mg^{2+} and the correlation with the results of Mg^{2+} retention tests was contradictory [97–99].

Hypomagnesemia develops late in the course of Mg^{2+} deficiency and intracellular Mg^{2+} depletion may be present despite normal serum Mg^{2+} levels. Due to the kidney's ability to sensitively adapt its Mg^{2+} transport rate to imminent deficiency, the urinary Mg^{2+} excretion rate is important in the assessment of the Mg^{2+} status. In hypomagnesemic patients, urinary Mg^{2+} excretion rates help to discern renal Mg^{2+} wasting from extrarenal losses. In the presence of hypomagnesemia, the 24-h Mg^{2+} excretion is expected to decrease below 1 mmol [100]. Mg^{2+}/creatinine ratios and fractional Mg^{2+} excretions have also been advocated as indicators of evolving Mg^{2+} deficiency [101, 102]. However, the interpretation of these results seems to be limited due to intra- and inter-individual variability [103, 104].

In patients at risk for Mg^{2+} deficiency but with normal serum Mg^{2+} levels, the Mg^{2+} status can be further evaluated by determining the amount of Mg^{2+} excreted in the urine following an intravenous infusion of Mg^{2+}. This procedure has been described as "parenteral Mg^{2+} loading test" and is still the gold standard for the evaluation of the body Mg^{2+} status [96, 98]. Normal subjects excrete at least 80% of an intravenous Mg^{2+} load within 24 h, whereas patients with Mg^{2+} deficiency excrete much less. The Mg^{2+} loading test, however, requires normal renal handling of Mg^{2+}.

Hereditary Disorders of Mg^{2+} Handling

Recent advances in molecular genetics of hereditary hypomagnesemia substantiated the role of a variety of genes and their encoded proteins in human epithelial Mg^{2+} transport (Table 37.8). The knowledge of underlying genetic defects helps to distinguish different clinical subtypes of hereditary disorders of Mg^{2+} homeostasis.

By careful clinical observation and additional biochemical parameters, the different disease entities can already be distinguished clinically and biochemically in many cases, even if there might be a considerable overlap in phenotypic characteristics (Table 37.9).

Table 37.8 Inherited disorders of renal Ca^{2+} and Mg^{2+} handling

Thick ascending limb (TAL)	**OMIM #**	**Inheritance**	**Gene**	**Protein**
Autosomal dominant hypoparathyroidism 1 (ADH1)	601198	AD	*CASR*	CaSR, Ca^{2+}/Mg^{2+} sensing receptor
Autosomal dominant hypoparathyroidism 2 (ADH2)	615361	AD	*GNA11*	G-protein α-11
Familial hypocalciuric hypercalcemia 1 (FHH1)	145980	AD	*CASR*	CaSR, Ca^{2+}/Mg^{2+} sensing receptor
Familial hypocalciuric hypercalcemia 2 (FHH2)	145981	AD	*GNA11*	G-protein α-11
Familial hypocalciuric hypercalcemia 3 (FHH3)	600740	AD	*AP2S1*	Adaptor protein 2 σ-subunit
Neonatal severe hyperparathyroidism (NSHPT)	239200	AR	*CASR*	CaSR, Ca^{2+}/Mg^{2+} sensing receptor
Familial hypomagnesemia with hypercalciuria/ nephrocalcinosis (FHHNC) (HOMG3)	248250	AR	*CLDN16*	Claudin-16, tight junction protein
Familial hypomagnesemia with hypercalciuria/ nephrocalcinosis (FHHNC) and severe ocular involvement (HOMG5)	248190	AR	*CLDN19*	Claudin-19, tight junction protein
Hypohidrosis, electrolyte imbalance, lacrimal gland dysfunction, ichthyosis, and xerodermia (HELIX)	617671	AR	*CLDN10*	Claudin-10, tight junction protein
Distal convoluted tubule (DCT)	**OMIM #**	**Inheritance**	**Gene**	**Protein**
Gitelman syndrome (GS)	263800	AR	*SLC12A3*	NCC, NaCl cotransporter
EAST/SeSAME syndrome	612780	AR	*KCNJ10*	KCNJ10, basolateral potassium channel
Hypomagnesemia with secondary hypocalcemia (HSH) (HOMG1)	602014	AR	*TRPM6*	TRPM6, ion channel subunit
Hypomagnesemia, seizures, and mental retardation 2 (HOMGSMR2)	618314	AD	*ATP1A1*	α1-subunit of the Na^+-K^+-ATPase
Isolated dominant hypomagnesemia (IDH) (HOMG2)	154020	AD	*FXYD2*	γ-subunit of the Na^+-K^+-ATPase
Hypomagnesemia, episodic ataxia/myokymia syndrome	160120	AD	*KCNA1*	Kv1.1, apical potassium channel
Isolated recessive hypomagnesemia (IRH)(HOMG4)	611718	AR	*EGF*	Pro-EGF (epidermal growth factor)
Hypomagnesemia, seizures, and mental retardation 1 (HOMGSMR1)	616418	AR, AD	*CNNM2*	CNNM2, Cyclin M2
HNF1α nephropathy	137920	AD	*HNF1B*	HNF1beta, transcription factor
Transient neonatal hyperphenylalaninemia	264070	AR	*PCBD1*	PCBD1, tetrahydrobioterin metabolism
Hypomagnesemia/metabolic syndrome	500005	mito	*MTTI*	Mitochondrial tRNA (Isoleucin)

Table 37.9 Clinical and biochemical characteristics of inherited Ca^{2+} and Mg^{2+} disorders

Disorder	Age at onset	Serum Mg^{2+}	Serum Ca^{2+}	Serum K^{+}	Blood pH	Urine Mg^{2+}	Urine Ca^{2+}	Nephro-calcinosis	Renal stones
Autosomal dominant hypoparathyroidism (ADH)	Infancy	↓	↓	N	N or ↑	↑	↑–↑↑	Yes[a]	Yes[a]
Familial hypocalciuric hypercalcemia (FHH)	Often asymptomatic	N to ↑	↑	N	N	↓	↓	No	?
Neonatal severe hyperparathyroidism (NSHPT)	Infancy	N to ↑	↑↑↑	N	N	↓	↓	No	?
Infantile hypercalcemia	Infancy	N	↑↑↑	N	N	?	↑↑	Yes	Yes
Familial hypomagnesemia with hypercalciuria/ nephrocalcinosis (FHHNC)	Childhood	↓	N	N	N or ↓	↑↑	↑↑	Yes	Yes
HELIX syndrome, CLDN10 tubulopathy	Childhood to adulthood	↑	N or ↑	↓	↑	↓	↓	No	No
Gitelman syndrome (GS)	Adolescence	↓	N	↓	↑	↑	↓	No	No
EAST/SeSAME syndrome	Infancy	↓	N	↓	↑	↑	↓	No	No
Hypomagnesemia with secondary hypocalcemia (HSH)	Infancy	↓↓↓	↓	N	N	↑	N	No	No
Hypomagnesemia, seizures, and mental retardation 2	Infancy	↓↓↓	N	N or ↓	N	↑↑	N	No	No
Isolated dominant hypomagnesemia (IDH)	Childhood	↓	N	N	N	↑	↓	No	No
Isolated recessive hypomagnesemia (IRH)	Childhood	↓	N	N	N	↑	N	No	No
Hypomagnesemia, seizures, and mental retardation 1	Infancy to adolescence	↓↓	N	N	N	↑	N	No	No
HNF1B nephropathy	Childhood	↓	N	N	N	↑	↓	No	No
Transient neonatal hyperphenylalaninemia	Adulthood	↓	N	N	N	↑	↓	No	No
Hypomagnesemia/metabolic syndrome	Adulthood	↓	N	↓	N	↑	↓	No	No

[a] Frequent complication under therapy with Ca^{2+} and vitamin D

Familial Hypomagnesemia with Hypercalciuria and Nephrocalcinosis

Familial hypomagnesemia with hypercalciuria and nephrocalcinosis (**FHHNC**) is an autosomal recessive disorder caused by mutations in two members of the claudin gene family which encode the tight junction proteins claudin-16 and claudin-19 [105, 106]. More than 150 patients have been reported to date, allowing a comprehensive characterization of the clinical spectrum [107–111]. Due to excessive renal Mg^{2+} and Ca^{2+} wasting, affected individuals almost uniformly develop the characteristic triad of hypomagnesemia, hypercalciuria, and nephrocalcinosis that gave the disease its name. Additional biochemical abnormalities include elevated PTH levels before the onset of chronic renal failure, hypocitraturia, and hyperuricemia. The majority of patients clinically present during early childhood with recurrent urinary tract infections, polyuria/polydipsia, nephrolithiasis, and/or failure to thrive. Clinical symptoms of severe hypomagnesemia such as seizures and muscular tetany are less common. The clinical course of FHHNC patients is complicated by the development of chronic renal failure (CRF) early in life. A considerable number of patients exhibit a marked decline in GFR (<60 mL/min per 1.73 m^2) already at the time of diagnosis and about one third of patients develops ESRD during adolescence. Hypomagnesemia may completely disappear with the decline of GFR due to a reduction in filtered Mg^{2+} that limits urinary Mg^{2+} losses. Whereas the renal phenotype is almost identical in carriers of *CLDN16* and *CLDN19* mutations, ocular involvement including severe myopia, nystagmus, or macular coloboma are observed only in patients with *CLDN19* mutations [106, 107, 109, 110].

Claudins are crucial components of tight junctions and the individual composition of tight junctions strands with different claudin members confers the characteristic properties of different epithelia regarding paracellular permeability and/or transepithelial resistance. Within the tight junction barrier, claudins positioned on neigbouring cells are thought to form charge-selective pores.

Claudin-16 and claudin-19 colocalize at tight junctions of the TAL [106]. Tight-junction strands in this part of the renal tubule also express other members of the claudin family including claudin-3, claudin-10 and claudin-18. These other claudins maintain the barrier function of the tight junction complex also in the absence of claudin-16 and -19, however, claudin-16 and -19 depleted tight junctions display a loss in cation permselectivity [112]. Mice deficient in Claudin-16 or claudin-19 also exhibit increased renal Na^+ and K^+ losses in addition to impaired renal Mg^{2+} and Ca^{2+} handling [113].

The majority of *CLDN16* and *CLDN19* mutations reported in FHHNC are missense mutations affecting the transmembrane domains and the extracellular loops with a particular clustering in the first extracellular loop. Within this domain, patients originating from Germany or Eastern European countries exhibit a common mutation (p.L151F) due to a founder effect [108].

Progressive renal failure in FHHNC is thought to be a consequence of massive urinary Ca^{2+} wasting and nephrocalcinosis. Individuals with bi-allelic loss-of-function mutations in *CLDN16* exhibit a younger age at manifestation and a more rapid renal function loss compared to patients with at least one allele with residual claudin-16 function [114]. Of note, first degree relatives of FHHNC patients appear to have a high incidence of hypercalciuria, nephrolithiasis and/or nephrocalcinosis [107, 108]. Moreover, a tendency towards mild hypomagnesemia has been observed in these heterozygous family members [115]. Thus, one might speculate that *CLDN16* mutations could be involved in idiopathic hypercalciuric stone formation. Finally, a homozygous *CLDN16* mutation (p.T303R) affecting the C-terminal PDZ domain has been identified in two families with isolated hypercalciuria and nephrocalcinosis without abnormalities in renal Mg^{2+} handling [116]. Notably, hypercalciuria disappeared during follow-up and urinary Ca^{2+} levels reached normal values beyond puberty.

In addition to oral Mg^{2+} supplementation, **therapy** aims at a reduction of Ca^{2+} excretion in order to prevent the progression of nephrocalcinosis and stone formation because the degree of renal calcifications has been correlated with progression of chronic renal failure [107]. In a short term study, thiazides effectively reduced urinary Ca^{2+} excretion in FHHNC patients [117]. However, these therapeutic strategies have not been shown yet to significantly influence the progression of renal failure. Supportive therapy is important for the protection of kidney function and should include provision of sufficient fluids and effective treatment of stone formation and bacterial colonization. As expected, renal transplantation is performed without evidence of recurrence of the disease because the primary defect resides in the kidney.

Gitelman Syndrome

Gitelman syndrome (GS) is the most frequent inherited salt wasting disorder with an estimated prevalence of approximately 1:40.000 [118]. It is caused by mutations in the *SLC12A3* gene coding for the thiazide-sensitive NaCl- cotransporter, NCC [119]. The NCC is exclusively expressed at the apical membrane of the DCT where it reabsorbs approximately 5–10% of the filtered NaCl (Fig. 37.5). The cardinal biochemical features of GS are persistent hypokalemia and metabolic alkalosis together with hypomagnesemia and hypocalciuria [120, 121]. Hundreds of GS patients have been reported to date, allowing for a thorough clinical description of the phenotype as well as for an extensive analysis of the mutational spectrum in the *SLC12A3* gene [122–126].

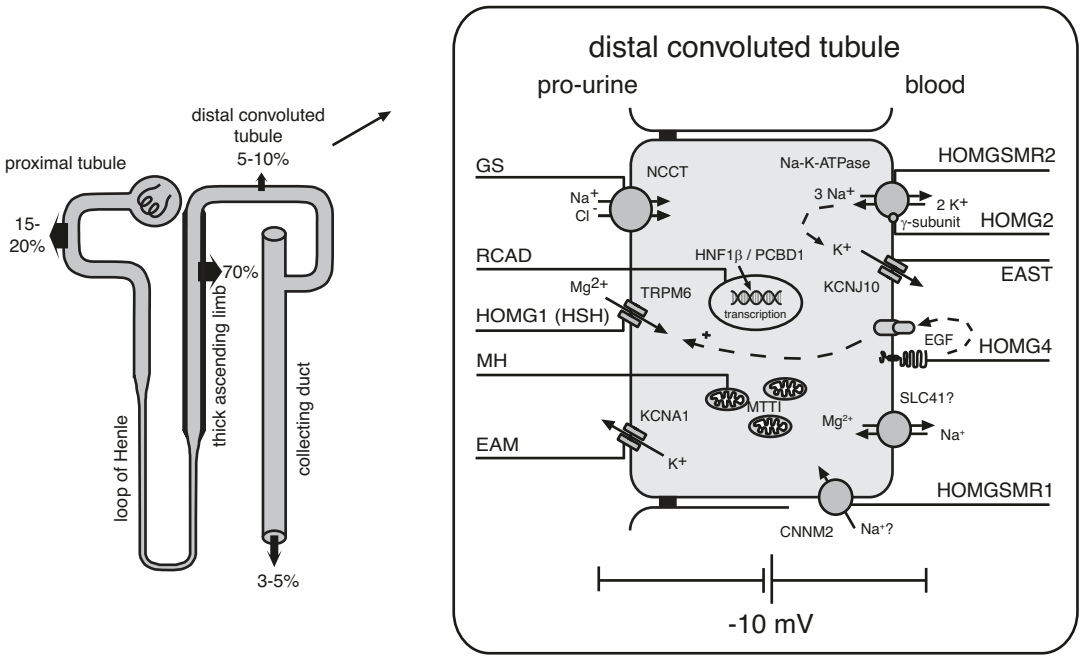

Fig. 37.5 Mg^{2+} reabsorption in the distal convoluted tubule. Mg^{2+} is actively reabsorbed via the transcellular pathway involving an apical entry step through a Mg^{2+}-permeable ion channel (TRPM6) and a basolateral exit, presumably mediated by a Na^+-coupled exchange mechanism. Mg^{2+} reabsorption in the DCT is dependent on transcellular salt reabsorption via NCC, basolateral Na^+/K^+-ATPase activity, and K^+ recycling. It is regulated by basolateral EGF and the putative Mg^{2+} sensor CNNM2. Furthermore, Mg^{2+} transport rates are influenced by cellular energy metabolism (mitochondrial function) and expression levels of transport proteins (regulated by HNF1B). The respective hereditary defects are indicated

A consensus and guidance document concerning the diagnostic criteria, clinical workup, genetic testing, treatment, and long-term follow-up of patients with GS was recently published to which the reader should refer if diagnosing or treating a GS patient [127].

The initial presentation of GS most frequently occurs at school age or later with the characteristic symptoms being muscular weakness, cramps, and fatigue [128]. However, severe manifestations with early onset of disease, growth retardation, chondrocalcinosis, tetany, rhabdomyolysis, seizures, or ventricular arrhythmia have been reported [122, 129]. On the other hand, patients are frequently diagnosed accidentally while searching medical consultation for unrelated reasons, because of growth retardation, constipation or enuresis. A thorough past medical history of such patients commonly reveals long-standing salt craving [122]. Typically, urinary concentrating ability is not affected. Laboratory examination shows the characteristic constellation of metabolic alkalosis, low-normal Cl^- levels, hypokalemia, and hypomagnesemia; urine analysis reveals hypocalciuria [130]. Family studies demonstrated electrolyte imbalances present since infancy despite the absence of obvious clinical signs and symptoms in the affected infant [131].

Of note, the combination of hypokalemia and hypomagnesemia exerts an exceptionally unfavorable effect on cardiac excitability, which puts these patients at high risk for cardiac arrhythmia [132, 133]. Therefore, next to appropriate potassium and magnesium supplementation, the recognition of other possible triggering mechanisms as well as the avoidance of drugs that aggravate K^+ and Mg^{2+} depletion or prolong the QT-interval appears to be critical. Respective tables of drugs that should be avoided are available online (www.crediblemeds.org).

The pathognomonic feature of Gitelman syndrome is the dissociation of renal Ca^{2+} and Mg^{2+} handling, with low urinary Ca^{2+} and high urinary Mg^{2+} levels. Subsequent hypomagnesemia causes neuromuscular irritability and tetany. Decreased renal Ca^{2+} elimination together with Mg^{2+} deficiency favors deposition of mineral Ca^{2+} as demonstrated by increased bone density as well as chondrocalcinosis [134].

Although the combination of hypomagnesemia and hypocalciuria is typical for NCC deficiency, this finding is neither specific nor universal. Clinical observations in NCC deficient patients disclosed intra- and inter-individual variations in urinary Ca^{2+} concentrations which can be attributed to gender, age-related conditions of bone metabolism, intake of Mg^{2+} supplements, changes in diuresis and urinary osmolality, respectively. Likewise, hypomagnesemia might not be present from the beginning. Renal Mg^{2+} loss can be balanced temporarily by Mg^{2+} release from bone and muscle stores as well as by an increase of intestinal Mg^{2+} reabsorption. The mechanisms compromising distal Mg^{2+} reabsorption and favoring reabsorption of Ca^{2+} are not yet completely understood.

In contrast to TAL defects, disturbed salt reabsorption along the DCT does not affect the tubulo-glomerular feedback and thus is not associated with increased renal prostaglandin synthesis [135]. Accordingly, NSAIDs are of little benefit in Gitelman syndrome. Substitution of KCl and Mg^{2+} is therefore of prime importance in the treatment of this disorder. As pointed out above, avoidance of factors which in addition to hypokalemia and hypomagnesemia might affect cardiac excitability (in particular QT-time prolonging drugs) is mandatory to prevent life-threatening cardiac arrhythmia.

EAST/SeSAME Syndrome

A clinical syndrome with autosomal recessive inheritance combining epilepsy, ataxia, sensorineural deafness and renal salt wasting with/without mental retardation was first described in 2009 under the acronyms EAST or SeSAME syndrome [136, 137]. Patients usually present early in infancy with generalized tonic–clonic seizures, speech and motor delay, as well as ataxia leading to an inability to walk, intention tremor, and dysdiadochokinesis. In addition, they exhibit a variable degree of hearing impairment [138]. The

neurological features and hearing impairment are usually non-progressive [139]. Renal salt wasting is often recognized later during the course of the disease. Closely resembling GS, the renal phenotype includes the combination of hypokalemic alkalosis, hypomagnesemia and hypocalciuria.

EAST/SeSAME syndrome is caused by loss-of-function mutations in the *KCNJ10* gene encoding the inwardly-rectifying K^+-channel KCNJ10 (Kir4.1) [136, 137]. The expression pattern of KCNJ10 fits to the disease phenotype with highest expression in brain, the stria vascularis of the inner ear, and in the distal nephron, especially in the DCT (Fig. 37.5). Here, KCNJ10 is localized at the basolateral membrane of DCT cells and supposed to function in collaboration with Na^+-K^+-ATPase as it might allow for a recycling of K^+ ions entering the tubular cells in countermove for the extruded Na^+ [137]. Loss of KCNJ10 function most likely leads to a depolarization of the basolateral membrane and thereby to a reduction of the driving force for basolateral anion channels as well as sodium-coupled exchangers. By this mechanism, KCNJ10 defects might also affect the putative Na^+/Mg^{2+} exchanger and possibly explain the Mg^{2+} wasting observed in EAST/SeSAME syndrome.

As in patients with Gitelman syndrome, medical treatment of EAST/SeSAME patients regarding the renal phenotype mainly comprises potassium and magnesium supplementation. In individual adult patients, persistent hypokalemia despite supplementation has been treated with spironolactone in order to prevent frequent hospitalisations [140]. Seizures typically respond well to initial anti-epileptic treatment but may recur at later age. Finally, ataxia remains a major debilitating disease feature.

Hypomagnesemia with Secondary Hypocalcemia

Hypomagnesemia with secondary hypocalcemia (HSH) is a rare recessive disorder that manifests in early infancy with generalized seizures or other symptoms of increased neuromuscular excitability [141]. Biochemical abnormalities include extremely low serum Mg^{2+} (about 0.2 mmol/L) and low serum Ca^{2+} levels. Hypocalcemia is thought to result from an impaired synthesis and/or release of PTH in the presence of severe hypomagnesemia [92]. The failure of the parathyroid gland to synthesize and secrete parathyroid hormone has been attributed to a defect in g-protein signalling within parathyroid cells required for CaSR-mediated stimulation of PTH release [93].

Transport studies in HSH patients pointed to a primary defect in intestinal Mg^{2+} absorption [142, 143]. However, in some patients an additional renal leak for Mg^{2+} was suspected [144]. In 2002, recessive loss of function mutations in TRPM6 encoding a member of the transient receptor potential (TRP) family of ion channels were discovered as the underlying genetic defect [145, 146]. To date, mutations in *TRPM6* have been identified in more than 50 families affected by HSH [145–149]. The mutational spectrum mainly comprises truncating mutations. In addition, a number of missense mutations have been described for which functional analyses also indicated a complete loss-of-function [145, 147, 149, 150].

TRPM6 is expressed along the intestine (duodenum, jejunum, ileum, colon) as well as in the DCT of the kidney (Fig. 37.5) [145].

In the intestine, intraluminal Mg^{2+} concentrations and rates of Mg^{2+} absorption show a curvilinear relationship presumably reflecting two transport processes working in-parallel: an active and saturable transcellular transport essential at low intraluminal Mg^{2+} concentrations and a passive paracellular Mg^{2+} absorption gaining importance at higher intraluminal Mg^{2+} concentrations [87]. The observation that in HSH patients the substitution of high oral doses of Mg^{2+} achieves at least subnormal serum Mg^{2+} levels supports the hypothesis of two independent intestinal transport systems for Mg^{2+}. By participating in the formation of apical magnesium-permeable ion channels, TRPM6 probably represents a molecular component of active transcellular Mg^{2+} transport. An increased intraluminal Mg^{2+} concentration (by increased oral intake) enables to compensate for the defect in active

transcellular transport by increasing absorption via the passive paracellular pathway (Fig. 37.1).

In the kidney, TRPM6 is expressed predominantly in the DCT arguing for an important role of renal Mg^{2+} wasting for the pathogenesis of HSH [151]. This is also supported by intravenous Mg^{2+} loading tests in HSH patients, which disclosed a considerable renal Mg^{2+} leak albeit still being hypomagnesemic [146].

Hypocalcemia in patients with HSH is resistant to treatment with Ca^{2+} or vitamin D. Relief of clinical symptoms, normocalcemia, and normalization of PTH levels are only achieved by administration of high doses of Mg^{2+} [152]. Oral magnesium supplementation is preferentially performed with organic magnesium compounds (i.e. Mg-citrate, -aspartate, or -gluconate), doses vary between 0.5 and 4 mmol/kg/day. Serum magnesium levels usually remain in the subnormal range despite adequate treatment, but usually enable an undisturbed physical and mental development. However, delayed diagnosis or noncompliance with treatment can be fatal or result in permanent neurological damage.

Hypomagnesemia, Refractory Seizures and Mental Retardation Type 2

Heterozygous de-novo mutations in the *ATP1A1* gene encoding the α1-subunit of Na^+K^+-ATPase have recently been described in three children with severe hypomagnesemia due to renal magnesium wasting [153]. The affected children presented in infancy with cerebral seizures that were refractory to antiepileptic medication and also did not respond to high dose magnesium supplementation. All three children developed a significant degree of mental retardation and global developmental delay. Serum magnesium concentrations remained low despite adequate oral supplementation.

The α1-subunit (ATP1A1) is one of four different α-subunits of Na^+K^+-ATPase in human, but it represents the exclusive α-subunit in kidney. Along the kidney tubule, the DCT represents the segment with the highest energy consumption and density of Na^+K^+-ATPase expression [154]. Here, basolaterally expressed Na^+K^+-ATPase generates favorable electrochemical gradients for transcellular salt and magnesium reabsorption (Fig. 37.5). The critical role of Na^+K^+-ATPase for magnesium reabsorption had previously been demonstrated by discovery of mutations in its γ-subunit (*FXYD2*) in patients with isolated dominant hypomagnesemia (see below). In the central nervous system, the α1-subunit is ubiquitiously expressed and thought to maintain neuronal housekeeping functions by generating the resting membrane potential and clearing extracellular K^+ during neuronal activity [155].

The ATP1A1 mutations discovered in hypomagnesemic children were shown to not only lead to a loss of ATPase function, but also to result in abnormal ion permeabilities and leak currents [153]. Therefore, the severe hypomagnesemia phenotype might not be the result of a simple ATP1A1 haploinsufficiency. Whereas a homozygous loss of ATP1A1 function is not compatible with life, heterozygous germline ATP1A1 mutations have also been described in patients with Charcot-Marie-Tooth disease type 2 and hereditary spastic paraplegia [156, 157]. Originally, somatic ATP1A1 mutations had been identified in patients with primary hyperaldosteronism due to aldosterone producing adenomas [158].

Isolated Dominant Hypomagnesemia

A first variant of isolated dominant hypomagnesemia **(IDH)** was described in 1999 in two related families by Meij et al. who discovered a mutation in the *FXYD2* gene encoding the γ-subunit of renal Na^+K^+-ATPase [159]. The γ-subunit of Na^+K^+-ATPase is a member of the FXYD family of small single transmembrane proteins that constitute regulatory, tissue-specific subunits of the Na^+K^+-ATPase. Along the kidney tubule, F*XYD2* is preferentially expressed in the DCT, where it is thought to increase the affinity of Na^+K^+-ATPase for ATP while decreasing its Na^+ affinity thereby providing a mechanism for balancing energy utilization and maintaining appropriate salt gradi-

ents [160, 161] The reported G41R mutation in the γ-subunit leads to retention of the γ-subunit within the cell.

Urinary Mg^{2+} wasting together with the expression pattern of the *FXYD2* gene point to a defect in transcellular Mg^{2+} reabsorption in the DCT in IDH patients. Affected children present with cerebral seizures and hypomagnesemia around 0.5 mmol/L. [162] Hypomagnesemia is due to renal losses while intestinal Mg^{2+} absorption is preserved or even stimulated [162]. Low Ca^{2+} excretion, hypokalemia and metabolic alkalosis reminiscent of Gitelman syndrome have also been reported in some families [163]. Selective magnesium supplementation partially corrects hypomagnesemia and normalizes serum potassium levels [163].

Hypomagnesemia, Episodic Ataxia/ Myokymia Syndrome

Another form of dominant hypomagnesemia was established by the identification of a heterozygous missense mutation in *KCNA1,* which encodes the voltage-gated potassium channel KCNA1 (Kv1.1) [164]. The clinical phenotype associated with the reported p.N255D mutation in Kv1.1 includes muscle cramps, tetany, tremor, and muscle weakness starting during infancy.

Originally, dominant *KCNA1* mutations had been identified in patients with episodic ataxia with myokymia (OMIM #160120), a neurologic disorder characterized by an intermittent appearance of incoordination and imbalance as well as myokymia, an involuntary, spontaneous, and localized trembling of muscles [165]. In addition to muscle cramps and tetany attributed to Mg^{2+} deficiency, these symptoms were also present in hypomagnesemic patients with the p.N255D mutant. Urine analyses in these patients revealed a renal Mg^{2+} leak without alterations in renal Ca^{2+} handling.

Co-expression studies of the mutant and wild-type Kv1.1 channel subunits indicate a dominant-negative effect of the mutant [166]. Kv1.1 is expressed at the apical membrane of the DCT. As Kit is co-localized there with TRPM6, Kv1.1 may allow for hyperpolarization of the apical membrane of DCT cells as a prerequisite for TRPM6-mediated Mg^{2+} entry (Fig. 37.4b), thereby linking Mg^{2+} reabsorption to K+ secretion in the DCT [164].

Isolated Recessive Hypomagnesemia

In the 1980s Geven et al. first reported a form of isolated hypomagnesemia in a consanguineous family indicating autosomal recessive inheritance [167]. Two affected sisters presented in infancy with generalized seizures. Unfortunately, a late diagnosis resulted in neurodevelopmental deficits in both girls. A thorough clinical and laboratory workup at 4 and 8 years of age, respectively, revealed serum Mg^{2+} levels around 0.5–0.6 mmol/L with no other associated serum electrolyte abnormality. Of note, renal Ca^{2+} excretion rates were in the normal range. A ^{28}Mg-retention test in one patient indicated a primary defect in renal Mg^{2+} conservation [167].

Groenestege et al. identified a homozygous missense mutation in the *EGF* gene leading to a nonconservative amino acid exchange in the encoded pro-EGF protein (pro-epidermal growth factor) in the two sisters [168]. In the kidney, co-expression with key proteins of transcellular Mg^{2+} reabsorption including TRPM6 in the DCT was demonstrated. Pro-EGF is a transmembrane protein that is inserted in both the luminal and basolateral membrane of polarized epithelia. After the soluble EGF peptide is cleaved, it binds to and activates specialized EGF receptors (EGFRs). In case of the DCT, these EGFRs are exclusively expressed at the basolateral membrane (Fig. 37.5). Their activation leads to increased trafficking of TRPM6 to the luminal membrane and increased Mg^{2+} reabsorption [169]. The mutation described in IRH (p.P1070L) disrupts the basolateral sorting motif in pro-EGF leading to a mistargeting of pro-EGF [168]. Therefore, the activation of basolateral EGFRs is compromised which ultimately causes impaired active transcellular Mg^{2+} reabsorption. Despite

acting in a paracrine fashion in the DCT, the authors speculate on a role of EGF as a first selectively acting magnesiotropic hormone [168].

Hypomagnesemia, Refractory Seizures, and Mental Retardation 1

Another form of hereditary Mg^{2+} wasting has been linked to mutations in *CNNM2* encoding the transmembrane protein CNNM2 or Cyclin M2 [170]. CNNM2 was identified by differential expression in murine DCT cells exposed to varying Mg^{2+} concentrations and by transcriptome studies in mice lacking claudin-16 [170, 171]. In addition, common variants in CNNM2 were found to be associated with serum Mg^{2+} levels in a genome-wide association study [172]. The precise physiological function of CNNM2 is still elusive. CNNM2 is ubiquitously expressed in mammalian tissues, most prominently in kidney, brain and lung [173, 174]. In the kidney, CNNM2 is expressed at the basolateral membrane of TAL and DCT. Whereas CNNM2 had initially been proposed as a Mg^{2+} transporter, more recent data point to a role in Mg^{2+}-sensing [171, 174].

CNNM2-associated disease shows a wide phenotypic as well as genetic spectrum. Most patients identified thus far carry heterozygous de novo mutations in *CNNM2*. These patients mainly present in infancy with generalized convulsions and display a mild to moderate degree of neurodevelopmental delay with a disturbed speech development and dysarthria as prominent features [175]. Cerebral seizures tend to subside during follow-up in the majority of patients. A considerable number of patients of both genders develops severe obesity as the most prominent extraneurological symptom [175]. Serum magnesium levels are typically in the range of 0.5–0.6 mmol/L and remain in the subnormal range upon oral magnesium supplementation.

A milder clinical phenotype without significant intellectual disability was originally described in members of two families with dominant inheritance [170]. By contrast, patients with bi-allelic *CNNM2* mutations exhibit profound hypomagnesemia and a severe neurological phenotype with refractory epilepsy, microcephaly, global developmental delay, and severe intellectual disability [176, 177]. Brain MR imaging in these patients demonstrate widened outer cerebrospinal liquor spaces indicative of cerebral atrophy as well as myelinization defects.

HNF1B Nephropathy

Hepatocyte nuclear factor 1β (HNF1B) is a transcription factor critical for the development of the kidney and the pancreas. *HNF1B* mutations are present in heterozygous state, either inherited or de novo, and comprise point mutations as well as whole-gene deletions [178]. *HNF1B* mutations were first implicated in a subtype of maturity-onset diabetes of the young (MODY5) [179]. Later, an association with anomalous kidney development was reported. The renal phenotype is highly variable comprising enlarged hyperechogenic kidneys, multicystic kidney disease, renal agenesis, renal hypoplasia, cystic dysplasia, as well as hyperuricemic nephropathy (see Chap. 8). Since neither a renal cystic phenotype nor diabetes are constant clinical findings, the neutral term HNF1B nephropathy has been introduced [180]. Interestingly, 25–50% of patients present with hypomagnesemia due to impaired renal Mg^{2+} conservation [181, 182]. As *HNF1B* regulates the expression of *FXYD2* (see above), defective basolateral Na^+K^+-ATPase function potentially explains renal Mg^{2+} wasting in patients with HNF1B mutations [182]. In agreement with this assumption, renal Mg^{2+} wasting in HNF1B nephropathy can be part of a Gitelman syndrome-like picture with hypokalemia, metabolic alkalosis and hypocalciuria, compatible with dysfunction of the DCT [182, 183].

The observed variable prevalence of hypomagnesemia in children with HNF1B nephropathy suggests that this phenotypic feature of HNF1B nephropathy may represent an age-dependent phenomenon and develop over time [184]. Indeed, a study from Poland reports an increasing fraction of hypomagnesemic HNF1B patients during follow-up [185].

Transient Neonatal Hyperphenylalaninemia

Renal Mg^{2+} wasting has been demonstrated in transient neonatal hyperphenylalaninemia due to recessive mutations in the *PCBD1* gene [186]. Affected patients developed hypomagnesemia and a MODY type diabetes in adulthood. Functional studies revealed that PCBD1 is an essential dimerization cofactor of HNF1B. Defective dimerization of PCBD1 with HNF1B abrogates the HNF1B-mediated stimulation of *FXYD2* promoter activity in the DCT [186].

Mitochondrial Hypomagnesemia

A mutation in the mitochondrial tRNA gene for Isoleucine, $tRNA^{Ile}$ or MTTI, has been discovered in a single large Caucasian kindred [187]. An extensive clinical evaluation of this family was prompted after the discovery of hypomagnesemia in the index patient. Pedigree analysis was compatible with mitochondrial inheritance as the phenotype was exclusively transmitted by affected females. The phenotype included hypomagnesemia, hypercholesterolemia, and hypertension. Of the adults on the maternal lineage, the majority of offspring exhibited at least one of the mentioned symptoms, approximately half of the individuals showed a combination of two or more symptoms, and around 1/6 had all three features [187]. Serum Mg^{2+} levels of family members on the maternal lineage greatly varied ranging from 0.3 to 1.0 mmol/L with approximately 50% of individuals being hypomagnesemic. Hypomagnesemic individuals showed higher fractional excretions (median around 7.5%) than their normomagnesemic relatives on the maternal lineage (median around 3%) clearly pointing to renal Mg^{2+} wasting as causative for hypomagnesemia. Interestingly, hypomagnesemia was accompanied by decreased urinary Ca^{2+} levels, a finding pointing to the DCT as the affected tubular segment. As ATP consumption along the tubule is highest in the DCT, energy metabolism of DCT cells may be impaired as a consequence of the mitochondrial defect which in turn could lead to disturbed transcellular Mg^{2+} reabsorption [187].

Acquired Hypomagnesemia

Cisplatin and Carboplatin

The cytostatic agent cisplatin and the newer antineoplastic drug, carboplatin, are widely used in various protocols for the therapy of solid tumors. Among different side effects, nephrotoxicity receives most attention as the major dose-limiting factor. Carboplatin has been reported to have less severe side effects than cisplatin [188–190].

Hypomagnesemia due to renal Mg^{2+} wasting is regularly observed in patients treated with cisplatin [189, 191]. The incidence of Mg^{2+} deficiency is greater than 30% and even increases to over 70% with extended cisplatin usage and greater cumulative doses. Notably, cisplatin-induced Mg^{2+} wasting is relatively selective [189]. Hypocalcemia and hypokalemia may be observed but only with prolonged and severe Mg^{2+} deficiency [192]. The influence of Mg^{2+} deficiency on PTH secretion and end-organ resistance is a possible explanation for enhanced urinary Ca^{2+} excretion and diminished mobilization resulting in low serum Ca^{2+} concentrations [193]. The effects on K^+ balance are more difficult to explain. The hypokalemia observed with Mg^{2+} deficiency is refractory to K^+ supplementation. The effects of cisplatin may persist for months or years, long after the inorganic platinum has disappeared from the renal tissue [194, 195].

In the rat model cisplatin treatment resulted in EGF and TRPM6 downregulation in the DCT [196]. Nephrotoxicity was effectively prevented by Mg^{2+} supplementation either during or even before cisplatin administration, demonstrating the close relationship between cisplatin-induced Mg^{2+} deficiency and nephrotoxicity [197].

Aminoglycosides

Aminoglycosides, such as gentamicin, induce renal impairment in up to 35% patients dependent on the dose and duration of administration. In addition, aminoglycosides cause hypermagnesiuria and hypomagnesemia [198]. As many as 25% of patients receiving gentamicin develop hypomagnesemia [198]. The hypermagnesiuric response occurs soon after the onset of therapy; it is dose-dependent and readily reversible upon withdrawal. As with adults, neonates also display an immediate increase of Ca^{2+} and Mg^{2+} excretion after gentamicin infusion [199, 200]. Mg^{2+} wasting is associated with hypercalciuria that may lead to diminished plasma Ca^{2+} concentrations. This would suggest that aminoglycosides affect renal Mg^{2+} and Ca^{2+} transport in the distal tubule where both are reabsorbed. The cellular mechanisms are not completely understood but hypermagnesiuria and hypercalciuria are observed in the absence of histopathological changes. Because gentamicin is a polyvalent cation it has been postulated that it may interfere with the function of the Ca^{2+}-sensing receptor (CaSR) [89, 201]. CaSR activation by polyvalent cations would inhibit passive absorption of Mg^{2+} and Ca^{2+} in the loop of Henle and active hormone-mediated transport in the DCT, leading to renal Mg^{2+} and Ca^{2+} wasting. The observation that gentamicin treatment results in an up-regulation of Ca^{2+} and Mg^{2+} transport proteins in the DCT, namely TRPV5, TRPM6 and calbindin-D28k, suggests that this adaptation represents an attempt to counter upstream losses, i.e. in the TAL [202]. This would be in accordance with the hypothesis that gentamicin affects Na^+ reabsorption in TAL leading to a reduced lumen-positive voltage and a subsequent reduction in Ca^{2+} and Mg^{2+} reabsorption.

Calcineurin Inhibitors

The calcineurin inhibitors cyclosporine and tacrolimus are widely prescribed as immunosuppressants to organ transplant recipients and in numerous immunologic disorders. Under this therapy, patients are at high risk of developing renal injury and hypertension. Tubular dysfunction with subsequent disturbance of mineral metabolism is another common side effect. Both drugs commonly lead to renal Mg^{2+} wasting and hypomagnesemia [203]. Unlike the other agents mentioned above, these drugs also cause modest hypercalcemia with hypercalciuria and hypokalemia [203]. The hypomagnesemic effect is probably attenuated by the fall in GFR and reduction in filtered Mg^{2+} but this defect appears to be specific for Mg^{2+}. Calcineurin inhibitor therapy is associated with an inappropriately high fractional excretion rate of Mg^{2+}, suggesting impaired passive reabsorption in the TAL or active Mg^{2+} transport in the DCT [204]. Cyclosporine reduces claudin-16 expression in the TAL [205]. Moreover, tacrolimus downregulates specific Ca^{2+} and Mg^{2+} transport proteins in the DCT. In an animal study, tacrolimus suppressed the expression of TRPV5, calbindin-D28k and TRPM6 [206]. In accordance with these observations, urinary EGF levels were found decreased in adult and pediatric hypomagnesemic renal allograft recipients treated with cyclosporin pointing to a defect of transcellular magnesium reabsorption in the DCT [207, 208].

EGF Receptor Antibodies

The EGF hormone axis has been implicated in renal Mg^{2+} handling by the identification of a homozygous mutation in the *EGF* gene in a family with isolated recessive hypomagnesemia (see below) [168]. The way for this discovery was paved by the observation that anticancer treatments with monoclonal antibodies against the EGF receptor (EGFR) resulted in renal Mg^{2+} wasting and hypomagnesemia [209]. Of note, patients treated with EGFR targeting antibodies (cetuximab, panitumumab) for colorectal cancer usually receive a combination therapy with platinum compounds potentially aggravating the effects on serum Mg^{2+} levels. A significant number of patients receiving such a chemotherapeutic regimen shows decreasing serum Mg^{2+} concentrations over time [209–211]. 24-h urine

collections as well as Mg^{2+} loading tests in single patients demonstrated defective renal Mg^{2+} conservation [209]. Together with the genetic findings in patients with isolated recessive hypomagnesemia due to a pro-EGF mutation, these observations imply a selective effect of EGF-receptor targeting on transcellular Mg^{2+} transport in the DCT. There, TRPM6 mediated Mg^{2+} uptake into DCT cells is stimulated by basolaterally secreted EGF via its receptor (EGFR) [168]. It is still controversial If the development of hypomagnesemia correlates with the efficacy of anti-EGF receptor treatment [212, 213].

Proton-Pump Inhibitors (PPIs)

Over the past two decades, PPIs for the reduction of gastric acidity have emerged to one of the most widely prescribed classes of drugs worldwide [214]. Symptomatic hypomagnesemia has been observed in a small but significant number of patients receiving PPIs [215]. A systematic review of the published cases showed severe hypomagnesemia (below 0.4 mmol/L) with concomitant hypocalcemia, a laboratory constellation reminiscent of Hypomagnesemia with Secondary Hypocalcemia (HSH) due to TRPM6 defects (see below) [214]. The initial report on hypomagnesemia following PPI treatment had already described suppressed PTH levels during episodes of severe hypomagnesemia as a probable cause of hypocalcemia [215]. Although a number of patients additionally receive diuretics, this finding does not explain the profound degree of Mg^{2+} deficiency observed in patients receiving PPIs.

Unfortunately, the molecular link between proton-pump inhibition and hypomagnesemia still remains unclear. Data regarding renal Mg^{2+} losses in hypomagnesemic patients receiving PPIs are inconclusive. Fractional Mg^{2+} excretion was reported to be low in face of profound hypomagnesemia, possibly pointing to an intact tubular Mg^{2+} reabsorption [215, 216]. However, as observed in HSH patients, a renal Mg^{2+} leak might only become apparent if serum Mg^{2+} levels reach a certain threshold. An alternative explanation could involve disturbed intestinal reabsorption of Mg^{2+}. Possible molecular mechanisms include an inhibition of TRPM6 leading to a combined intestinal and renal defect, but also a disturbance of ATPases or ATPase-subunits other than gastric H^+-K^+-ATPase involved in epithelial Mg^{2+} transport.

It is recommended to monitor serum Mg^{2+} levels patients receiving PPIs, particularly on those with concomitant cardiac disease at risk for arrhythmia.

Miscellaneous Agents

A number of antibiotics, tuberculostatics, and antiviral drugs may result in renal Mg^{2+} wasting [198]. The cellular basis and molecular mechanisms by which these agents lead to abnormal Mg^{2+} reabsorption are largely unknown. Many are associated with general cytotoxicity. Amphotericin B may lead to an acquired distal tubular acidosis which in turn reduces renal Mg^{2+} reabsorption. Pamidronate used in the treatment of acute symptomatic hypercalcemia of various origin has also been reported to cause transient hypomagnesemia. Again, the cellular mechanisms are difficult to predict since this drug is used in patients with hypercalcemia that may aggravate renal Mg^{2+} wasting [217].

Particular attention should be given to these medications in patients with pre-existing hypomagnesemia and especially hereditary disorders of magnesium metabolism.

Therapy of Hypomagnesemia

The substitution of Mg^{2+} in patients with hypomagnesemia is primarily aimed at the relief of clinical symptoms. Unfortunately, in patients with renal Mg^{2+} wasting, normal values for total serum Mg^{2+} are hardly achieved by oral substitution without considerable side effects, mainly resulting from the cathartic effects of Mg^{2+} salts.

The primary route of administration depends on the severity of the clinical findings. Acute intra-

venous infusion is usually reserved for patients with symptomatic hypomagnesemia, i.e. with cerebral convulsions or tetany [218]. Intravenous administration should be preferred to painful intramuscular injections, especially in children.

In neonates and children, the initial treatment usually consists of 25–50 mg Mg^{2+} sulphate (0.1–0.2 mmol Mg^{2+}) per kilogram body weight slowly given intravenously (over 20 min) (up to a maximum of 2 g Mg^{2+} sulphate, which is the adult dosage). This dose can be repeated every 6–8 h or can be followed by a continuous infusion of 100–200 mg Mg^{2+} sulphate (0.4–0.8 mmol Mg^{2+}) per kilogram body weight given over 24 h [178, 219].

In the presence of hypocalcemia, this regimen can be continued for 3–5 days. When Mg^{2+} is administered intravenously, Ca^{2+} gluconate (i.v.) should be available as an antidote. Control of blood pressure, heart rate, and respiration is important as well as a close monitoring of serum Mg^{2+} levels. Before administration, normal renal function has to be ascertained.

In asymptomatic hypomagnesemic or Mg^{2+}-deficient patients, oral replacement represents the preferred route of administration. Exact dosages required to correct Mg^{2+} deficiency are largely unknown. For the pediatric population, 10–20 mg Mg^{2+} (0.4–0.8 mmol) per kg body weight given three to four times a day have been recommended to correct hypomagnesemia [220]. Dosages for maintenance therapy (i.e. in hereditary disorders (see below)) vary between 10 and 100 mg Mg^{2+} (0.4–4 mmol) per kg per day.

Due to the laxative effect of oral magnesium and due to rapid renal excretion especially in case of high peak serum levels, the required daily amount should be given in two to four divided doses preferentially with meals. Solubility, intestinal absorption, and side effects greatly differ depending on the Mg^{2+} salt used for oral treatment. The bioavailability and pharmacokinetics of diverse Mg^{2+} salts have been reviewed [221]. Considering solubility, intestinal absorption and bioavailability, organic Mg^{2+} salts such as Mg^{2+} citrate or aspartate appear most suitable for oral replacement therapy. In addition, the laxative effect of these preparations seems to be less pronounced compared with inorganic Mg^{2+} salts. Moreover, slow-release formulations might be used if available. In our personal experience continuous administration of Mg^{2+}, for example dissolved in mineral water, has proven useful, as peak Mg^{2+} blood levels are avoided. When initiating therapy, dosages should be titrated based on blood levels and intestinal tolerance.

In addition to replacement therapy, the use of certain diuretics has been proposed for the reduction of renal Mg^{2+} excretion. The aldosterone antagonist spironolactone, as well as K^+ -sparing diuretics such as amiloride, exert Mg^{2+}-sparing effects [222, 223]. Studies in patients with hereditary Mg^{2+} wasting disorders showed beneficial effects of these diuretics on renal Mg^{2+} excretions, serum Mg^{2+} levels, and clinical manifestations [224, 225].

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Disorders of Phosphorus Metabolism

38

Dieter Haffner and Siegfried Waldegger

Introduction

The underlying pathophysiological mechanisms of hypophosphatemic disorders have been unraveled during the last decade, although some puzzles remain to be solved. In 1937, Albright first reported on a patient with rickets and severe hypophosphatemia not responding to high doses of vitamin D. The term vitamin D resistant rickets was coined, and later the disease was named X-linked hypophosphatemic rickets respective X-linked hypophosphatemia (XLH) [1].

In recent years several underlying genes have been identified in distinct forms of hypophosphatemic rickets [2, 3]. Rickets is a disease of the growth plate and therefore only growing children are affected [4]. Whereas in the past rickets was thought to be a disease of calcium and vitamin D metabolism, there is growing evidence that rickets is due to insufficient availability of phosphate which is required for normal bone metabolism [5].

In principle, phosphate deficiency may result from inappropriate absorption in the gut or reabsorption in the kidney. The latter situation can be further divided between defects in the tubular reabsorption apparatus and abnormalities of circulating factors which regulate phosphate reabsorption [6]. The major breakthrough in our understanding of hypophosphatemic disorders was the discovery of fibroblast growth factor 23 (FGF23), a member of the FGF family, which mediates the combined renal tubular defects in phosphate reabsorption and altered vitamin D metabolism observed in patients with hypophosphatemic rickets [7].

Currently, specific disease-causing mutations in genes involved in the regulation of phosphate homeostasis can be identified in approx. 85% of familial or sporadic cases of hypophosphatemic rickets [8–11]. This chapter focuses on the etiology, pathogenesis, clinical presentation, differential diagnosis and treatment of hypophosphatemic disorders due to a reduction of renal tubular reabsorption.

Phosphate Homeostasis

Inorganic phosphate (Pi) is a key player in cellular metabolism and skeletal mineralization. It accounts for about 0.6 and 1% of body weight of a neonate and an adult, respectively. Approximately 85% of total body Pi content is

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deposited as hydroxyl-apatite [$Ca_5(PO_4)_3OH$] in the skeleton and the teeth. About 14% distributes within the intracellular compartment. There, Pi participates in as diverse cellular processes as cell membrane function (phospholipids), energy metabolism (ATP), cell signaling (phosphorylation by kinases), and DNA- or RNA-biosynthesis (phosphorylated nucleotides). Only 1% of the total body Pi content is found as a soluble fraction in the extracellular compartment. There, Pi contributes to the acid-base buffering capacity of the plasma and even more important of the urine. Solely this tip of the iceberg is amenable to conventional laboratory investigations from blood and urine samples. Moreover, circulatory Pi is the central mediator between bone, the parathyroid glands, the gut and the kidneys, which are fine-tuned by numerous hormonal signals to keep the serum phosphate concentration within close limits.

In a steady state condition, as it is the case after completion of skeletal growth, the serum Pi concentration is determined by the balance between intestinal absorption of phosphate from the diet (16 mg/kg per day), bone-turnover of phosphate in the skeleton (3 mg/kg per day), and excretion of phosphate through the urine (16 mg/kg per day), (Fig. 38.1). In growing individuals, the balance of Pi must be positive to meet the needs of skeletal growth and consolidation. A typical Western diet commonly provides plenty of alimentary Pi, most of which provided by protein-rich foods like meat, milk and eggs. In contrast to plant-derived phosphate in the form of phytate (hexa-phospho-inosite), the animal derived phosphate is easily absorbed by the intestine, where roughly two thirds of the ingested phosphate are absorbed. Intestinal Pi absorption in growing infants is higher than in adults and can exceed 90% of dietary intake. Absorbed Pi first distributes in the extracellular compartment and then equilibrates with the bone and intracellular compartment. Within the kidney, Pi is freely filtered at the glomerular capillaries and is reabsorbed mainly along the proximal nephrons according to the actual requirements of the organism. The majority of the transepithelial Pi transport in the intestine

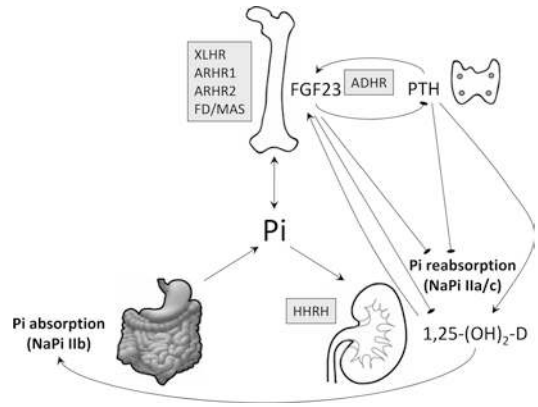

Fig. 38.1 Regulation of phosphate (P_i) homeostasis. FGF23 and PTH reduce renal tubular phosphate reabsorption via a decrease in apical expression of the sodium-phosphate cotransporters NaPi IIa and NaPi IIc. In contrast to FGF23, which inhibits 1,25-$(OH)_2$D synthesis, PTH stimulates 1,25-$(OH)_2$D production. 1,25-$(OH)_2$D increases intestinal absorption of dietary P_i via increased expression of NaPi IIb and activates FGF23 production. PTH and FGF23 affect each other's production through a negative feedback loop that is not yet fully elucidated. The mode of action of the therapeutic antibody burosumab is indicated. Note, burosumab is currently licensed for treatment of XLH and tumor induced osteomalacia (TIO), only. The sites of defect of the different genetic forms of hypophosphatemic disorders are given. *XLH* X-linked hypophosphatemia, *ARHR1/2* autosomal recessive hypophosphatemic rickets 1/2, *Raine S.* Raine syndrome, *FD/MAS* Fibrous dysplasia/McCune-Albright syndrome, *HHRH* hereditary hypophosphatemic rickets with hypercalciuria, *ADHR* X-linked dominant hypophosphatemic rickets. *FGF23 protein resistant to degradation

and the kidney is mediated by the type II family of sodium-coupled phosphate transporters, i.e. NaPi-IIa (NPT2a; *SLC34A1*), NaPi-IIb (NPT2b; *SLC34A2*), and NaPi-IIc (NPT2c; *SLC34A3*) [12]. NaPi-IIa is primarily localized at the brush border of proximal tubular epithelial cells and accounts for roughly 90% of renal phosphate reabsorption. Reabsorption of the remaining 10% is accomplished by NaPi-IIc exclusively expressed along proximal tubules of deep nephrons. Its critical contribution to Pi homeostasis is demonstrated by loss-of-function mutations, which leads to renal phosphate wasting resulting in the rare syndrome of hypophosphatemic rickets with hypercalcemia (HHRH) [13].

In contrast to NaPi-IIa and NaPi-IIc, NaPi-IIb shows a broader expression pattern including

pulmonary alveolar type II cells, where it participates in Pi uptake from the alveolar fluid for surfactant production. *SLC34A2* mutations cause pulmonary alveolar microlithiasis, a disease characterized by the deposition of calcium-phosphate crystals throughout the lungs [14]. In the intestine, NaPi-IIb is expressed in the brush border membrane of enterocytes and mediates absorption of ingested phosphate.

Regulators of Phosphate Homeostasis

The amount of intestinal phosphate absorption directly correlates with dietary supply. Only 30% of intestinal Pi absorption occurs in a regulated, 1,25-dihydroxyvitamin D [1,25-$(OH)_2$D] dependent manner. This poor regulation at the uptake level contrasts with the meticulous regulation of phosphate excretion within the kidney. Proximal tubular reabsorption of phosphate, mainly via regulation of expression of NaPi-IIa and NaPi-IIc within proximal tubular brush border membranes, thus plays a key role in maintaining serum phosphate homeostasis. The amount of renal phosphate reabsorption is tightly regulated primarily by dietary Pi intake, parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23). Other factors like insulin, human growth hormone, and possibly FGF7 also affect renal phosphate handling, but their actions are less well understood [15].

Dietary Pi intake directly affects the amount of renal phosphate reabsorption. An increase or decrease in dietary Pi induces an increase or decrease, respectively, in renal Pi excretion independent of vitamin D, PTH or FGF23. Part of this effect might be explained by a phosphate-responsive element in the promoter of the *SLC34A1* gene [16].

Parathyroid hormone is a major hormonal regulator of proximal tubular Pi reabsorption. PTH synthesis and secretion are up-regulated by low serum calcium and increased serum phosphate levels, and down-regulated by increased serum calcium and 1,25$(OH)_2$D levels. Its binding to proximal tubular PTH receptors results in an inhibition of NaPi-cotransport through mechanisms that involve rapid clearance of NaPi-IIa from the tubular epithelial brush border membrane [17]. On the other hand, PTH stimulates the synthesis of 1,25-$(OH)_2$D. The net effect of these actions is an increase of serum calcium levels and a decrease in serum phosphate levels.

Fibroblast Growth Factor 23 is a glycoprotein primarily synthesized in osteocytes and osteoblasts. FGF23 expression is induced by increased serum phosphate and 1,25$(OH)_2$D levels. In the presence of Klotho, a membrane bound protein with ß-glucuronidase activity, FGF23 binds with high affinity to the FGF receptor FGFR1 [15]. Klotho/FGFR1 mediated renal effects of FGF23 result in inhibition of proximal tubular Pi reabsorption and 1,25$(OH)_2$D synthesis. Its net effect thus is a reduction in serum phosphate and 1,25-$(OH)_2$D levels, which may result in hypocalcemia (Fig. 38.1).

Hypophosphatemic Disorders

Clinical, Biochemical, and Radiological Manifestations

Clinical Findings

The clinical and radiological features of the various types of hypophosphatemic rickets are similar, although not identical. In children, the primary clinical symptoms are rickets, skeletal pain and deformity, disproportionate short stature, and dental abscesses' (Figs. 38.2 and 38.3) [19]. In adults, osteomalacia, bone pain and stiffness, pseudofractures, enthesopathy and poor dental condition including periodontitis are typical findings. With medical therapy these abnormalities can be improved, but usually do not entirely resolve [18, 20]. Most children with hypophosphatemic rickets are identified in the first year of life if there is a known family history of the disorder. By 6 months of age, classic skeletal deformities may already appear including frontal bossing with flattening at the back of the head. In the absence of a family history, children often present at 2–3 years of age with delayed walking, muscle weakness, a waddling gait and

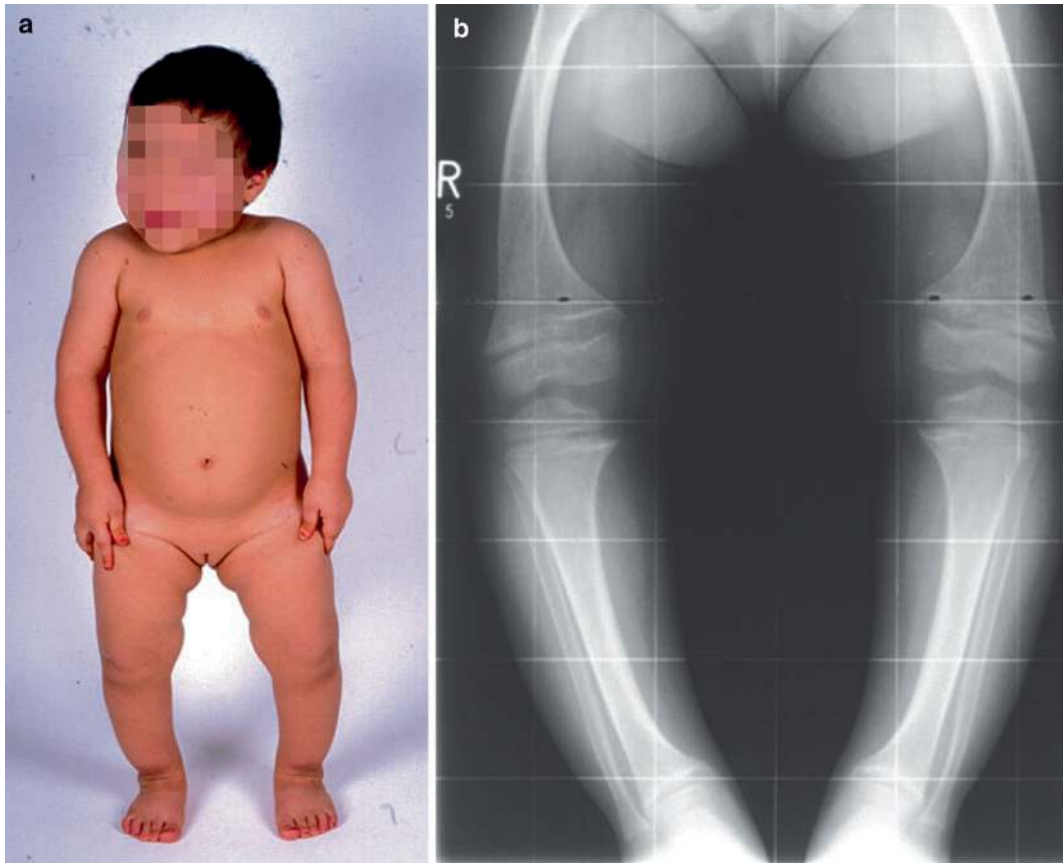

Fig. 38.2 Photograph (**a**) and radiograph of the lower extremities (**b**) of a 3-year-old girl with X-linked hypophosphatemia. The patient shows disproportionate stunting with bowed legs. The radiograph reveals severe leg bowing, partial fraying and irregularity of the distal femoral and proximal tibial growth plates

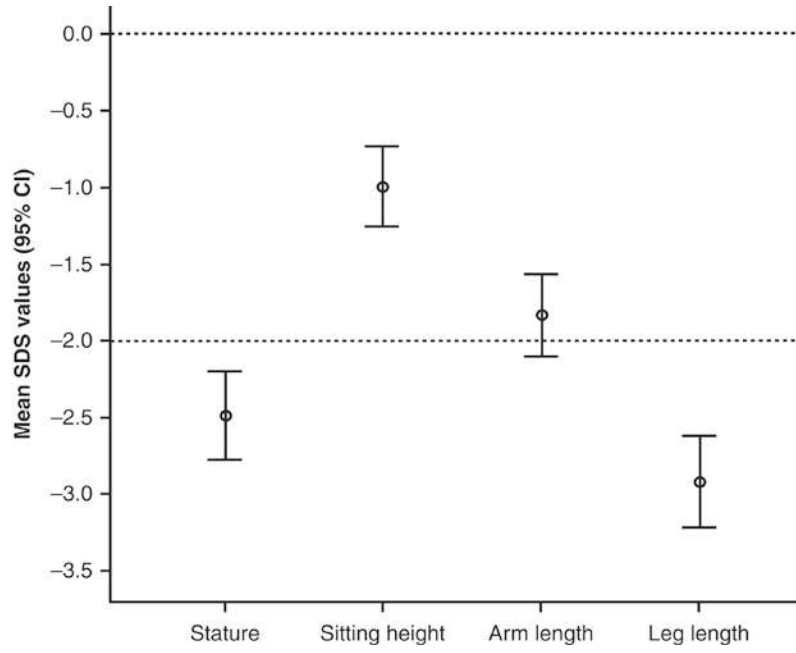

Fig. 38.3 Mean standard deviation scores (SDS) and 95% confidence intervals (CI) of stature, sitting height, and arm and leg length in 76 children with X-linked hypophosphatemia. Patients showed disproportionate stunting. Leg length was the most impaired and trunk length the most preserved linear body dimension (Used with permission of Nature/Springer from Zivicnjak et al. [18])

progressive lower extremity deformities (bow-legged). Leg growth is often more impaired than trunk growth resulting in disproportionate short stature [18]. Patients may present with abnormal skull shape, i.e. dolichocephaly, characterized by frontal bossing, parietal flattening and widened sutures due to premature cranial synostosis [21]. Common misdiagnoses are metaphyseal dysplasia and nutritional rickets. Long-term outcomes are substantially better when treatment is applied at an early age, i.e. in the first year of life [22–24]. In adults, the occurrence of pseudofractures of the long bones and enthesopathies may result in additional pain and substantially compromised quality of life [25].

Bone histology is influenced by the pathophysiology of the disease. In general, pure hypophosphatemia results in accumulation of unmineralized osteoid (Fig. 38.4), while the concomitant presence of hyperparathyroidism adds the component of enhanced bone resorption by osteoclasts [27]. However, establishing a diagnosis of phosphopenic osteomalacia requires histopathological proof that the abundant osteoid results from abnormal mineralization and not increased osteoid production. Thus, histopathological detection of an increase in the bone-forming cell surface by incompletely covered mineralized osteoid, an increase in osteoid volume and thickness and a decrease in the mineralization front (the percentage of osteoid-covered bone-forming surface undergoing calcification) or the mineral apposition rate is a prerequisite [27].

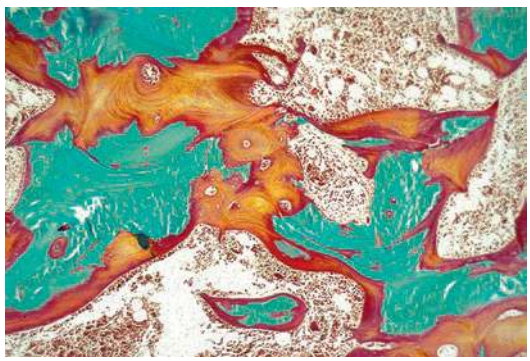

Fig. 38.4 Bone biopsy in a patient with X-linked hypophosphatemia showing abundance of unmineralized osteoid (orange), and decreased mineralization of trabecular and cortical bone (Used with permission of Nature/Springer from Schnabel and Haffner [26])

In addition to these skeletal defects, dental abnormalities contribute to considerable clinical morbidity. Tooth eruption may be delayed and teeth may exhibit inadequate dentine calcification [28–32]. As a result, the pulp chambers expand, and the overall barrier to external pathogens is compromised, thereby predisposing to dental abscesses. The prevalence of dental abscesses is about 25% in children and more than 85% in adults [33]. Individuals who present with one abscess usually develop multiple abscesses during follow-up, indicating that the development of one abscess predicts future abscesses. It is important to note that dental abscesses usually occur in the absence of caries. Finally, hypertension, left ventricular hypertrophy, nephrocalcinosis, and hearing loss have been identified in patients suffering from hypophosphatemic rickets, although it is not clear if these abnormalities are due to the disease itself or to the treatment [34–36].

Radiological Findings

The rachitic abnormalities in children with phosphopenic disease result in a variety of characteristic radiological findings in the skeleton. The growth plates of the long bones are cupped and show increased thickness and irregular, hazy appearance at the diaphyseal line (Fig. 38.2). The latter is due to an irregular invasion of recently calcified cartilage in bone tissue. These abnormalities preferentially occur at sites of rapid growth. Therefore, widening of the forearm at the wrist and thickening of the costochondral junctions frequently occur. For clinical confirmation, an x-ray of the knees and/or the wrist is usually sufficient to diagnose rickets [19]. In addition, other typical signs of rickets such as rachitic rosary and Harrison's groove may also develop.

Biochemical Findings

In general, the primary diagnosis of rickets is based on typical clinical and radiological findings (see above) in combination with an elevated serum alkaline phosphatase activity. Physicians often overlook the latter abnormality in children,

since normal levels in young children are high. On the other hand, in some affected patients, normal alkaline phosphatase activity might be observed. Moreover, in adults, the alkaline phosphatase levels are often inexplicably normal. For differential diagnosis of the various forms of rickets, additional biochemical parameters are needed. Phosphorus is the common denominator of all types of rickets [5, 6]. Therefore, the diagnostic approach focuses on the mechanisms leading to hypophosphatemia, i.e. (1) high PTH activity, (2) inadequate phosphate absorption from the gut, or (3) renal phosphate wasting. The latter may be due to either intrinsic tubular defects or high circulating FGF23 levels (Fig. 38.5) [5, 6, 19].

Renal phosphate loss can be evaluated through the tubular reabsorption of phosphate per glomerular filtration rate (TmP/GFR) as: $TmP/GFR = P_p - (U_p \times P_{cr}/U_{cr})$, where P_p, U_p, P_{cr} and U_{cr} refer to plasma and urine concentration of phosphate and creatinine, respectively. All values must be expressed in the same units, e.g. in milligrams per deciliter [37]. The normal range of TmP/GFR in infants and children (6 months – 6 years) ranges from 1.2 to 2.6 mmol/L and in adults from 0.6 to 1.7 mmol/L. An important pitfall to recognize is that in patients with insufficient intake or absorption of phosphate from the gut TmP/GFR might be falsely low when serum phosphate levels have not been restored to normal. This can usually be assumed in the presence of low urinary phosphate levels. In such cases, TmP/GFR should only be calculated after phosphate supplementation when serum and urine phosphate concentrations are raised.

Typically, in cases of hypophosphatemic rickets due to renal tubular abnormalities or elevated serum levels of FGF23, serum concentrations of calcium and PTH are normal before initiation of treatment. Circulating $1,25(OH)_2D$ levels are low or inappropriately normal in the setting of hypophosphatemia. Plasma FGF23 levels are usually elevated with the exception of HHRH patients. Measurement of serum $1,25(OH)_2D$ levels may be a useful tool for diagnosis in HHRH patients. In Table 38.1, the main biochemical features of the various forms of hypophosphatemic rickets due to perturbations in proximal tubule phosphate reabsorption compared to vitamin D defi-

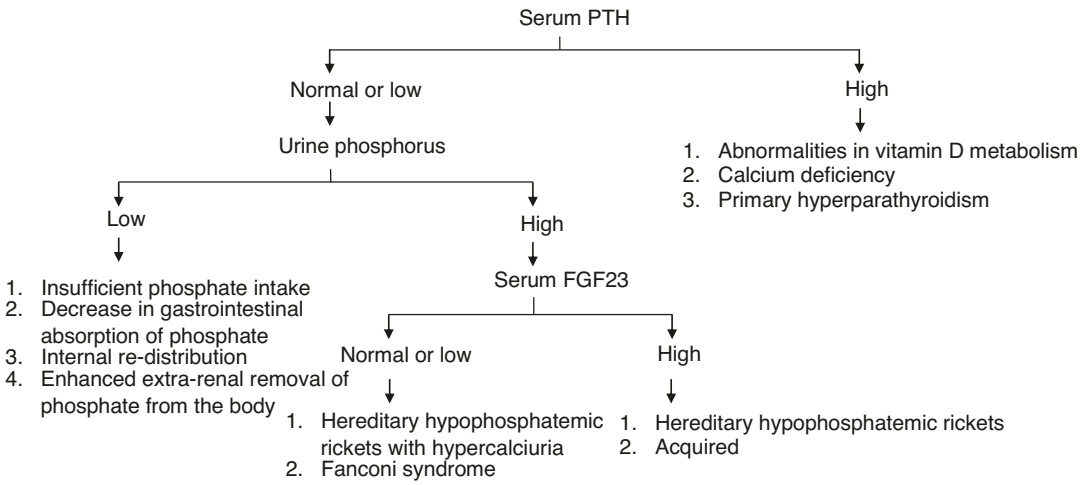

Fig. 38.5 Algorithm for the evaluation of the child with rickets presenting with hypophosphatemia. The differential diagnoses are based on the mechanisms leading to hypophosphatemia, namely high parathyroid hormone (PTH) activity, inadequate phosphate absorption from the gut, or renal phosphate wasting. The latter may be due to either primary tubular defects or high levels of circulating FGF23. Further details of individual entities can be found in Table 38.1; *FGF-23* fibroblast growth factor 23 (Used with permission of Nature/Springer from Haffner et al. [19])

Table 38.1 Molecular genetic and biochemical characteristics of inherited or acquired causes of phosphopenic rickets in comparison to calcipenic rickets. (Adapted from [19])

Disorder (abbreviation; OMIM#)	Gene (location)	Ca	P	ALP	U_{Ca}	U_P	TmP/GFR	FGF23	PTH	25 (OH) D[a]	1,25 $(OH)_2D$	Pathogenesis
Rickets and/or osteomalacia with high PTH levels (calcipenic rickets)												
Nutritional rickets (vitamin D and/or calcium deficiency)	NA	N, ↓	N, ↓	↑↑↑	↓	Varies	↓	N	↑↑↑	↓↓, N	varies	Vitamin D deficiency
Vitamin D dependent rickets type 1A (VDDR1A; OMIM#264700)	*CYP27B1* (12q14.1)	↓	N, ↓	↑↑↑	↓	Varies	↓	N, ↓	↑↑↑	N	↓	Impaired synthesis of 1,25 $(OH)_2D$
Vitamin D dependent rickets type 1B (VDDR1B; OMIM#600081)	*CYP2R1* (11p15.2)	↓	N, ↓	↑↑↑	↓	Varies	↓	N	↑↑↑	↓↓	varies	Impaired synthesis of 25 (OH) D
Vitamin D dependent rickets type 2A (VDDR2A; OMIM#277440)	*VDR* (12q13.11)	↓	N, ↓	↑↑↑	↓	Varies	↓	N, ↓	↑↑↑	N	↑↑	Impaired signaling of the VDR
Vitamin D dependent rickets type 2B (VDDR2B; OMIM#264700)	HNRNPC	↓	N, ↓	↑↑↑	↓	Varies	↓	N	↑↑↑	N	↑↑	Impaired signaling of the VDR
Vitamin D dependent rickets type 3 (VDDR3; OMIM# pending)	CYP3A4	↓	↓	↑↑↑	↓	Varies	↓	?	↑↑↑	↓	↓	↑ inactivation of 1,25 $(OH)_2D$
Phosphopenic rickets												
Rickets and/or osteomalacia due to dietary phosphate deficiency or impaired bioavailability												
Breastfed very low birthweight infants Use of elemental or hypoallergenic formula diet or parental nutrition Excessive use of phosphate binders Gastrointestinal surgery or disorders	NA	N, ↑	↓	↑, ↑↑	?	↓	N[b]	N, ↓	N	N	N, ↑	Phosphate deficiency
Rickets and/or osteomalacia with renal tubular phosphate wasting due to elevated FGF23 levels and/or signaling												
X-linked hypophosphatemia (XLH; OMIM#307800)	*PHEX* (Xp22.1)	N	↓	↑, ↑↑	↓	↑	↓	↑, N	N, ↑[c]	N	N[d]	↑ FGF23 expression in bone and impaired FGF23 cleavage
Autosomal dominant hypophosphatemic rickets (ADHR; OMIM#193100)	*FGF23* (12p13.3)	N	↓	↑, ↑↑	↓	↑	↓	↑, N	N, ↑[c]	N	N[d]	FGF23 protein resistant to degradation
Autosomal recessive hypophosphatemic rickets 1 (ARHR1; OMIM#241520)	*DMP1* (4q22.1)	N	↓	↑, ↑↑	↓	↑	↓	↑, N	N, ↑[c]	N	N[d]	↑ FGF23 expression in bone
Autosomal recessive hypophosphatemic rickets 2 (ARHR2; OMIM#613312)	ENPP1 (6q23.2)	N	↓	↑, ↑↑	↓	↑	↓	↑, N	N, ↑[c]	N	N[d]	↑ FGF23 expression in bone
Raine syndrome associated (ARHR3; OMIM#259775)	*FAM20C* (7q22.3)	N	↓	↑, ↑↑	?	↑	↓	↑, N	N, ↑[c]	N	N[d]	↑ FGF23 expression in bone

(continued)

Table 38.1 (continued)

Disorder (abbreviation; OMIM#)	Gene (location)	Ca	P	ALP	U_{Ca}	U_P	TmP/GFR	FGF23	PTH	25 (OH) D[a]	1,25 $(OH)_2D$	Pathogenesis
Fibrous dysplasia (FD; OMIM#174800)	*GNAS* (20q13.3)	N, ↓	↓	↑, ↑↑	↓	↑	↓	N, ↑	N, ↑[c]	N	N[d]	↑ FGF23 expression in bone
Tumor induced osteomalacia (TIO)	NA	N, ↓	↓	↑, ↑↑	↓	↑	↓	N, ↑	N, ↑[c]	N	N[d]	↑ FGF23 express-ion in tumoral cells
Cutaneous skeletal Hypophosphatemia syndrome (SFM; OMIM#163200)	*RAS* (1p13.2)	N, ↓	↓	↑, ↑↑	↓	↑	↓	N, ↑	N, ↑[c]	N	N[d]	?
Osteoglophonic dysplasia (OGD) (OMIM#166250)	*FGFR1* (8p11.23)	N	↓	↑, N	N	↑	↓	N	N, ↑[c]	N	N[d]	↑ FGF23 expression in bone
Hypophosphatemic rickets and hyperparathyroidism (OMIM#612089)	*KLOTHO* (13q13.1)	N	↓	↑, ↑↑	↓	↑	↓	↑	↑↑	N	N[d]	Unknown; translocation of the KLOTHO promoter
Rickets and/or osteomalacia due to primary renal tubular phosphate wasting												
Hereditary hypophosphatemic rickets with hypercalciuria (HHRH; OMIM#241530)	*SLC34A3* (9q34.3)	N	↓	↑ (↑↑)	N, ↑	↑	↓	↓	Low N, ↓	N	↑↑	Loss of function of NaPi2c in the proximal tubule
X-linked recessive hypophosphatemic rickets (OMIM#300554)	*CLCN5* (Xp11.23)	N	↓	↑ (↑↑)	N, ↑	↑	↓	varies	varies	N	↑	Loss of function of CLCN5 in the proximal tubule
Hypophosphatemia and nephrocalcinosis (NPHLOP1; OMIM#612286) Fanconi reno-tubular syndrome 2 (FRTS2; OMIM#613388)	*SLC34A1* (5q35.3)	N	↓	↑ (↑↑)	↑	↑	↓	↓	varies	N	↑	Loss of function of NaPi2a in the proximal tubule
Cystinosis (OMIM#219800) and other hereditary forms of Fanconi syndrome)	*CTNS* (17p13.2)	N, ↓	↓	↑ (↑↑)	N, ↑	↑	N, ↓	N, ↑[e]	N, ↑[e]	N	N[d]	Cysteine accumulation in the proximal tubule
Iatrogenic proximal tubulopathy	NA	N	↓	↑ (↑↑)	varies	↑	↓	↓	varies	N	↑	Drug toxicity

N normal, ↑ elevated, ↑↑ or ↑↑↑ very elevated, ↑ (↑↑) may range widely: Ca, serum levels of calcium, *P* serum levels of phosphate, *ALP* alkaline phosphatase, U_{Ca} urinary calcium excretion, *TmP/GFR* maximum rate of renal tubular reabsorption of phosphate normalized to the glomerular filtration rate, *FGF23* fibroblast growth factor 23, *PTH* parathyroid hormone, *1,25(OH)₂D* 1,25-dihydroxyvitamin, *25(OH)D* cholecalciferol, *NA* not applicable

[a] Cave: prevalence of vitamin D deficiency was reported to be up-to 50% in healthy children

[b] Normal after restoration of P, but falsely reduced before restoration

[c] PTH may be moderately elevated

[d] Decreased relative to the serum phosphate concentration

[e] Depending on the stage of chronic kidney disease

ciency rickets are summarized. Extended molecular genetic analysis may be required to establish diagnosis in unclear cases [38].

Hypophosphatemic Disorders with Increased FGF23 Activity

X-Linked Hypophosphatemia (*PHEX* Mutation)

X-linked hypophosphatemia (XLH) is the most frequent inherited phosphate wasting disorder, accounting for about 80% of familial cases with an incidence of 1:20,000 individuals. It typically presents within the first 2 years of life. Males usually show the full manifestation of the disease and females show a wide spectrum ranging from one identical to males to one with no clinical symptoms but only isolated hypophosphatemia. The characteristic laboratory results are hypophosphatemia, hyperphosphaturia, hyperphosphatasia and normocalcemia. Serum 25(OH)D and $1,25(OH)_2D$ levels are in the normal range [19]. However, the level of the latter appears to be decreased relative to the diminished serum phosphate concentrations. Elevated serum levels of FGF23 and mutations in the *PHEX* gene (phosphate regulating gene with homologies to endopeptidases on the X-chromosome) are found in most patients [11, 19, 39]. Of note, serum levels of phosphate, TmP/GFR and alkaline phosphatase activity might be in the normal range within the first 3 months of life in XLH patients. Therefore, in the case of a positive family history, affected patients should undergo mutation analysis of the *PHEX* gene in order to establish the diagnosis and treatment of XLH as early as possible [19]. More than 350 different PHEX mutations have been reported so far including nonsense, missense, frame shift, splice site, deletion and duplication mutations. Mutations were reported in all 22 *PHEX* exons without a hot spot and no genotype-phenotype correlations were found in children with XLH [8].

Although the genetic cause of XLH is well established, the exact pathogenic mechanisms of how mutations in the *PHEX* gene result in elevated plasma FGF23 levels remains to be elucidated. *PHEX* encodes for a membrane-bound endopeptidase and is primarily expressed in osteoblasts, osteocytes, odontoblasts, muscle, lung and ovary [40]. The finding that hypophosphatemia recurs in XLH patients undergoing kidney transplantation with prior parathyreoidectomy strongly suggested a circulating factor causing phosphate wasting in the kidney transplant [41]. Likewise, studies in *Hyp* mice, an orthologic animal model of XLH employing parabiosis and cross-transplantation of the kidneys between *Hyp* and normal mice, also showed recurrence of hypophosphatemia [42]. After it became clear that FGF23 is the cause of ADHR, FGF23 levels were measured in patients with XLH and *Hyp* mice. The majority of XLH patients as well as *Hyp* mice show elevated FGF23 levels [43, 44]. The normal FGF23 serum levels found in some XLH patients might still be viewed as inappropriately high in relation to the degree of hypophosphatemia. Administration of neutralizing antibodies to FGF23 corrects hypophosphatemia and decreases $1,25(OH)_2D$ levels in *Hyp* mice and patients with XLH and thus confirms the pathogenic role of FGF23 in XLH [45, 46]. Although PHEX is an enzyme, it is thought that *PHEX* affects the expression of FGF23 rather than its degradation [7, 47]. PHEX may regulate serum FGF23 indirectly via cleavage by proprotein convertases such as subtilisin/kexin-type 2 (PC2). In addition, *PHEX* malfunction results in increased skeletal synthesis of osteopontin and acid serine aspartate-rich-MEPE-associated protein (ASARM) peptide, both of which also contribute to impaired bone mineralization in XLH [7, 48]. Thus, XLH results from a complex osteoblast/odontoblast defect. Therefore, it remains to be seen whether therapeutic FGF23 blockade in patients with XLH (*vide infra*) will result in complete healing of bone and teeth abnormalities as well as prevention of rare complications like sensorineural hearing loss.

ADHR (*FGF23* Mutation)

Autosomal-dominant hypophosphatemic rickets (ADHR) is a rare disorder that was first described by Bianchine et al. in 1971 [49]. ADHR and XLH have marked clinical similarities but differ in their modes of inheritance. ADHR is due to activating mutations of the *FGF23* gene. The mutated

protein is resistant to cleavage by proteolytic activity, which in turn leads to elevated, circulating FGF23 level [50]. Elevated FGF23 levels result in phosphaturia, hypophosphatemia, and inappropriately low levels of $1,25(OH)_2D$. The penetrance of ADHR is incomplete, with a highly variable phenotype and, thus, variable symptomatology and biological findings. In contrast to XLH, patients suffering from ADHR may become symptomatic in adolescence or even during adulthood. During childhood, the clinical symptoms are similar to that observed in XLH, i.e. rickets with bone deformities, disproportionately short stature, and dental abnormalities (abscesses) [51]. If patients become symptomatic after puberty, complications due to osteomalacia, e.g. weakness, fatigue, bone pain and pseudofractures are the major symptoms. Adults present with symptoms similar to those with tumor induced osteomalacia (TIO), as discussed below. Interestingly, some children show improvement of phosphate wasting during puberty [52]. Studies in ADHR mice and humans suggest that iron status is an important regulator of FGF23 metabolic pathways [52–54]. Therefore, the onset of ADHR is the product of gene-environment interactions. Serum iron levels are negatively associated with FGF23 plasma level both in ADHR patients and in healthy subjects, indicating increased expression of FGF23 in the setting of a low iron status. A recent study in iron deficient adult ADHR patients demonstrated normalization of both serum FGF23 and phosphate levels by oral iron repletion [55]. Therefore, the standard approach to ADHR patients should also include the recognition and correction of iron deficiency.

ARHR (1, *DMP1* Mutation; 2, *ENPP1* Mutation)

The finding of hypophosphatemic rickets in consanguineous kindreds suggested an autosomal recessive form of hypophosphatemia (ARHR) [56]. Clinical symptoms are similar to those observed in XLH patients and affected individuals present with elevated FGF23 serum levels, renal phosphate wasting and inappropriately normal levels of $1,25(OH)_2D$. One characteristic radiological feature of this disorder is the relatively high bone density of the vertebral bodies. ARHR is either due to mutations in the *DMP1* gene encoding for dentin matrix acidic phosphoprotein 1 (ARHR1) or to mutations in the *ENPP1* gene encoding for ectonucleotide pyrophosphatase/phosphodiesterase 1 (ARHR2) [57–60].

DMP1 is a member of the short integrin-binding ligand interacting N-linked glycoprotein (SIBLING) family of skeletal matrix proteins and is highly expressed in mineralized tissues, e.g. in osteoblasts and osteocytes. It is an important regulator of the development of bone, cartilage, and teeth. How mutations in *DMP1* result in elevated FGF23 serum levels in ADHR1 patients is unclear.

Levi-Litan et al. identified an inactivating mutation in the *ENPP1* gene (later named ARHR2) that caused ARHR in a Bedouin family [60]. ENPP1 is a cell surface protein that catalyzes phosphoester cleavage of adenosine triphosphate, generating the mineralization inhibitor pyrophosphate. Mutations in *ENPP1* were initially reported in patients with infantile arterial calcifications [61] but clinical and biochemical manifestations can markedly differ even in patients presenting with the same biallelic *ENPP1* pathogenic variants and include enthesopathy and primary hyperparathyroidism [62]. It remains to be elucidated how inactivating *ENPP1* gene mutations result in increased FGF23 synthesis from bone.

Raine Syndrome Associated (*FAM20C* Mutation)

Raine syndrome is a rare skeletal disorder with highly variable clinical phenotype ranging from lethal to isolated teeth and/or bone phenotypes [63]. It is caused by biallelic mutations in the *FAM20C* gene coding for a protein kinase which phosphorylates FGF23 and thereby promotes FGF23 cleavage. Therefore, *FAM20C* mutations result in high serum FGF23 concentrations causing renal phosphate wasting with consecutive hypophosphatemic rickets [64, 65]. The majority of patients die in early life from respiratory failure [63].

Tumor-Induced Osteomalacia and Tumor-Induced Rickets (TIO)

Tumor-induced osteomalacia (TIO), also called tumor-induced rickets, is a rare disorder

characterized by hypophosphatemia, hyperphosphaturia, low $1,25(OH)_2D$ serum levels, and osteomalacia which develops in previously healthy individuals [66–68]. Therefore, in any patients presenting with hypophosphatemic rickets beyond the second year of life TIO should be excluded. Clinical symptoms are similar to that in XLHR or ADHR patients. TIO is caused by usually small, often difficult to locate, tumors. Most histologic diagnoses have been classified as phosphaturic mesenchymal tumors of the mixed connective tissue type. A characteristic histologic feature of these tumors is a background of spindle cells that tend to have low mitotic activity [66, 68]. Once the tumor is removed, the clinical symptoms quickly resolve, which has led to the notion that circulating factors produced by the tumor (phosphatonins) are causing renal phosphate loss in these patients. Later, TIO tumors were shown to contain high levels of FGF23 mRNA and protein, and TIO patients revealed elevated circulating FGF23 levels, which rapidly declined after removal of the tumor in parallel with resolution of clinical symptoms. The tumors are benign, but may recur. The paranasal sinuses, neck and mandible are common sites of these tumors. Newer imaging techniques such as nuclear magnetic resonance and positron emission tomography are helpful in establishing diagnosis of TIO. If tumor resection is not possible, treatment with the anti-FGF23 antibody Burosumab results in improvement of bone lesions [68].

Hypophosphatemic Rickets and Hyperparathyroidism

This is an extremely rare disorder caused by increased synthesis of α-Klotho. Patients reveal both hypophosphatemic rickets and hyperparathyroidism due to parathyroid hyperplasia. It is caused by *de novo* translocation resulting in elevated plasma α-Klotho levels [69]. Recently it was demonstrated that α-Klotho enhances FGF23-stimulated FGF receptor activation, and consequently inhibition of sodium phosphate transporter and hyperphosphaturia [70]. It is thought that the concomitant suppression of calcitriol production by α-Klotho/FGF23 action may cause increased production of FGF23 and PTH in these patients.

Fibrous Dysplasia (FD) and McCune-Albright Syndrome (MAS)

Fibrous dysplasia (FD) is characterized by fibrous skeletal lesions and localized mineralization defects. Patients may present with solitary or multiple bone lesions. When the skeletal findings occur in combination with abnormal skin pigmentation (e.g. café-au-lait spots), premature sexual development and/or thyrotoxicosis, the disease is called McCune-Albright syndrome (MAS) [71]. FD/MAS is a rare disorder due to post-zygotic gain-of-function mutations in the *GNAS1* gene, encoding for the α subunit of a stimulatory G protein [72]. G proteins function to couple specific receptors to intracellular signaling molecules. Phosphate wasting is due to increased secretion of FGF23 from bone lesions and the severity of hypophosphatemia correlates with the number of fibrous dysplasia lesions. However, how *GNAS1* mutations result in elevated FGF23 secretion is currently unknown.

Hypophosphatemic Disorders with Normal or Suppressed FGF23 Activity

HHRH (*SLC34A3* Mutation)

In 1985 Tieder et al. described an unusual case of hypophosphatemic rickets in a consanguineous Bedouin tribe [73]. In contrast to XLH, hypophosphatemia and phosphate wasting was associated with elevated $1,25(OH)_2D$ serum levels resulting in hypercalciuria and suppressed PTH plasma concentrations. The disorder was later shown to be caused by mutations in the *SLC34A3* gene encoding for the NaPi-IIc renal phosphate cotransporter in the proximal tubule, and many more patients were identified [74]. The reported mutations were all loss-of-function mutations and, most likely, reduced renal phosphate absorption through decreasing the apical membrane expression of NaPi-IIc or the uncoupling of sodium-phosphate co-transport in the proximal tubule [75]. Thus, in contrast to XLH and ADHR hypophosphatemia is not due to enhanced FGF23 serum levels in HHRH. The normal physiological reaction to hypophosphatemia resulting in increased serum elevated $1,25(OH)_2D$ levels results in increased

intestinal calcium absorption, hypercalciuria and suppression of PTH. Consequent to hypercalciuria, patients developed nephrocalcinosis and nephrolithiasis. Milder forms may be under-diagnosed and therefore careful evaluation of urinary calcium excretion before and during medical treatment is strongly recommended in all patients with hypophosphatemic rickets, especially in those without a proven underlying genetic cause [76].

Hypophosphatemia and Nephrocalcinosis (*SLC34A1* Mutation)

The *SLC34A1* gene is located on chromosome 5q34 and encodes for the NaPi-IIa renal phosphate cotransporter in the proximal tubule. *SLC34A1* mutations can lead to a wide range of clinical phenotypes including infantile hypercalcemia, kidney stones and rickets, all of which are accompanied by reduced serum phosphate levels [77, 78].

Nephrolithiasis and Osteoporosis Associated with Hypophosphatemia

Two different heterozygous mutations (A48P and V147M) in *NPT2a*, a gene encoding a sodium dependent phosphate transporter, have been reported in patients suffering from urolithiasis or osteoporosis and persistent hypophosphatemia due to decreased tubular phosphate reabsorption [79].

Fanconi Syndrome

Fanconi syndrome is characterized by generalized proximal tubular dysfunction with impaired ability to absorb water, phosphate, glucose, urate, amino acids and low molecular weight proteins. Other common features include increased excretion of sodium, potassium, calcium and bicarbonate. Clinical consequences result mainly from hypophosphatemia and metabolic acidosis, i.e. rickets and osteomalacia. The syndrome may be due to both genetic defects and acquired disorders (see Chap. 31).

Treatment of XLH

For more than four decades treatment of XLH patients was based on oral supplementation of inorganic phosphate salts and treatment with active vitamin D metabolites (calcitriol or alfacalcidol) [80]. Recently, a fully humanized anti-FGF23 antibody (burosumab) has become available for the treatment for XLH, which represents a major breakthrough and paradigm shift in the treatment of this disorder (Fig. 38.1). The advantages of burosumab over conventional treatment are (1) the removal of the burden to take medications several times a day as required with conventional treatment, which frequently leads to non-adherence particularly in adolescents and adults, (2) its greater efficacy in ameliorating rickets compared to conventional treatment, and (3) its excellent safety profile and removal of the typical side effects of conventional therapy such as diarrhea and nephrocalcinosis.

However, one has to keep in mind that the disease spectrum, and thus the medication requirement, is heterogeneous—some individuals are only minimally affected even without treatment [81]. Adults need less treatment than children or even no treatment depending on the clinical symptoms. Other forms of FGF23-dependent hypophosphatemic rickets like ADHR are usually treated with phosphate salts and active vitamin D, whereas FGF23-independent forms require disease specific therapeutic approaches *(vide infra)*.

The European Medicines Agency (EMA) and the U.S. Food and Drug Administration (FDA) approved burosumab to treat children ≥1 year and adults with XLH and TIO showing radiographic evidence of bone disease. If available, burosumab treatment should be considered in children with XLH ≥ 1 year and in adolescents with growing skeletons in the following situations: radiographic evidence of overt bone disease; disease that is refractory to conventional therapy; complications related to conventional therapy; or patient's inability to adhere to conventional therapy, presumed that adequate monitoring is feasible [19]. Evidence-based recommendations for the treatment of XLH were recently published and will be outlined below [19].

Phosphate Supplementation and Active Vitamin D Metabolites

Children in affected families should be screened for abnormal serum and urine phosphorus levels

and serum alkaline phosphatase activity within the first month of life and at 3 and 6 months, and if available should undergo *PHEX* gene mutation analysis [19]. In case of confirmed diagnosis, therapy with active vitamin D metabolites and phosphate should be started immediately. The required dosages differ largely depending on the severity of rickets (*vide infra*). In patients with negative family history for XLH, hypercalciuria should be excluded before initiation of calcitriol treatment. The latter would suggest the diagnosis of HHRH where calcitriol treatment is contraindicated.

Administration of phosphate increases the plasma phosphate concentration, which lowers the plasma ionized calcium concentration, and further reduces the plasma calcitriol concentration (by removing the hypophosphatemic stimulus of its synthesis). This causes secondary hyperparathyroidism [19, 82]. The latter can aggravate the bone disease and increase urinary phosphate excretion, thereby defeating the aim of phosphate therapy. Secondary hyperparathyroidism can be prevented by additional treatment with calcitriol. Calcitriol increases intestinal calcium absorption, and to a lesser degree phosphate, and consequently suppresses secondary hyperparathyroidism. In addition, it also directly suppresses PTH release.

The recommended starting dose of phosphate based on elemental phosphorus is 20–60 mg/kg/day (0.7–2.0 mmol/kg per day) in infants and preschool children, which should be adjusted according to the improvements of rickets, growth, alkaline phosphatase and parathyroid hormone levels [19]. Doses above 80 mg/kg per day should be avoided to prevent gastrointestinal discomfort and hyperparathyroidism. In infants diagnosed before they develop bone changes, the goal of the treatment is to prevent rickets. Important to note, serum phosphate levels increase rapidly after oral intake but return to baseline levels within 1.5 h. Therefore, phosphorus should be administered at least four times a day. Especially in infants and young children, a nighttime dose may be required to achieve satisfactory results. Liquid formulations may improve adherence and allow for more precise dosing in young children. Powders and crushed tablets may also be employed. Powders/tablets can be dissolved in water and the child may drink the solution at intervals during the day. It is important not to administer the phosphate preparation with dairy products, since their calcium content interferes with intestinal phosphate absorption. In general, phosphorus absorption is slower in capsule or tablet formulations than in liquid ones. Therefore, when possible, it is better to use the former. The recommended starting dose of calcitriol and alfacalcidiol is 20–30 ng/kg body weight and 30–50 ng/kg body weight daily respectively. Alternatively, treatment may be started empirically at 0.5 μg daily of calcitriol or 1 μg daily of alfacalcidiol in patients aged above 12 months and adjusted on the basis of clinical and biochemical response. In addition, supplementation with native vitamin D is recommended in case of vitamin D deficiency [19].

Monitoring and Dose Adjustments During Conventional Treatment

The primary goals of treatment are to correct or minimize rickets/osteomalacia, as assessed by clinical, biochemical and radiological findings. Serum alkaline phosphatase activity is a useful surrogate marker for bone healing. With adequate treatment, serum levels of alkaline phosphatase decrease, reaching normal or slightly elevated levels. A common misconception is that successful conventional treatment requires normalization of serum phosphate concentration, which is not a practical goal in these patients, since this could only be reached by excessive phosphate doses and paying the price of severe side effects like nephrocalcinosis and secondary hyperparathyroidism. Therefore, important measures of therapeutic efficacy include enhanced growth velocity, improvement in lower extremity bowing and associated abnormalities, and radiological evidence of epiphyseal healing.

Children should be seen every 2–4 months to monitor growth, serum concentrations of calcium, phosphate, alkaline phosphatase activity, creatinine, PTH and urinary calcium excretion. A random "spot" urine collection can be used to monitor urinary calcium excretion. The goal is to maintain a spot calcium/creatinine ratio < 0.3 mg/mg. Renal ultrasound should be performed at yearly intervals to detect nephrocalcinosis. The etiology of nephrocalcinosis, shown by kidney

biopsies to be composed of calcium phosphate precipitates, was thought to be due either to hypercalciuria, hyperphosphaturia, hyperoxaluria, hyperparathyroidism or any combination of these [19, 83–85]. However, the reported prevalence of nephrocalcinosis in XLH patients ranges between 17 and 80% and is clearly related to the dose of phosphate medication [18, 86, 87]. In addition, other soft-tissue calcifications, e.g. ocular, myocardial, and aortic valve calcifications, have been reported in XLH patients with persistent hyperparathyroidism and/or high dose treatment [34, 88]. Therefore, the calcitriol doses should be adjusted according to the serum levels of PTH and urinary calcium excretion. A high PTH level requires an increase in the calcitriol dose and/or a decrease of the phosphate dose.

The main side effect of an excessive dose of calcitriol is the development of hypercalciuria. In the presence of hypercalciuria the calcitriol dose should be reduced or thiazide diuretics added. The latter not only reduces urinary calcium excretion but also raises the TmP/GFR, most likely secondary to some degree of volume contraction.

Calcitriol and phosphate treatment increases FGF23 levels, thereby further stimulating urinary phosphate excretion [34]. Therefore, high dose treatment should not only be avoided to prevent hypercalciuria and nephrocalcinosis but also to prevent a vicious circle of therapy driven phosphate wasting. When secondary hyperparathyroidism cannot be adequately controlled by calcitriol treatment, i.e. persistent hypercalcemia and/or hypercalciuria, autonomous (tertiary) hyperparathyroidism can occur, necessitating surgical intervention.

It is often necessary to increase dosages of phosphate and calcitriol during the pubertal growth spurt as this may result in greater mineral demands and worsening of bowing defects so that a transient increase in dosage can be advantageous. Therapy with phosphate and calcitriol is maintained as long as the growth plates are open.

Burosumab

Efficacy and Safety

The efficacy and safety of burosumab has been investigated in three clinical trials in children with XLH [46, 89, 90]. In two open-label uncontrolled trials in a total of 65 children aged 1–12 years with severe XLH treated for 12–16 months, burosumab resulted in normalized TmP/GFR, near normal serum phosphate levels, increased $1,25(OH)_2$ vitamin D levels and significantly improved radiological rickets, bone pain and functional abilities [46, 91]. A head-on comparison between burosumab and conventional treatment was performed in an open-label phase 3 trial including 61 children aged 1–12 over 64 weeks [90]. After 40 weeks children treated with burosumab had significantly greater improvement in rickets severity scores than patients on conventional therapy (Fig. 38.6). Biochemical parameters such as serum alkaline phosphatase and serum phosphate levels were also rapidly normalized during burosumab treatment. Two-weekly dosing was superior to four-weekly dosing with respect to normalization of serum levels of phosphate and radiological improvement of rickets. The most common adverse reactions observed with burosumab were injection site reactions, headache and pain in the extremities.

Two open-label, uncontrolled trials and one randomized, double blind, placebo-controlled studies (including a total of 176 patients) have investigated burosumab in adult XLH patients, the majority of whom presented with skeletal pain associated with XLH and/or osteomalacia [91–95]. Four-weekly doses of burosumab given for 4–12 months yielded the following outcomes: significantly increased TmP/GFR and consequently raised serum levels of phosphate into the lower normal range and increased $1,25(OH)_2$ vitamin D levels; healed osteomalacia, and accelerated healing of active fractures and pseudofractures; and significantly reduced stiffness. It is important to note that conventional treatment with oral phosphate and active vitamin D metabolites should be stopped at least one week before the start of burosumab therapy for wash-out and to prove that fasting serum phosphate levels are below the normal reference for age.

Monitoring and Dose Adjustments During Burosumab Treatment

Burosumab should be started at a dose of 0.8 mg/kg body weight, given every 2 weeks subcutane-

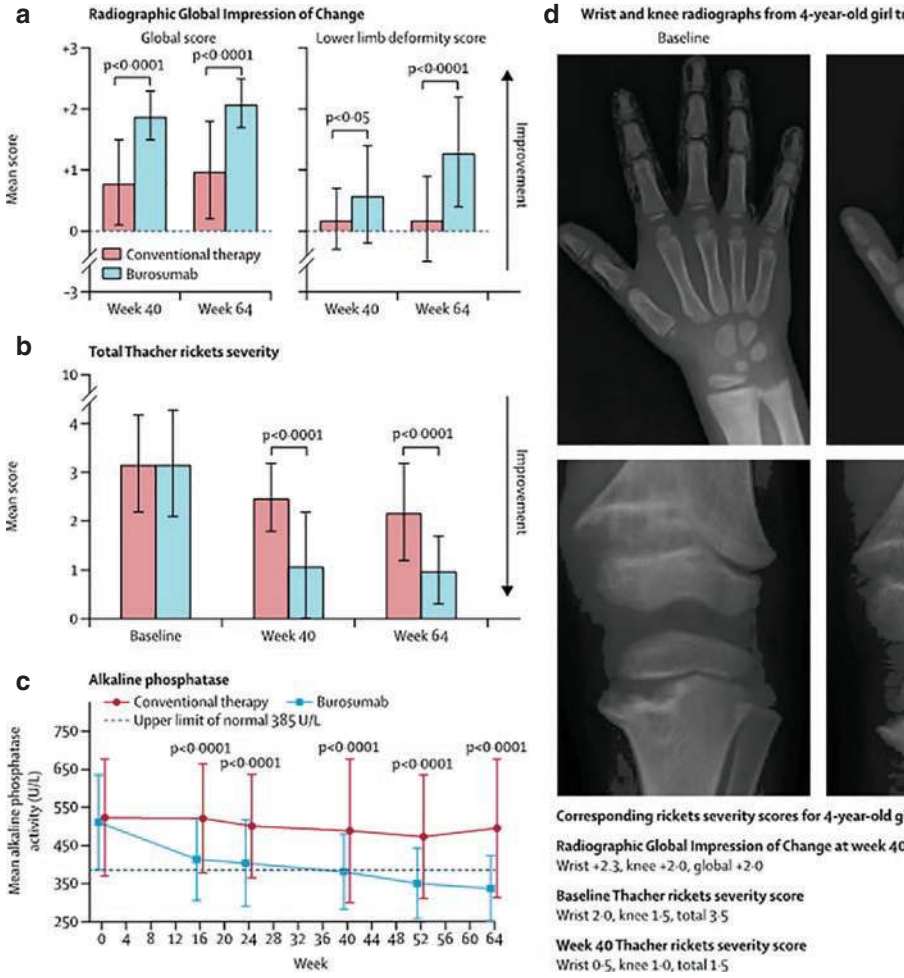

Fig. 38.6 Improvements in rickets severity in children on burosumab versus conventional therapy with phosphate and active vitamin D metabolites. Data in panels **a**, **b**, and **c** are reported as mean (SD). p values are based on the comparison between treatment groups in the least squares mean change from baseline, using the ANCOVA model for the week 40 Radiographic Global Impression of Change global score and week 40 Thacher rickets severity score assessments, and the generalized estimating equation model for alkaline phosphatase assessments, lower limb deformity assessments, and week 64 rickets assessments. The upper limit of normal for alkaline phosphatase varies by age and sex: girls aged 1–4 years 317 U/L, 4–7 years 297 U/L, 7–10 years 325 U/L, and 10–15 years 300 U/L; boys aged 1–4 years 383 U/L, 4–7 years 345 U/L, 7–10 years 309 U/L, and 10–15 years 385 U/L. These ranges were provided by Covance laboratories. Radiographs in panel **d** show improvement in rickets with burosumab in a 4-year-old girl who previously received conventional therapy for approximately 26 months (Used with permission of Elsevier from Imel et al. [90])

ously. Burosumab should be titrated in 0.4 mg/kg increments to raise fasting serum phosphate levels to the lower end of the normal reference range for age, to a maximum dosage of 2 mg/kg body weight (maximum dose 90 mg).

The half-life of burosumab is approximately 19 days and the peak serum concentration of burosumab occurs at 7–11 days after injection. Therefore, it is recommended to monitor fasting serum phosphate levels during the titration period

between injections, ideally 7–11 days after last injection in order to detect hyperphosphatemia. After achievement of a steady-state, which can be assumed after 3 months of a stable dosage, fasting serum phosphate levels should be assessed directly before injections in order to detect hypophosphatemia. In some patients burosumab might initially improve TmP/GFR while serum level of phosphate is still below the normal range owing to the high demand for phosphate of the bone. Therefore, TmP/GFR should be monitored together with fasting serum phosphate levels as a measure of drug efficacy. Serum levels of $1,25(OH)_2$ vitamin D might increase under burosumab therapy and should be monitored together with urinary calcium excretion.

Burosumab should be withdrawn if fasting serum phosphate level is above the upper range of normal. Burosumab can be restarted at approximately half of the previous dose when serum phosphate concentration is back within the normal range. Important to note, burosumab must not be given in conjunction with conventional treatment, when fasting phosphate levels are within the age-related normal range before initiation of treatment, or in the presence of severe renal impairment.

Adjunctive Therapies

Growth Hormone

Adult height is reduced in up to 60% of XLH patients and burosumab did not substantially improve longitudinal growth in pediatric XLH patients in clinical trials [19]. Although an impairment of the somatotropic hormone axis is not the primary cause of short stature in XLH patients, the physiological antiphosphaturic effect of growth hormone (GH) by stimulation of phosphate retention may be a useful adjunct to conventional treatment in improving growth in poorly growing XLH patients. Several mostly uncontrolled studies and a placebo-controlled randomized trial have documented sustained increases in age-standardized height during treatment periods of up to 3 years, and prepubertal patients responded better to GH than pubertal patients [96–102]. In a randomized trial on 3-year rhGH treatment in severely short prepubertal XLH patients, standardized height increased in the rhGH group by 1.1 SD scores with no change in body disproportion, whereas no significant change in standardized height was noted in controls [102]. In line with previous uncontrolled studies, a transient rise in TmP/GFR, and consequently of serum phosphate concentrations, was noted during the first 6 months of GH treatment but not in controls. Importantly, the degree of leg bowing tended to be higher in GH treated patients. Long-term follow-up of the same study failed to show significant benefits on the adult height, most likely due to the low number of patients followed up to adult height. In another study mean final height was significantly increased compared to non-randomized controls [97, 103]. Therefore, administration of rhGH should be considered in XLH patients with persistent short stature despite adequate metabolic control [19].

Calcimimetics

The calcimimetic compound cinacalcet reduces PTH levels in XLH patients, leading to increases in TmP/GFR and serum phosphate [104, 105]. The cinacalcet-induced decrease in serum PTH was accompanied by a slight decrease in serum-ionized calcium concentrations and was not associated with clinical symptoms. Similarly, Geller et al. proved the efficacy of cinacalcet in increasing serum phosphate levels in patients with TIO [106]. It has been suggested that long-term adjunctive treatment of XLH patients with cinacalcet may allow for lower doses of phosphate and calcitriol and thus may minimize the risk of secondary hyperparathyroidism, hypercalcemia, hypercalciuria and nephrocalcinosis caused by high-dose phosphate and calcitriol treatment [19]. However, randomized controlled trials on the long-term efficacy and safety of this drug in XLH patients are lacking.

Surgical Management

Many patients require surgical corrections of severe bowing, regardless of adequate medical treatment [19, 107]. In general, it is recommended that patients presenting with persisting

deformity despite optimized medical treatment and/or the presence of symptoms interfering with function should be considered for surgical treatment [19, 107–109]. The age of the child should be considered as an important factor in the decision-making process. Corrective osteotomies are not usually performed in children aged less than 6 years, as medical therapy often improves bone deformities in this age group. Newer, less-invasive approaches include epiphysiodesis, which induces differential corrective growth of the growth plate. Guided growth techniques depend on the remaining growth potential of the child and must therefore be carried out at least 2–3 years before skeletal maturity (age 14 in girls and age 16 in boys). By contrast, the complications associated with osteotomy reduce when the surgery is performed later in childhood or after skeletal maturity. Calcitriol medication should be adjusted during times of immobilization to avoid hypercalcemic episodes [19].

Dental Care

Children and adult patients with XLH might present with spontaneous endodontic infections on apparently intact teeth [19, 30]. Dental abscesses can develop on deciduous as well as on permanent teeth. The endodontic infection might be asymptomatic for months or years or it might evolve into dental abscesses, causing pain and swelling. Dental complications in patients with XLH are secondary to poorly mineralized dentin. On dental radiographs, the pulp chambers of deciduous or permanent teeth are larger than usual with long pulp horns extending to the dentino-enamel junction. In addition, the frequency and severity of periodontitis is increased in adult patients with XLH and can lead to tooth loss.

Conventional treatment improves dentin mineralization, reduces the number of dental abscesses, and decreases the frequency and severity of periodontitis [110, 111]. The effect of burosumab on dental health in XLH patients is currently unknown.

In children, in addition to standard preventative care, dental visits every 6 months are recommended and sealing of pits and fissures with flowable resin composite on both temporary and permanent teeth should be considered, as soon and as frequently as required. In adults, twice-yearly visits are recommended to perform conventional supportive periodontal therapy. Visits should include a thorough clinical investigation searching for pulp necrosis (color changes, fistula, swelling, abscess, cellulitis or pain), and performance of retrocoronal and/or periapical radiographs or orthopantomogram to search for enlarged pulp chambers and periapical bone loss depending on findings from a clinical examination. Finally, optimized conventional medical treatment of XLH is recommended before initiation of orthodontic treatment [19].

Expected Outcomes

Children with XLH have normal length at birth and show growth retardation, hypophosphatemia and rickets during the first year of life [112, 113]. With conventional treatment, growth and skeletal deformities generally improve. Height velocity commonly increases during the first year of treatment. Leg deformities may correct spontaneously obviating the need for surgery, although this is not always the case. Despite general growth improvement during treatment, correction is limited and adult height is often compromised. Median adult height in XLH patients on calcitriol and phosphate supplements (median age at start 2.3 years) published during the last two decades amounted to −2.3 SDS (range −2.7 to −1.2 SDS) [20, 22, 24, 103, 112–115]. However, growth outcome is significantly better if treatment is initiated early (<1 year). In three studies the mean standardized height after average treatment periods of 10 years was substantially higher in XLH patients with early compared to late treatment (−0.7 SDS versus −2.0 SDS, $p < 0.01$; −1.3 SDS versus −2.0 SDS, $p = 0.06$; Fig. 38.7; −0.7 SDS versus −2.0 SDS, $p < 0.01$) [22–24]. However, even early treatment does not completely normalize skeletal development, and the main effect of early treatment was the prevention of a severe height deficit during early childhood.

A recent North American cross-sectional study in 232 adult XLH patients treated with conventional therapy during childhood and adoles-

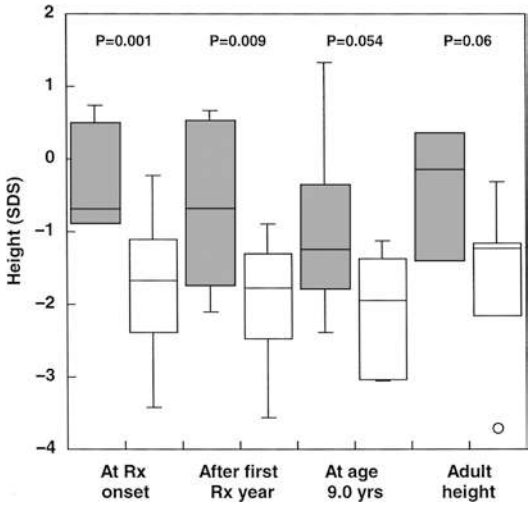

Fig. 38.7 Height z-scores in XLH patients started on calcitriol and phosphate treatment within the first 12 months of life 1 (■) and after the age of 12 months (□) at treatment onset, at the end of first treatment year, at age 9 years, and at final height (adult height or predicted adult height). The bottom of each box indicates the 25th, the cross line the 50th, and the top the 75th percentile; the bottom and top lines indicate the minimum and maximum values. P values refer to the difference between groups. *Rx* Treatment (Used with permission of Makitie et al. [23])

cence revealed a high prevalence of short stature (80%), bone or joint pain/stiffness (97%), and history of pseudofractures (44%) [116]. In addition, osteophytes, enthesopathy and spinal stenosis were reported in 46%, 27%, and 19% of patients, respectively. Similar results were reported in several European studies [25, 117, 118]. Reduced quality of life was shown in 84% of adult patients and associated with age and presence of structural lesions such as enthesopathy [25].

Zivicnjak et al reported on age-related stature and linear body dimensions in 76 children with XLH [18]. Despite calcitriol and phosphate treatment XLH patients showed progressive stunting and body disproportion during childhood, which was mainly due to diminished growth in the legs; growth of the trunk was less affected (Fig. 38.8). This resulted in an ever-increasing sitting height index (i.e. ratio between sitting height to stature).

Treatment with burosumab results in substantial improvement of ricketic bone lesions and degree of leg deformities but only minor changes in standardized height in children aged 1–12 years treated for up to 16 months. The observed limited growth improvement may be due to rather short observation periods and/or the primary osteoblast defect in XLH. Long-term outcome data on the impact of burosumab on linear growth and final height, dental health and rare complications such as hearing loss are urgently required, and multinational patient registries have been initiated to address this issue.

Treatment of Other Forms of Hypophosphatemic Rickets

Limited experience is available regarding the treatment of other forms of hypophosphatemic rickets. In general, hypophosphatemic conditions associated with elevated FGF23 and low/inappropriately low $1,25(OH)_2D$ levels, i.e. **ADHR and ARHR** are treated similarly as XLH. However, burosumab is currently not licensed for treatment of these FGF23 driven diseases.

In patients with **FD/MAS**, treatment with bisphosphonates results in decreased serum FGF23 levels and improves TmP/GFR [18, 119]. Treatment with bisphosphonates in combination with cabergoline, a synthetic ergot alkaloid which acts as a long-acting D2-selective dopamine agonist, successfully arrested both dysplastic bone growth and endocrine malfunction in a female patient with FD/MAS and severe facial involvement [119, 120]. This approach might be a suitable option in order to circumvent surgical interventions that might be of particular risk in patients suffering from polyostotic FD involving the skull base.

In patients with **TIO**, tumor removal usually results in a rapid clinical improvement but tumors might recur. Before surgery, patients are treated similarly to XLH patients. Cinacalcet was successfully used in TIO patients resulting in increased TmP/GFR and serum phosphate concentrations, thereby allowing the use of significantly lower doses of phosphate and calcitriol before surgery [106]. If available, patients may

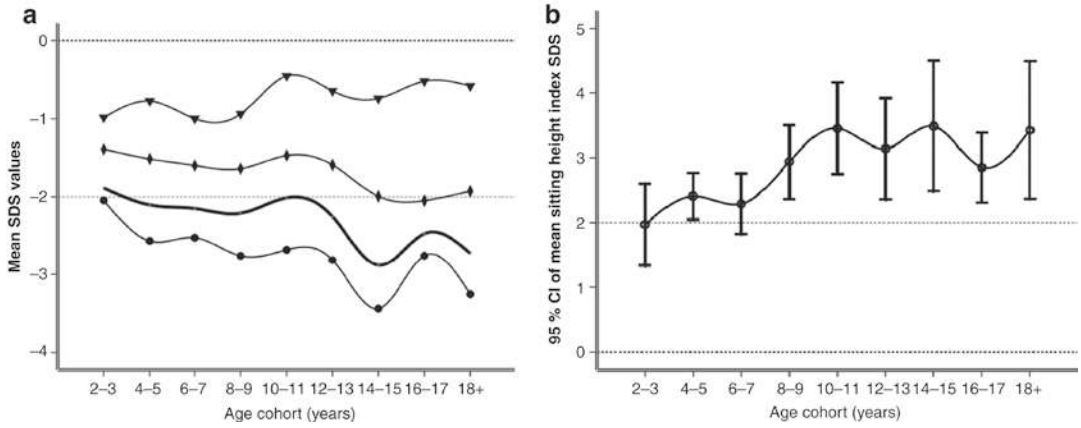

Fig. 38.8 Stature, sitting height, and arm and leg length (**a**) as well as the sitting height index (**b**) as a function of age in 76 children with XLH on conventional treatment. To assess age-related changes in body dimensions, all measurements were grouped according to age at the time of examination. Each age cohort comprised eight up to 26 children with 16 up to 38 measurements. A spline function was used to fit age-related changes. Unmarked solid line Stature, solid line with filled circles leg length, solid line with filled rhombuses arm length, solid line with filled inverted triangles sitting height. The 95% CI is indicated for sitting height index [18]

also be effectively treated by burosumab, which should be the preferred treatment option if tumor resection is not possible [68].

Treatment of **HHRH** usually requires the administration of phosphate salts alone. In fact, treatment with calcitriol may lead to nephrocalcinosis and chronic renal failure. The treatment goal is to provide sufficient phosphorus to improve mineralization of osteoid. In addition, oral phosphate supplementation is supposed to normalize decreased FGF23 levels and thereby suppress elevated circulating $1,25(OH)_2D$ level and consequently reduce intestinal calcium resorption and hypercalciuria [19, 121].

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Renal Tubular Acidosis

39

R. Todd Alexander and Detlef Bockenhauer

Introduction

Plasma pH is maintained in a very tight range, from 7.35 to 7.45, via multiple regulatory mechanisms including buffering, altered respiration and ultimately fine regulation by the kidney. This tight control of plasma pH is essential for many physiological functions including but not limited to: proper folding and functioning of proteins, neural transmission and cardiac contractility. Typically, perturbations in plasma pH are caused by alterations in respiration or the presence of exogenous acids, which overwhelm the remarkable underlying capacity of the body to regulate plasma pH. However, less commonly, excess loss of bicarbonate from the gut or kidney, or the failure of the renal tubule to excrete acid is at fault. It is these less common causes of metabolic acidosis that are the subject of this chapter.

In order to understand the molecular pathogenesis of renal tubular acidosis, it is a prerequisite to know both the role of the kidney in the maintenance and regulation of plasma pH and its interrelation to other processes that participate in acid-base homeostasis. Proteins and phosphate mediate buffering in the cell. In the extracellular compartment bicarbonate is the predominant buffer preventing decreases in pH in response to an acid load. This is followed by an increase in respiratory rate and depth (respiratory compensation). Finally, the kidneys respond by increasing bicarbonate reabsorption, a process that leads to increased ammoniagenesis and ultimately facilitates increased acid secretion from the distal nephron. A detailed discussion of buffering and respiratory control of acid-base status is beyond the scope of this chapter. However, in order to inform the discussion of the pathophysiology of altered tubular handling of acid, we will begin by detailing the current understanding of how the kidney participates in acid-base homeostasis.

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Physiology of Renal Acid-Base Handling

Bicarbonate Reabsorption

A significant amount of bicarbonate is filtered and consequently reabsorbed daily in order to preserve extracellular buffering capacity [1, 2]. Assuming a GFR of 120 mL/min in the average adult male and a plasma bicarbonate concentration of 24 mM, this amounts to approximately

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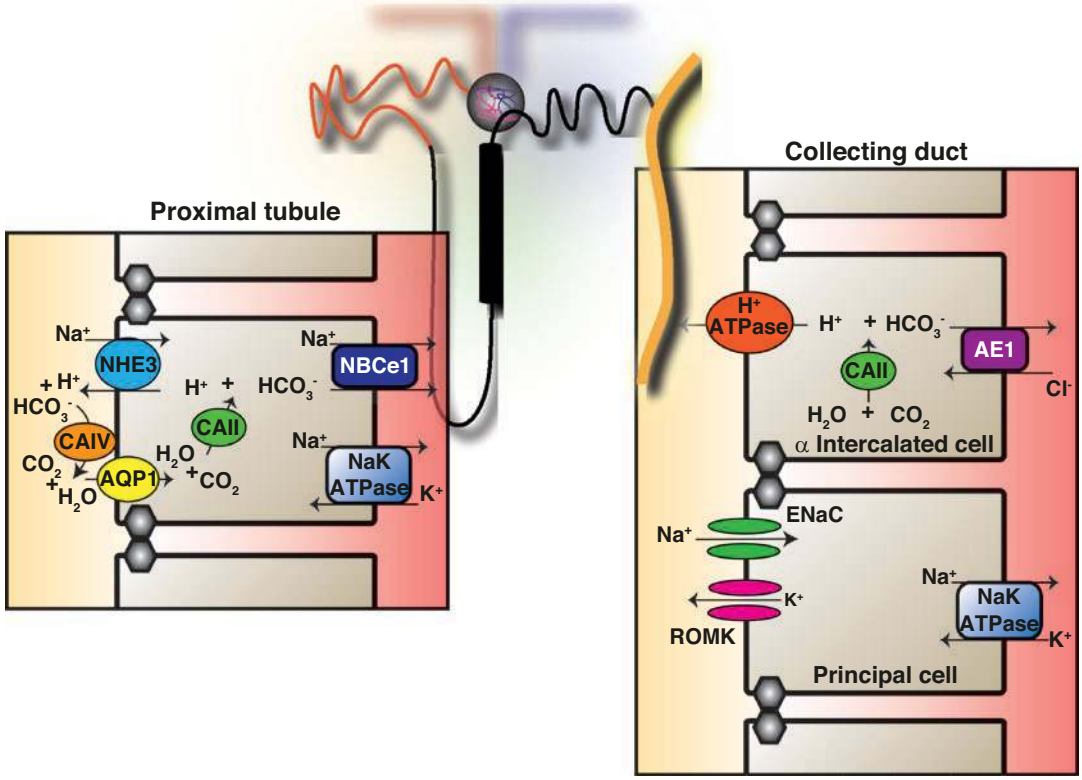

Fig. 39.1 Renal handling of bicarbonate and acid. The majority of filtered bicarbonate (HCO_3^-) is reabsorbed from the proximal tubule, after enzymatic (carbonic anhydrase IV, CAIV) conversion to water and carbon dioxide (CO_2). This is facilitated by proton excretion through the sodium proton exchanger isoform 3 (NHE3). In the cytosol carbonic anhydrase II (CAII), converts the water and CO_2 back into a proton, which is recycled through NHE3, and HCO_3^-, which is effluxed back into the blood through the sodium bicarbonate cotransporter NBCe1. In the collecting duct, H^+ is secreted from the α-intercalated cell via the apically expressed H^+ATPase. The proton is generated via the catalysis, by CAII, of water and CO_2. This also generates HCO_3^-, which is exchanged for Cl^- by the basolateral anion exchanger (AE1). In principle, cell sodium is reabsorbed through the epithelial sodium channel (ENaC). As this is an electrogenic process, either potassium (K^+) excretion trough ROMK or proton secretion via the H^+ATPase is required to maintain a permissive potential difference across the luminal membrane

4150 mmoles of bicarbonate that is filtered daily. Under normal circumstances the urine is free of bicarbonate, which means that all filtered bicarbonate is reabsorbed by the nephron [3] (Fig. 39.1). The vast majority is reabsorbed from the proximal tubule [4, 5]. Animal experiments provide evidence for bicarbonate reabsorption also in the thick ascending limb and the collecting duct, albeit to a much lesser degree [6–11]. The relative amount of bicarbonate absorbed from these sites varies depending on physiological status but in normal physiologic conditions is never more than a fraction of the proximal tubule's contribution to this process [3].

Proximal Tubule

There is no known bicarbonate transporter in the apical membrane of the proximal tubule. Consequently filtered bicarbonate is converted to water and carbon dioxide by a brush border membrane carbonic anhydrase [12]. Carbonic anhydrase IV and likely also the transmembrane isoform carbonic anhydrase XIV mediate this function [13–15]. The water channel, aquaporin-1, permits the rapid influx of water into the proximal tubular epithelial cell [16–19] and may also facilitate the permeation of carbon dioxide [20–22], in addition to diffusion across the membrane down its concentration gradient [23]. Cytoplasmic

carbonic anhydrase II, which interacts directly with aquaporin-1 enhancing water flux, then converts intracellular water and carbon dioxide back into bicarbonate and a proton (through the intermediate carbonic acid) [24–29]. The proton is exchanged for a sodium ion, predominantly by the apical sodium proton exchanger isoform 3 (NHE3) [30–33], while the bicarbonate is extruded back into the circulation across the basolateral membrane via the electrogenic sodium dependent bicarbonate transporter (NBCe1) [34, 35]. Yet, while mutations in *SLC4A4* (encoding NBCe1) are associated with a severe form of inherited pRTA, mutations in *SLC9A3* (encoding NHE3) cause congenital sodium diarrhea with only mild renal bicarbonate wasting [36–39]. This likely reflects redundancy provided by other NHE isoforms, such as NHE8 [40].

There is also a proton pump, H^+ ATPase which effluxes protons into the lumen of the proximal tubule [41, 42], although its contribution to proximal tubular bicarbonate reabsorption is minimal relative to that of NHE3 [32]. The majority of bicarbonate transport occurs in the first part of the proximal tubule [43]. This results in a slightly lumen negative transepithelial potential difference which is believed to be responsible for driving paracellular chloride reabsorption from the latter part of the proximal tubule [44, 45].

Thick Ascending Limb of Henle's Loop

NHE3, the epithelial sodium proton exchanger, is also expressed in the apical membrane of the thick ascending limb of Henle's loop (TAL) [46]. Consequently, bicarbonate reabsorption from this segment is felt to occur via a similar process as in the proximal tubule [47, 48]. The absence of aquaporin-1 from this segment may explain the decreased efficiency of bicarbonate reabsorption from the thick ascending limb relative to the proximal tubule [16]. Efflux across the basolateral membrane is mediated by the anion exchanger isoform 2 [49–51].

Collecting Duct

Any remaining bicarbonate reaching the collecting duct is reabsorbed by an analogous process [7, 8]. Efflux of a proton into the lumen permits the titration of bicarbonate back into carbonic acid and then carbon dioxide and water. Proton efflux is achieved via a luminally situated V-type H^+ ATPase, also called a proton pump [52]. Apically expressed proton pumps are only found in α-intercalated cells [41, 53, 54], whose apical membranes are largely impermeable to water, although carbon dioxide could diffuse into the epithelial cells of the collecting duct including α-intercalated cells. Intracellular carbon dioxide is hydrated, generating a proton and bicarbonate. This is achieved by cytosolic carbonic anhydrase II [54, 55]. The *de novo* generated bicarbonate is then extruded into the circulation via the basolateral anion-exchanger (AE1) [56]. This is the same mechanism permitting the trapping of ammonium in the distal nephron described below.

Acid Secretion

Acid secretion is achieved in the collecting duct via the mechanism described above for the titration of bicarbonate. The luminal H^+ ATPase in α-intercalated cells secretes a proton that is trapped by converting ammonia into ammonium, or by titrating another acid [57, 58]. These so-called titratable acids include: bicarbonate, phosphate and sulphate. The proton is generated from cytosolic carbon dioxide and water via the action of carbonic anhydrase II [24, 55]. The bicarbonate generated by this process is extruded back into the circulation in exchange for a chloride via AE1 [59, 60]. Moreover, there is a potassium-proton pump in the apical membrane of collecting duct α-intercalated cells, which also participates in acid secretion, although to a lesser extent [61–63].

Beta-intercalated cells exhibit essentially reverse polarity to α-intercalated cells and thus have an opposite function, as they can generate and secrete bicarbonate into the urine and a proton back into the circulation [8, 41, 53]. They contain a chloride bicarbonate exchanger in their apical membrane, Pendrin, and a proton pump in the basolateral membrane [54, 64–66]. They also contain a cytosolic carbonic anhydrase, CAII [60, 64]. Importantly, recent evidence has implicated

this cell type in sodium and chloride reabsorption [67, 68]. ß-intercalated cells nearly completely lack the expression of the Na^+/K^+ ATPase, instead the basolateral H^+ ATPase appears to drive transport across this cell type [69]. This facilitative role of the H^+ ATPase may contribute to the polyuria and volume contraction often observed in patients with distal renal tubular acidosis due to mutations in this pump [70, 71].

Ammonia Genesis and Recycling

Ammonia (NH_3) is the major urinary buffer and consequently its protonation to ammonium (NH_4^+) is essential for the efficient urinary excretion of acid [57, 72].

Ammonia Genesis

Ammonium is produced in the proximal tubule from glutamine and then secreted into the lumen [73]. Free circulating glutamine in the plasma enters the proximal tubular epithelial cell across the basolateral membrane via the LAT2 amino acid transporter [74]. Glutamine in the cytosol then enters the mitochondria via an electroneutral uniporter [75] where it is first converted to glutamate by glutaminase, then to ammonium by a glutamate dehydrogenase [76]. These enzyme-catalyzed reactions produce bicarbonate, which is returned to the circulation via the sodium-dependent bicarbonate transporter NBCe1. Of note, hyperkalemia inhibits the generation of ammonia [77].

Ammonia Recycling

Ammonium has been thought to be secreted into the proximal tubule by substituting for sodium on the apical sodium proton exchanger NHE3 [78–81]. However, a study employing a proximal tubular *Nhe3* knockout mouse argues against this role for the exchanger [39]. Alternatively, another EIPA (Ethylisopropyl-amiloride) sensitive sodium proton exchanger that is expressed in the apical membrane of the proximal tubule such as NHE8 may play this role [32, 82, 83], or free ammonia may diffuse into the lumen where proton excretion may trap it by conversion to ammonium.

Luminal ammonium then travels down the thin descending limb. In TAL, a nephron segment largely impermeant to ammonia, luminal ammonium is absorbed into the tubular epithelial cell across the apical membrane via the sodium potassium chloride co-transporter NKCC2 [84]. Ammonium is approximately the size of potassium and consequently can substitute for it. Thus hyperkalemia can inhibit ammonium excretion, as increased luminal concentrations of potassium will compete with ammonium for the transport site on NKCC2, preventing its influx into the tubular epithelial cell [85, 86]. Once transported across the luminal membrane of TAL cells, it diffuses across the basolateral membrane into the interstitium in the form of ammonia. Accumulation in the interstitium occurs as the apical membrane is impermeable to ammonia, preventing a back leak of ammonia into the tubular lumen [87].

This medullary interstitial accumulation of ammonia is essential to the efficient excretion of acid as it provides a concentration difference, from interstitium to lumen of the collecting duct for ammonia diffusion into the lumen. Consequently, ammonia diffuses into the lumen of the collecting duct where it is trapped via the excretion of protons as ammonium [88, 89]. The collecting duct is relatively impermeable to ammonium [90]. There is evidence implicating RhCG, a rhesus glycoprotein homologue, in this process as it forms a pore in the apical and basolateral membranes of the collecting duct epithelial cells permitting the passage of ammonia, but not ammonium [91–94]. Proton excretion is achieved as described above via apically expressed ATPases, predominantly the V-type H^+ ATPase and to a lesser extent the H^+K^+-ATPase. Ultimately, ammonium generation in the proximal tubule, recycling in the loop of Henle and finally trapping in the collecting duct permit significant and efficient proton excretion in the urine (Fig. 39.2).

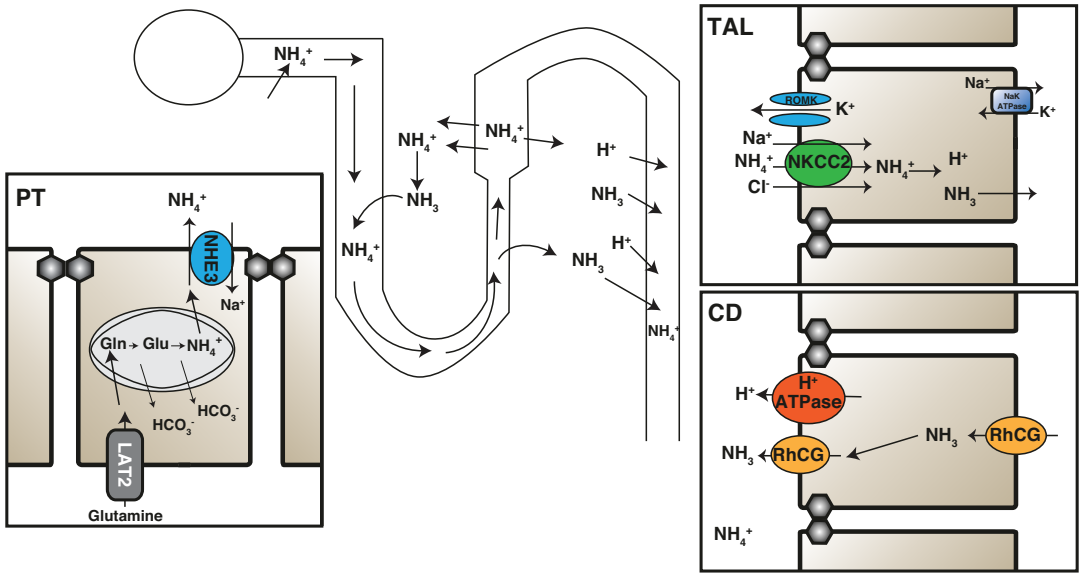

Fig. 39.2 Ammonia generation and recycling. Ammonium is generated in the proximal tubule (PT) and secreted into the lumen in exchange for sodium (Na^+) by the sodium proton exchanger isoform 3 (NHE3). It then travels down the nephron to the thick ascending limb (TAL) where it is reabsorbed by the sodium potassium chloride cotransporter, (NKCC2). Ammonia then diffuses into the interstitium where it either undergoes recycling in the loop or permeates the collecting duct through RhCG and is trapped by a proton secreted through the H^+-ATPase

Pathophysiology of Renal-Acid Base Handling

Definitions

The principles of renal tubular acid-base handling discussed above provide the foundation of the classification of renal tubular acidosis. Defects in bicarbonate reabsorption, which occurs predominantly in the proximal nephron, are referred to as proximal renal tubular acidosis, or pRTA [95]. Historically, pRTA has also been classified as type II RTA. Defects in distal tubular proton secretion are referred to as distal renal tubular acidosis, dRTA [95]. This type of RTA has also been referred to as Type I. Clinical syndromes with features of both proximal and distal renal tubular acidosis, *i.e.* a failure to reclaim both filtered bicarbonate and excrete acid in the urine, are known as mixed renal tubular acidosis or type III RTA. All these types of RTA are typically accompanied by normal or low plasma potassium levels, and are distinguished by the presence of bicarbonaturia and/or the failure to acidify the urine in the presence of an acid load. Due to the molecular link between sodium and acid-base homeostasis, RTA can also occur as a secondary consequence of impaired sodium reabsorption in the collecting duct, as sodium uptake by ENaC provides a favorable electrical gradient for proton secretion. The salt-wasting tubulopathy related to impaired ENaC activity is also referred to as aldosterone insufficient or resistant RTA, or type IV RTA. Since ENaC activity also facilitates potassium secretion, this type of RTA is distinguished from the other types by being associated with hyperkalemia. All forms of RTA are associated with a non-anion gap acidosis, consistent with loss of bicarbonate. For pRTA, this is obvious, as bicarbonate reabsorption is impaired. In dRTA the mechanism is indirect: bicarbonate is consumed for buffering the protons the distal tubule fails to excrete.

Proximal Renal Tubular Acidosis (Type II RTA)

Clinical Presentations

Isolated proximal renal tubular acidosis is a rare condition, which is almost exclusively due to a single gene defect in the sodium dependent bicarbonate transporter, NBCe1 (*SLC4A4*) [96–98]. This genetic form of pRTA is inherited in an autosomal recessive pattern [99]. Given that the renal isoform of NBCe1 is also expressed in the eye it is not surprising that affected individuals commonly display eye abnormalities, such as band cataracts, glaucoma or band keratopathy [99–101]. As with all untreated renal tubular acidosis, failure to thrive and hypomineralized bones are common at presentation [99]. There are also pancreatic and brain isoforms of NBCe1 and consequently, some patients with mutations affecting these isoforms also have increased circulating amylase levels (without evidence of pancreatitis) and there are reports of associated intellectual impairment [99].

Autosomal dominant pRTA has been described in two separate families. The responsible gene accounting for this syndrome has yet to be determined. The first family identified is Costa Rican. One affected brother presented with short stature, bilateral coloboma and subaortic stenosis, while another brother showed limited clinical symptoms, except metabolic acidosis with a urinary pH < 5.0 as did the affected sibling [102]. They both had evidence of hypomineralized bones. The other family described includes a father and all 4 of his children who have metabolic acidosis with increased bicarbonate excretion following bicarbonate loading. Despite sequencing a number of candidate genes the cause of their disease is yet to be defined [97]. As detailed above, mutations in NHE3 have not been reported to cause pRTA, but instead congenital sodium diarrhea [38]. Interestingly, affected patients have either absent or only mild acidosis. Similarly, mice with proximal tubule-specific knock out of Nhe3, also only have marginally lower serum bicarbonate levels compared to wild type [39]. There are some data suggesting compensatory upregulation of Nhe8 [103].

Other genetic and acquired forms of pRTA usually occur as part of the renal Fanconi syndrome [104], which is characterized by complex proximal tubular dysfunction due to genetic defects or proximal tubular toxicity from a drug, toxin or metabolite. Renal Fanconi syndrome causes are listed and described in Chap. 31.

Diagnosis

Patients with pRTA typically demonstrate a hyperchloremic non-anion gap metabolic acidosis, accompanied by hypokalemia. However, since the ability to acidify urine is preserved, patients with pRTA can lower their urine pH to less than 5.5 when plasma bicarbonate levels are lower than their renal threshold for tubular bicarbonate absorption. This distinguishes pRTA from dRTA. A definitive means of diagnosing pRTA is by assessing renal tubular bicarbonate absorption (or the fractional excretion of bicarbonate) across a range of plasma bicarbonate levels. A fractional excretion of bicarbonate greater than 15% in the context of a metabolic acidosis definitively diagnoses pRTA [104, 105]. Some authors have suggested an even lower cut off level, i.e. 5% [41]. In case of doubt, the diagnosis can be ascertained by assessing tubular bicarbonate handling as an alkali is given to the patient, resulting in a gradual increase in plasma pH [106]. Practically this can be done by measuring the fractional excretion of bicarbonate repeatedly while normalizing plasma bicarbonate levels with increasing doses of alkali (e.g., administration of intravenous bicarbonate at a rate predicted to increase blood bicarbonate by 2 mmol/L/h, until urine pH is >6.8 [103]). A marked increase in urinary bicarbonate excretion normally occurs at a specific serum bicarbonate level. This is called the bicarbonate threshold. A normal bicarbonate threshold is around 22 mmol/L in infants and 25 mmol/L in older children/adults [107]. A bicarbonate threshold less than 20 indicates pRTA. In practical terms, a pRTA patient with a bicarbonate threshold of 16 will stop wasting bicarbonate and be able to acidify the urine to <5.5 at a plasma bicarbonate at or below 16 [108].

In clinical practice, the fractional excretion of bicarbonate is rarely performed, due to technical

considerations especially relevant in children: when urine is exposed to air, CO_2 diffuses out, lowering urinary bicarbonate concentration, while at the same time increasing the pH ($CO_2 + H_2O$ <<<=> $H^+ + HCO_3^-$). Thus, fresh urine should be obtained and ideally under oil to minimize CO_2 loss. This is obviously challenging in non-toilet trained children, unless a catheter is inserted. Similarly, the lab should be alerted to handle these samples urgently and not every biochemical lab will measure urinary bicarbonate. From a practical point of view: isolated pRTA is exceedingly rare and virtually always associated with eye abnormalities. Otherwise, pRTA occurs in the context of a more generalized proximal tubulopathy. Thus, if pRTA is suspected, an eye examination and a screen for renal Fanconi syndrome (glycosuria, phosphaturia, low molecular weight proteinuria, amino- and organic aciduria, see Chap. 31) should be performed and is usually sufficient to establish a diagnosis. If typical abnormalities are present, a genetic diagnosis can be established by sequencing *SLC4A4* [96]. An algorithm for the diagnosis of RTA is presented in Fig. 39.3.

Treatment

For treatment of renal Fanconi syndrome, please see Chap. 31. For isolated pRTA, alkali replacement is the main line of therapy [109, 110]. This is especially important in prepubertal children as persistent metabolic acidosis will impair growth [109]. Given the pathophysiology, with bicarbonate wasting when plasma bicarbonate levels are above the threshold, it is not surprising that even with prodigious quantities (5–15 mEq/kg/day) of bicarbonate supplementation, plasma levels may remain low [104]. This is another distinguishing factor from dRTA, where normal plasma bicarbonate levels can be achieved with doses of usually 2–5 mEq/kg/day [111]. It is recommended to administer smaller doses of alkali replacement frequently, as the larger the dose, the higher the peak in subsequent plasma bicarbonate concentration which, in turn, will increase urinary bicarbonate losses [112]. The use of long-acting alkali replacement formulations has not been reported in pRTA, but should help to maintain more consistent plasma bicarbonate levels, while minimizing the frequency of daily doses.

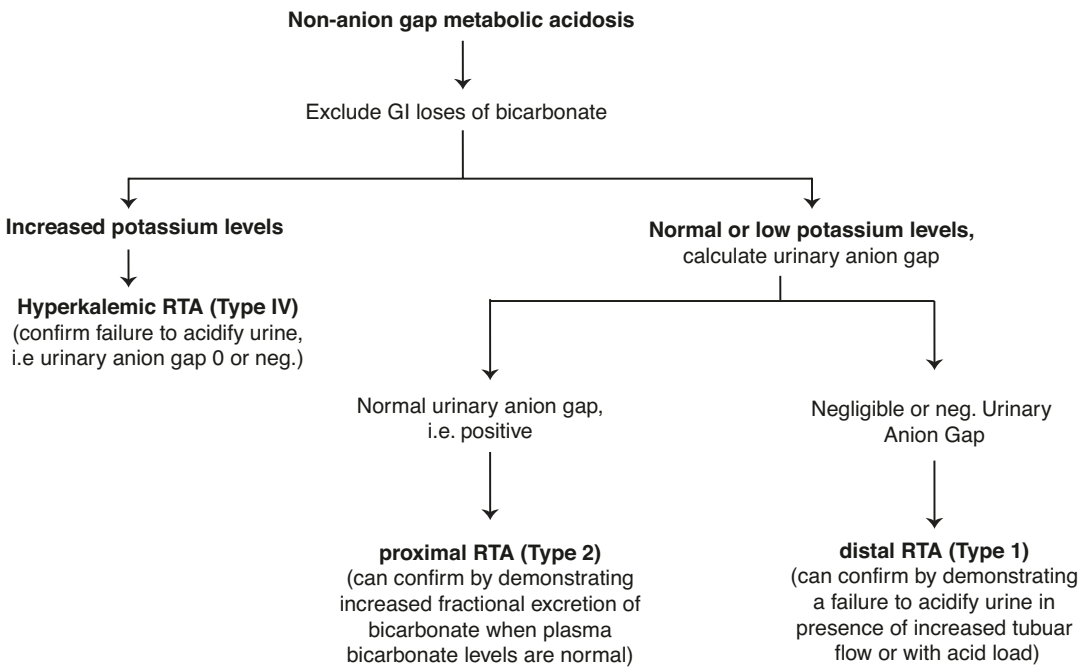

Fig. 39.3 Algorithm for diagnosis of RTA

Prognosis

Due to the extreme rarity of the disease, little information on the long-term outcomes of children with isolated pRTA is available. So far, renal failure has not been reported in contrast to dRTA, and growth can be improved with alkali supplementation [109, 113].

Distal Renal Tubular Acidosis (Type I RTA)

Clinical Presentation

Distal renal tubular acidosis is more common than the proximal form. Biochemically, it also presents as a hyperchloremic non-anion-gap metabolic acidosis, typically in association with hypokalemia, hypercalciuria and nephrocalcinois, although these features are not always present [114]. Some patients will also display renal cysts, although these typically develop later [115].

Mutations in at least 5 different genes have been identified and found to cause dRTA [116]. Moreover, a number of conditions, drugs and toxins can cause dRTA (see Table 39.1), yet this is more commonly seen in adults. In particular drugs targeting the distal nephron, autoimmune diseases and conditions characterized by hypercalciuria can be associated with dRTA [117].

Table 39.1 Causes of distal renal tubular acidosis

Genetic
H^+ATPase, α4 (*ATPV0A4*)
H^+ATPase, ß1 (*ATPV1B1*)
AE1 (*SLC4A1*)
FOXI1 (*FOXI1*)
WDR72 (*WDR72*, tryptophan-aspartate repeat domain 72)
CAII (*CA2*)[a]
Autoimmune
Cryoglobulinemia
Sjorgren syndrome
Thyroiditis
HIV-nephropathy
Chronic active hepatitis
Primary bilary cirrhosis
Polyarthritis nodosa
Hypercalciuria/nephrocalcinosis
Primary hyperparathyroidism
Hyperthyroidism
Medullary sponge kidney
Drug induced
Amphotericin B
Cyclamate
Vanadate
Ifosfamide
Toluene
Mercury
Lithium
Foscarnet
Analgesic nephropathy
Miscellaneous causes
Sickle cell disease
Marfan syndrome
Ehlers-Danlos syndrome
Carnitine palmitoyltransferase deficiency

[a]CAII deficiency causes a combined pRTA and dRTA i.e. type III RTA

Distal RTA with Mutations in AE1 (SLC4A1)

Mutations in the anion exchanger isoform 1, AE1, have been found to cause dRTA [118–120]. The encoding gene, *SLC4A1*, is expressed in both red blood cells as well as in α-intercalated cells of the collecting duct [121]. Some mutations in AE1 cause congenital forms of anemia including hereditary spherocytosis and ovalocytosis [122–126]. These mutations are predominantly found in regions of Southeast Asia with a historically high incidence of *plasmodium falciparum* infections, against which ovalocytosis provides protection [127]. Interestingly, mutations in AE1 that cause blood dyscrasias generally do not result in dRTA and conversely, mutations causing dRTA often do not cause anemia. Most mutations associated with dRTA are missense variants leading to aberrant trafficking (e.g. to the apical membrane) or ER retention. AE1 functions as a dimer, so that these mutations can have a dominant negative effect [127–129]. Since red cells are not polarized, aberrant trafficking is less of a problem. Moreover, red cells express glycophorin and other chaperones responsible for trafficking AE1 to the plasma membrane that are absent in renal epithelial cells. This explains how some mutations cause dRTA and not anemia [130]. In contrast, mutations causing isolated red cell dis-

orders typically affect transport function, suggesting a susceptibility of red cells to AE1 haploinsufficiency [128]. In addition, the isoforms of AE1 expressed in red cells and kidney are different; the latter lacks the first 65 amino acids, so that mutations in this region are not expected to cause kidney disease [131]. In a minority of patients both dRTA and anemia are present [132–136].

Mutations in AE1 were originally reported to only be transmitted in an autosomal dominant fashion [118]. Such families have been reported globally [118–120, 133, 137, 138]. Autosomal dominant mutations can cause complete or incomplete dRTA (note incomplete dRTA is evidence of a failure to acidify urine when challenged with an acid load in an individual *without* metabolic acidosis at baseline) [139]. Typically, patients with autosomal dominant dRTA present in adolescence or even at adult age. In general, biochemical abnormalities are less severe than in patients with recessive disease due to mutations in subunits of the H^+-ATPase, potentially explaining the later presentation [115]. Hypercalciuria, nephrocalcinosis and nephrolithiasis have been associated with this disease [120, 130, 138]. The frequency of these associations appears to increase with patient age.

Later, patients with mutations in AE1 inherited in an autosomal recessive fashion were reported [125, 130, 140–144]. Such recessive mutations typically cause a milder trafficking defect, so that both alleles need to be mutated to cause a clinically relevant problem. Patients who are compound heterozygous for one mutation causing ovalocytosis and one causing dRTA can have both disorders [128].

Distal RTA with Mutations in Proton Pump Subunits a4 (ATP6V0A4) and B1 (ATP6V1B1)

Mutations in at least two of the subunits of the vacuolar H^+-ATPase also cause dRTA [70, 145–149]. This 14-subunit proton pump is expressed in the luminal membrane of the α-intercalated cell and is responsible for the secretion of protons into the collecting duct. Mutations in the a4 (*ATP6V0A4*) and B1 (*ATP6V1B1*) subunit have been reported to cause complete dRTA [149]. These mutations are inherited in an autosomal recessive pattern, although a specific mutation in the B1 subunit (p. Arg394Gln) has been reported recurrently in heterozygous form without an identified mutation on the other allele [111, 150]. Both the a4 and B1 subunits are also expressed in the inner ear, so it is not surprising that they are also associated with sensorineural hearing loss [149]. Nevertheless, some genotype-phenotype correlations exist: mutations in *ATP6V0A4* are associated with more severe dRTA, as assessed by a trend to earlier presentation, increased frequency of nephrocalcinosis and higher prescribed daily alkali dose [111, 115]. Yet, only about a third of patients with mutations in the a4 subunit have documented sensorineural hearing loss, compared to almost 90% in patients with B1 mutations [111]. Moreover, age at diagnosis of hearing loss and prescription of hearing aids or cochlear implants is significantly younger in patients with *ATP6V1B1* mutations compared to *ATP6V0A4*. [146, 149, 151]. The vast majority of patients with mutations in the a4 and B1 subunits have nephrocalcinosis (98% with *ATP6V1B1* and 90% with *ATP6V0A4*), which is typically present already at diagnosis [116].

Distal RTA with Mutations in FOXI1

FOXI1 is a transcription factor expressed in acid secreting epithelia important for the regulation of several genes involved in acid secretion, including *SLC4A1*, *ATP6V1B1* and *ATP6V0A4* [152]. Foxi1 knock out mice had already been reported in 2004 to have dRTA with sensorineural deafness [153]. It took another 14 years until two families with recessive mutations in FOXI1 were identified, suggesting that this is a very rare cause of dRTA [154]. Reported patients also have sensorineural deafness with massive enlargement of the endolymphatic sac [154]. Of note, male Foxi1 knock out mice are infertile, due to insufficient acidification of the luminal fluid in the epididymis with consequent pathologic post-testicular

sperm maturation [153]. Whether male infertility is also a clinical problem in human FOXI1-related dRTA remains to be seen.

Distal RTA with Mutations in WDR72

WDR72 (tryptophan-aspartate repeat domain 72) is a protein presumed to be involved in intracellular vesicle transport that was initially identified as a cause of autosomal recessive amelogenesis imperfecta [155]. In 2018, two families with dRTA were reported with recessive mutations in WDR72 [156]. Subsequently, more families have been reported [157, 158]. Whether some families only exhibit dRTA, others only amelogenesis imperfecta and others both features is not clear. Potentially, the report of only one manifestation may betray the focus of the reporting specialist (dentist versus nephrologist). Interestingly, in one report, reverse phenotyping of patients with WDR72 mutations and a clinical diagnosis of dRTA revealed that all patients also had findings of amelogenesis imperfecta [158]. Interestingly, not all family members with *WDR72*-associated amelogenesis imperfecta have clear evidence of dRTA [157]. This suggests that *WDR72*-associated dRTA is typically milder than the forms associated with the other recessive disease genes.

Candidate Genes

Currently, about 60–80% of families with a clinical diagnosis of inherited dRTA have identifiable pathogenic variants in the known disease-causing genes described above, suggesting that there are more yet unidentified dRTA disease genes [115, 150, 158]. Further, subunits of the vacuolar H^+-ATPase expressed in the kidney, such as C2, G3 and d3 have been suggested as candidates, but thus far, evidence definitively linking them to dRTA is limited [158–160].

Diagnosis

The presence of a hypokalemic, hyperchloremic, non-anion gap metabolic acidosis in the absence of intestinal losses and in the presence of a normal GFR should make one suspect RTA [161, 162]. Notably, substantially reduced GFR (CKD stage >3) can cause a normal anion gap metabolic acidosis due to insufficient nephron mass to excrete sufficient protons to maintain normal plasma pH. This cause of metabolic acidosis due to renal insufficiency is not traditionally labeled as dRTA, and can be easily distinguished by assessment of global renal function and the presence of the other clinical consequences of kidney disease.

Urinary pH will be inappropriately elevated with dRTA. Unfortunately, this finding can also be observed with pRTA when plasma pH is above the bicarbonate threshold. Definitive diagnosis of dRTA is made by demonstrating an inability to acidify the urine. Since ammonium is the major component of acid secretion it should be measured, ideally directly. However, most clinical laboratories do not offer urinary ammonium determination, but excretion can be estimated indirectly, using the **urine anion gap** [163]. This is in analogy to the plasma anion gap, although it would better be termed "cation gap", as it serves to estimate the unmeasured cation ammonium [164]. It is calculated using the formula: $U_{AG} = [Na^+]_U + [K^+]_U - [Cl^-]_U$.

The unmeasured urinary anions sulphate and phosphate are generally constant and at low levels. Moreover bicarbonate is not typically present in urine. Similarly, the urinary excretion of calcium and magnesium is relatively low and constant relative to sodium and potassium. Consequently, the urinary excretion of ammonium (NH_4^+) represents the greatest unmeasured urinary cation, making this equation a useful estimate of ammonium excretion. In the presence of metabolic acidosis there should be significant urinary ammonium excretion and consequently a negative anion gap. An alkaline urine pH in the presence of a negative urinary anion gap is consistent with the diagnosis of pRTA. An anion gap >10 mmol/L reflects the inappropriate absence of ammonium excretion and supports a diagnosis of dRTA [165]. Alternatively (or additionally), the urinary osmolality gap can be used [166].

In clinical practice, in the absence of intestinal losses of bicarbonate, hypokalaemic hyperchloremic metabolic acidosis with elevated urine pH is most likely caused by dRTA. In contrast, generalized proximal tubular dysfunction is consistent with pRTA. It is important to remember though, that approximately a third of patients with dRTA have evidence of proximal tubular dysfunction at presentation, confounding an accurate diagnosis [115]. This is likely related to the acidosis, as proximal dysfunction resolves with resolution of the acidosis. The complete **normalization of plasma bicarbonate levels with alkali supplementation** suggests a diagnosis of dRTA. In contrast, the ongoing bicarbonate wasting present in pRTA usually **precludes complete normalization of plasma bicarbonate levels. Further, a diagnosis of dRTA is confirmed by the later resolution of proximal dysfunction with adequate alkali treatment. The diagnosis can be secured by genetic testing in the vast majority of cases.**

The diagnosis can be challenging in milder (incomplete) forms, as these patients typically have normal plasma biochemistries at baseline. Further **diagnostic procedures to assess distal acidification** can help. Two such procedures are commonly employed to this end. The first is the administration of an acid load typically as ammonium [167]. This is to induce an actual metabolic acidosis, so that appropriate urinary acidification can be assessed. Unfortunately, ammonium has a foul taste and may not be tolerated by patients. Alternatively, distal acidification can be stimulated by co-administration of a loop diuretic and mineralocorticoid, such as furosemide and fludrocortisone [168, 169]. Sodium reabsorption via the epithelial sodium channel ENaC (see Fig. 39.1) provides a favorable electrical gradient for acid secretion in the collecting duct. In patients with normal distal tubular function this results in an acidification of the urine (as sodium absorption stimulates the secretion of a counter ion, *i.e.* H^+ and K^+). Patients with dRTA will not acidify their urine under these conditions either. The tests are described in Fig. 39.4.

Once a diagnosis of dRTA has been made, identification of clinical conditions and/or toxins causing dRTA is important and their treatment or removal often effective therapy. In pediatric prac-

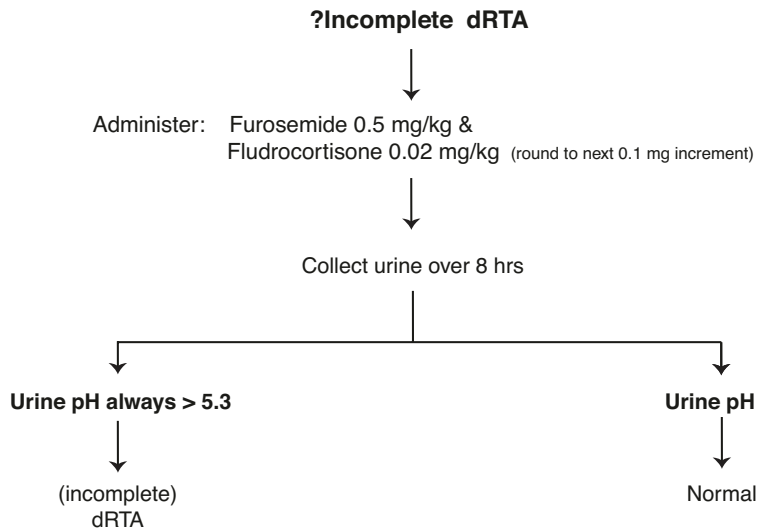

Fig. 39.4 Approach to diagnosis of incomplete dRTA. Note that one can substitute 100 mg/kg NH_4Cl for furosemide and fludrocortisone, however the described test is better tolerated and consequently preferable. Urine pH will be increased by sample exposure to air, due to diffusion of CO_2 and NH_3 from the sample. Thus, the test is difficult to do in non-toilet trained children, unless catheterised. Samples are ideally collected under oil and need to be analyzed straight away

tice, the etiology is almost always primary dRTA. A formal **hearing examination** is recommended. Patients with mutations in AE1 typically have normal hearing, patients with mutations in carbonic anhydrase II may have conductive hearing loss while patients with mutations in the H^+ ATPase often have sensorineural hearing loss, especially those with mutations in the B1 subunit [70, 111, 170].

Treatment

The goal of therapy is to ensure adequate growth, heal or prevent bony abnormalities and normalization of urinary calcium excretion to prevent worsening of nephrocalcinosis and the (re) occurrence of nephrolithiasis [161]. Treatment is simple in principle: provision of sufficient doses of alkali supplementation. Yet, in a large international study of 340 patients with primary dRTA, more than half had inadequate metabolic control, as judged by plasma bicarbonate and urinary calcium at last follow-up [111]. Importantly, inadequate metabolic control was associated with lower final height and lower eGFR at last follow-up, highlighting the importance of good treatment. Impediments to proper treatment likely include the need for frequent dosing, lack of approved alkali supplementation and poor taste of available preparations. Of note, more than 30 different preparations were prescribed and there was no apparent difference in outcome whether bicarbonate or citrate preparations were employed [165].

The key to optimal therapy is to provide a sufficient quantity of alkali frequently enough to maintain sustained control of the acidosis. The dose of alkali equivalents to compensate alkalosis decreases with age and is typically 3–6 mEq/kg/day below the age of 6 years (but up to 10 mEq/kg/day in infancy) and declines to 1–2 mEq/kg/day in adulthood [103, 115, 161]. Alkali supplementation reflects the renal acid load, which is derived primarily from dietary protein, specifically from sulfur-containing amino-acids which are metabolized to sulfuric acid [171]. Animal proteins have a higher content of sulfuric amino acids than plant proteins and consequently, a reduction in animal protein intake reduces the dose of alkali supplementation required for buffering. Nevertheless, sufficient protein intake (the recommended daily intake) is needed for normal growth and development in children.

In pRTA, because of the ongoing bicarbonate wasting, sustained normal plasma bicarbonate levels may not be achievable, despite often enormous prescribed doses. As detailed above, more frequent provision of smaller doses will provide more stable plasma levels and less wastage. However, this may be difficult for some patients and there families. Finally, the management of kidney stones, coexisting hearing loss or anemia may require other subspecialty support.

Prognosis

In dRTA, if the diagnosis is made early and alkali therapy provided consistently the prognosis is good, with final height in the normal range, albeit slightly below average [111]. While mild CKD is common, ESKD is exceedingly rare and was not observed in the cohort of 340 dRTA patients, which included 83 adults up to the age of 70 years [163]. Unfortunately, due to the non-specific nature of presenting symptoms, diagnosis is often delayed [151] and in these or non-compliant patients progression to renal failure and or significant growth impairment can occur [172, 173]. Importantly, alkali therapy does not prevent hearing loss [174].

When pRTA occurs in association with renal Fanconi syndrome, the prognosis depends primarily on the underlying condition. For isolated pRTA with eye findings, not enough patients have been reported to make meaningful statements on prognosis.

Mixed Proximal and Distal Renal Tubular Acidosis (Type III RTA)

Carbonic anhydrase II (CAII) is a metalloenzyme that catalyzes the reversible hydration of carbon dioxide into a proton and bicarbonate. This cytosolic enzyme is expressed in renal tubular epithelial cells along the nephron. The highest level of expression is in the intercalated cells with reduced

levels of expression in the proximal tubule and thick ascending limb. In both the proximal tubule and the α-intercalated cells it provides protons for secretion into the lumen (via NHE3 and the H^+-ATPase respectively). Its other product bicarbonate is effluxed into the peritubular interstitium via NBCe1 and AE1 respectively. Mutations in CAII lead to mixed proximal and distal RTA, or Type III RTA, which can be variable in severity and is often associated with nephrocalcinosis and nephrolithiasis [37, 175]. Carbonic anhydrase II is also essential for osteoclast function, and loss-of-function is therefore associated with excessive mineralization (osteopetrosis) accompanied by cerebral calcification, developmental delay, facial dysmorphism (low set ears, hypertelorism and a depressed nasal bridge), conductive hearing loss and cognitive impairment [145, 176–178]. This rare condition is inherited in an autosomal recessive fashion [179]. Bone marrow or stem cell transplantation has been used to prevent the progression of osteopetrosis although it may not be completely curative [180]. Alkali supplementation remains the mainstay of treatment for metabolic acidosis.

Hyperkalemic Renal Tubular Acidosis (Type IV RTA)

Clinical Presentation

Hyperkalemic renal tubular acidosis is typically the result of actual or functional (pseudo-)hypoaldosteronism [181]. This condition is primarily a salt-wasting tubulopathy of the collecting duct. Since sodium reabsorption and potassium and proton secretion are coupled in this nephron segment, hyperkalemic acidosis occurs as a secondary consequence. Aldosterone is required for sodium reabsorption through the epithelial sodium channel, ENaC, which generates the negative transmembrane potential required to drive both potassium and proton secretion across this nephron segment. While several rare genetic disorders are associated with hyperkalemic RTA, this type of RTA more commonly results from acquired causes such as renal damage from obstructive uropathy, due to an autoimmune disorder, drug therapy or interstitial renal disease (Table 39.2). Drugs such as amiloride, triamterene and spironolactone either directly or indirectly inhibit sodium absorption from the collecting duct through ENaC. Due to the coupling of sodium absorption to either potassium or proton excretion in this segment, these drugs also cause hyperkalemic metabolic acidosis. Ascending infections and urinary obstruction disproportionately affect collecting duct function resulting in Type 4 RTA, while autoimmune diseases can produce autoantibodies targeting col-

Table 39.2 Causes of hyperkalemic renal tubular acidosis

Genetic	
Pseudohypoaldosteronism type 1	MR
	ENaC, α, ß and γ subunits
Pseudohypoaldosteronism type 2	WNK1 (PHA2)
	WNK4 (PHA2)
Bartter syndrome type 2[a]	KCNJ1/ROMK (Bartter Type 2)
Congenital adrenal insufficiency	21 hydroxylase deficiency
Drug induced	
Spironolactone	
Heparin	
Amiloride	
Prostaglandin inhibitors	
Triamterene	
ACE inhibitors and ARBs	
Calcineurin inhibitors	
Methicillin	
Intrinsic renal disease	
Obstructive uropathy	
Pylonephritis	
Interstitial nephritis	
Nephrosclerosis	
Post renal transplant	
Lupus nephritis	
Renal amyloidosis	
Miscellaneous causes	
Addison's disease	
Diabetes	
Gout	
Renal venous thrombosis	

[a] Note KCNJ1/ROMK mutations can cause type IV RTA in infancy that evolves into a hypokalemic metabolic alkalosis as a young child

lecting duct epithelial cells thereby causing the disease.

Notably, hyperkalemia in type IV RTA is not only a consequence of impaired distal sodium reabsorption, due to aldosterone insufficiency or resistance. Hyperkalemia also appears to inhibit ammonia production in the proximal tubule, thereby reducing ammonium excretion and exacerbating the renal tubular acidosis [182, 183].

An important cause of hyperkalemic RTA is **pseudohypoaldosteronism (PHA**), both type 1 and type 2 [184]. **Type 1** is associated with lower blood pressure, hyponatremia and renal salt wasting, despite elevated circulating levels of aldosterone and renin. The condition results from mineralocorticoid resistance. Patients typically present as infants with dehydration, hypotension, weight loss and vomiting. There are two clinically and genetically distinct subtypes of this disorder. A milder renal-limited form, which is inherited in an autosomal dominant fashion, is due to loss-of-function mutations in the mineralocorticoid receptor [185, 186]. These patients typically present in the first few months of life with growth failure and subsequent work-up reveals the hyperkalaemic acidosis. Interestingly, plasma electrolytes spontaneously normalize with age, although renin and aldosterone levels usually remain elevated life-long [184, 187]. Genetic analysis of parents in the absence of a family history suggests that some cases may go undetected [150].

The more severe form of PHA type 1 is inherited in an autosomal recessive fashion and often displays evidence of multiple organ dysfunction, including increased sodium concentration in sweat, saliva and airway liquid with consequent pulmonary manifestations that can mimic cystic fibrosis [188]. This form of pseudohypoaldosteronism type 1 is caused by mutations in one of the three epithelial sodium channel subunits, α, ß or γ [189–192]. Affected patients typically present in the first days of life with failure to gain weight. Electrolyte abnormalities can be severe, with plasma potassium levels around 10 mmol/L or higher at presentation [193].

Pseudohypoaldosteronism type II, also known as hyperkalemic hypertension or Gordon's syndrome, describes a disorder characterized by hyperkalemic renal tubular acidosis and hypertension with variable aldosterone and low renin levels. In contrast to the salt-wasting hypovolemic disorder PHA type 1, type 2 is characterized by hypervolemia due to salt-retention in the distal convoluted tubule [194]. The consequent lack of delivery of sodium to the collecting duct causes the hyperkalemic acidosis. Consequently, PHA type 2 is highly sensitive to therapy with thiazide diuretics, whose target is the apically expressed sodium chloride co-transporter, NCC, which is expressed in the apical membrane of the distal convoluted tubule. Mutations in several genes have been identified to cause this disorder, including *WNK1*, *WNK4*, *CUL3* and *KLHL3* [195–197], (Table 39.3). Most patients with inherited PHA type 2 present during adolescence or even adulthood and especially hypertension may not be present until adulthood [194]. However, mutations in KLHL3 can be dominantly or recessively inherited and the latter ones can present already in infancy. Moreover, mutations in CUL3 appear to be associated with a more severe course with earlier presentation, early hypertension and failure-to-thrive [196].

Investigations into PHA have provided fascinating insights into the so-called "aldosterone paradox", i.e. the fact that the two key functions of aldosterone, regulation of volume and of potassium, can sometimes be competing. For instance, in a fasting state, the body may need to conserve volume (sodium) without sacrificing potassium. Conversely, in the presence of a high serum potassium, aldosterone is released to enhance potassium excretion, but without necessarily changing salt reabsorption [198]. The answer to this conundrum was provided by the identification of the WNK kinases and their associated regulatory proteins. If volume needs to be preserved without affecting potassium secretion, salt reabsorption is shifted to the distal convoluted tubule, so less sodium is delivered to the collecting duct where it could be exchanged for potassium. If potassium needs to be excreted without affecting sodium reabsorption, the process is shifted to the collecting duct, such that less sodium is reabsorbed in the distal convoluted

Table 39.3 Genetic causes of RTA

Gene name	Protein name	MIM #	Inheritance	Typical clinical features	Type of RTA
SLC4A4	NBCe1	603345, 604278	AR	Glaucoma, cataracts, band keratopathy	pRTA, Type II
?	?	?179830	AD	Short stature?	pRTA, Type II
ATP6V1B1	B1 subunit of the H^+ATPase	267300	AR	Sensorineural hearing loss, nephrocalcinosis or nephrolithiasis	dRTA, Type I
ATP6V0A4	A4 subunit of H^+ATPase	602722, 605239	AR	Late onset sensorineural hearing loss, nephrocalcinosis or nephrolithiasis	dRTA, Type I
SLC4A1	AE1	109270, 179800, 611590	AD (less commonly AR)	Nephrocalcinosis, osteomalacia, hemolytic anemia with AR Inheritence	dRTA, Type I
FOXI1	FOXI1	601093	AR	Deafness with enlarged vestibular aquaduct	dRTA, Type I
WDR72	WDR72	613214	AR	Amelogenesis imperfecta	dRTA, Type I
CA2	CAII	611492, 259730	AR	Osteopetrosis	Mixed or Type III
NR3C2	Mineralo-corticoid receptor	600983, 177735	AD	Pseudo-hypoaldosteronism Type 1	Hyperkalemic RTA, Type IV
SCNN1A, SCNN1B, SCNN1G	α, ß or γ subunit of ENaC	264350, 600228, 600761, 600760	AR	Pseudo-hypoaldosterism Type 1	Hyperkalemic RTA, Type IV
WNK1	WNK1	6052323, 614492	AD	Pseudo-hypoaldosterism Type 2, Hypertension	Hyperkalemic RTA, Type IV
WNK4	WNK4	145260	AD	Pseudo-hypoaldosterism Type 2, Hypertension	Hyperkalemic RTA, Type IV
CUL3	Cullin 3	603136, 614496	AD	Pseudo-hypoaldosterism Type 2, Hypertension	Hyperkalemic RTA, Type IV
KLHL3	Kelch-Like 3	614495	AD or AR	Pseudo-hypoaldosterism Type 2, Hypertension	Hyperkalemic RTA, Type IV

[a] Mutations of the genes in grey are primarily disorders of renal sodium handling and may not always cause RTA

tubule and therefore available for exchange with potassium. Mutations causing PHA type 2 shift this regulatory response towards salt reabsorption in the distal convoluted tubule, leading to hypervolameia and hyperkalaemic acidosis. The actual regulation is quite complicated, involving further kinases, such as SPAK and OSR1 [199, 200]. The final consequence of the mutations identified in affected individuals is increased phosphorylation and cell surface expression of NCC with increased sodium reabsorption. The volume expansion caused by increased NCC activity suppresses renin and the reduced delivery of sodium to the collecting duct impairs the distal secretion of potassium and protons leading to a hyperkalemic metabolic acidosis. Aldosterone levels can be variable, as aldosterone is also stimulated by hyperkalaemia. While inherited PHA type 2 is rare, a much more common acquired form, associated with calcineurin inhibitor treatment, has been described [201]. Typically, this complication occurs with large doses of tacrolimus, as commonly prescribed for instance in heart transplants. The exact mechanism is unclear, but in the mouse model, tacrolimus exposure leads to increased abundance of SPAK and WNK4 kinases and consequent increased phosphorylation and surface expression of NCC, analogous to inherited PHA type 2 [201]. Most imnportantly, this acquired form of PHA type 2 responds just as well to thiazides as the inherited form (see "treatment" below), providing an elegant and simple treatment, if reduction of tacrolimus is deemed clinically unacceptable.

Diagnosis

The initial diagnosis of type IV RTA is rather straightforward. The combination of hyperchloremic normal anion gap metabolic acidosis with hyperkalemia is pathognomonic for the diagnosis. In the context of clinical hypovolemia and elevated aldosterone levels, this is PHA type 1, whereas normal or elevated blood pressure is consistent with type 2. The diagnosis of type 2 PHA can be further confirmed by the excellent response to thiazide diuretics.

Investigations to determine the underlying cause are guided by patient history and physical examination and may include serum and urinary biochemistry to screen for nephritis, as well as renal ultrasound to assess urinary tract obstruction. Potential inciting drugs should be identified. Ambiguous genitalia point to congenital adrenal hyperplasia. Genetic testing should be sought whenever available to confirm hereditary disorders. As most mutations causing PHA are inherited in a dominant fashion, a careful family history should be obtained and frequently, further family members are identified, who have often been given a diagnosis of treatment-resistant hypertension or may even have suffered complications, such as stroke or myocardial infarction.

Treatment

The treatment of type IV RTA is largely etiology dependent. Offending drugs should be discontinued and any underlying renal disease treated. This often leads to complete resolution of the hyperkalemic metabolic acidosis. In the case of obstructive uropathy adequate urinary flow should be achieved by catheterization or an appropriate urologic procedure. Unfortunately, the metabolic acidosis associated with some cases of congenital obstruction may be irreversible, especially if associated with marked GFR impairment, and continued alkali supplementation is required.

In PHA type 1, especially the recessive form, immediate supplementation with sodium chloride, such as infusion of 0.9% saline will help stabilize intravascular volume. Correction of the acidosis with sodium bicarbonate will also lower plasma potassium levels. For longer term control, oral supplementation with sodium chloride and bicarbonate is often needed in doses >10 mmol/kg/day. Sodium resonium is helpful to eliminate potassium and provide sodium supplementation [193]. In the dominant form, oral supplementation with sodium chloride and/or bicarbonate is typically needed only in the first year of life or so.

In the case of pseudohyperaldosteronism type 2, thiazide diuretics are very effective and resolve the biochemical abnormalities, as well as the hypertension [202–204].

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Diabetes Insipidus

40

Detlef Bockenhauer and Daniel G. Bichet

History

Diabetes insipidus derives from the Greek word *diabinein* for "flow-through" and the Latin word *insapere* for "non-sweet tasting", separating it from another polyuric disorder, diabetes mellitus ("like honey"). A familial form affecting "chiefly males on the female side of the house" was first described by McIlraith in 1892 [1]. De Lange in 1935 reported a family with diabetes insipidus and no male-to-male transmission unresponsive to injections of posterior lobe extracts [2]. Forssman [3] and Waring [4] in 1945 recognized the disorder in these families as a renal problem. In 1947 Williams and Henry established the unresponsiveness to arginine-vasopressin (AVP) in these patients and coined the term nephrogenic diabetes insipidus (NDI) [5]. In 1969 the "Hopewell Hypothesis" was proposed by Bode and Crawford, proposing that most cases of NDI in the USA and Canada could be traced to descendants of Ulster Scots, who arrived on the ship Hopewell in Novia Scotia in 1761 [6]. Bichet later refuted this by molecular analysis [7]. In 1992 the *AVPR2* gene encoding the AVP2 receptor was cloned and mutations identified in patients with X-linked NDI [8–11]. Shortly after, the *AQP2* gene encoding the vasopressin-regulated water channel aquaporin-2 (*AQP2*) was cloned [12, 13] and in 1994 mutations in *AQP2* were found to underlie autosomal recessive DI [14].

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Clinic

Presentation in Infancy

Patients with congenital NDI typically present in the first weeks to months of life with dehydration [15]. Sometimes, patients receive repeated investigations for sepsis, as the dehydration can be associated with low-grade temperatures, until a set of serum electrolytes is obtained, revealing hypernatraemia. Failure-to-thrive with irritability are further symptoms. Often, patients suck vigorously, but develop vomiting shortly after starting to feed. Vomiting may be due to reflux exacerbated by the large volumes of fluid necessary to compensate for the renal losses. Interestingly, breast-fed infants with NDI typically thrive better

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than formula-fed ones, as breast milk presents a lower osmolar load than most standard formulas (see below). Of note, pregnancies with babies afflicted with NDI are not complicated by polyhydramnios, since the AVP-dependent mechanisms for urinary concentration are not fully developed until after birth and the osmolar load is cleared by the placenta [16].

Clinical Features and Long-Term Prognosis

Symptoms typically improve with advancing age, especially once food intake has changed to mainly solids, so that caloric and fluid intake are separated. Free access to water allows for self-regulation of serum osmolality. Patients remain polyuric, however and the frequency of voiding and drinking, especially during the night is useful information to assess the severity of the problem. Constipation is a common complication, presumably due to maximal extraction of water from the gut and should be discussed with the families and treated appropriately. Nocturnal enuresis is a frequent concern in childhood, obviously aggravated by the large urine volumes (parents often refer to it as "bed flooding" rather than bed wetting) and the average age at which patients achieve nocturnal continence is between 10 and 12 years [17, 18]. Parents also often report problems with concentration and attention span in their children and in one study almost half the patients were diagnosed with attention deficit hyperactivity disorder [19]. The reason for this is unclear, but maybe partly due to the constant need to drink and void. In an international multi-centre study of 315 patients with primary NDI, 16% were reported to have ADHD and an additional 20% to have other mental health problems [17]. Nevertheless, with treatment, patients with NDI can function well and while the proportion of adults with a university degree (27%) was lower than in the overall population, the rate of full-time employment and independent living in adulthood was similar [17, 19]. Impaired mental development with intracranial calcifications used to be a common feature in NDI [20–22]. This likely reflected repeated episodes of severe hypernatraemic dehydration and with appropriate treatment of NDI this devastating complication is essentially no longer seen. And while failure to thrive is a common problem in infancy, final height is typically in the normal range [17]. Indeed, a surprising finding from the international cohort study was the increased incidence of obesity in adult NDI patients (41%), which may be an unintended consequence of the intense efforts in childhood to maximise caloric intake [17].

Some patients develop dilatation of the urinary tract from the high urinary flow, especially if they have poor voiding habits [20, 23, 24]. However, in those with hydronephrosis, anatomic causes of obstruction need also be considered, as these are potentially remediable and even minor impediments to flow can cause severe dilatation in this polyuric disorder [25]. In the international cohort study, 38% of patients had evidence of flow uropathy which may contribute to the increased prevalence of CKD Stage ≥2 in children (32%) and adults (48%) [17].

Physiologic Principles

Tubular Concentration/Dilution Mechanism (Countercurrent Mechanism with Figure)

The kidney creates a concentration gradient via a so-called countercurrent multiplication system [26, 27]. The tonicity (osmolality) of urine as it proceeds along the nephron is depicted in Fig. 40.1. In the proximal tubule the urine remains isotonic to plasma because of the high water permeability of this segment, mediated by aquaporin 1 (AQP1) [28–30]. Urine then enters the tubular segment most important for countercurrent multiplication: the loop of Henle. First, urine is concentrated as it descends the thin descending limb (TDL). The precise mechanism of concentration are still debated: initially, it was thought that the TDL also expresses AQP1, allowing water exit into the medullary interstitium [31]. More recent data, however, show that only about 10–15% of TDL (the "long-looped" nephrons) express AQP1, whereas the other ones do not [32].

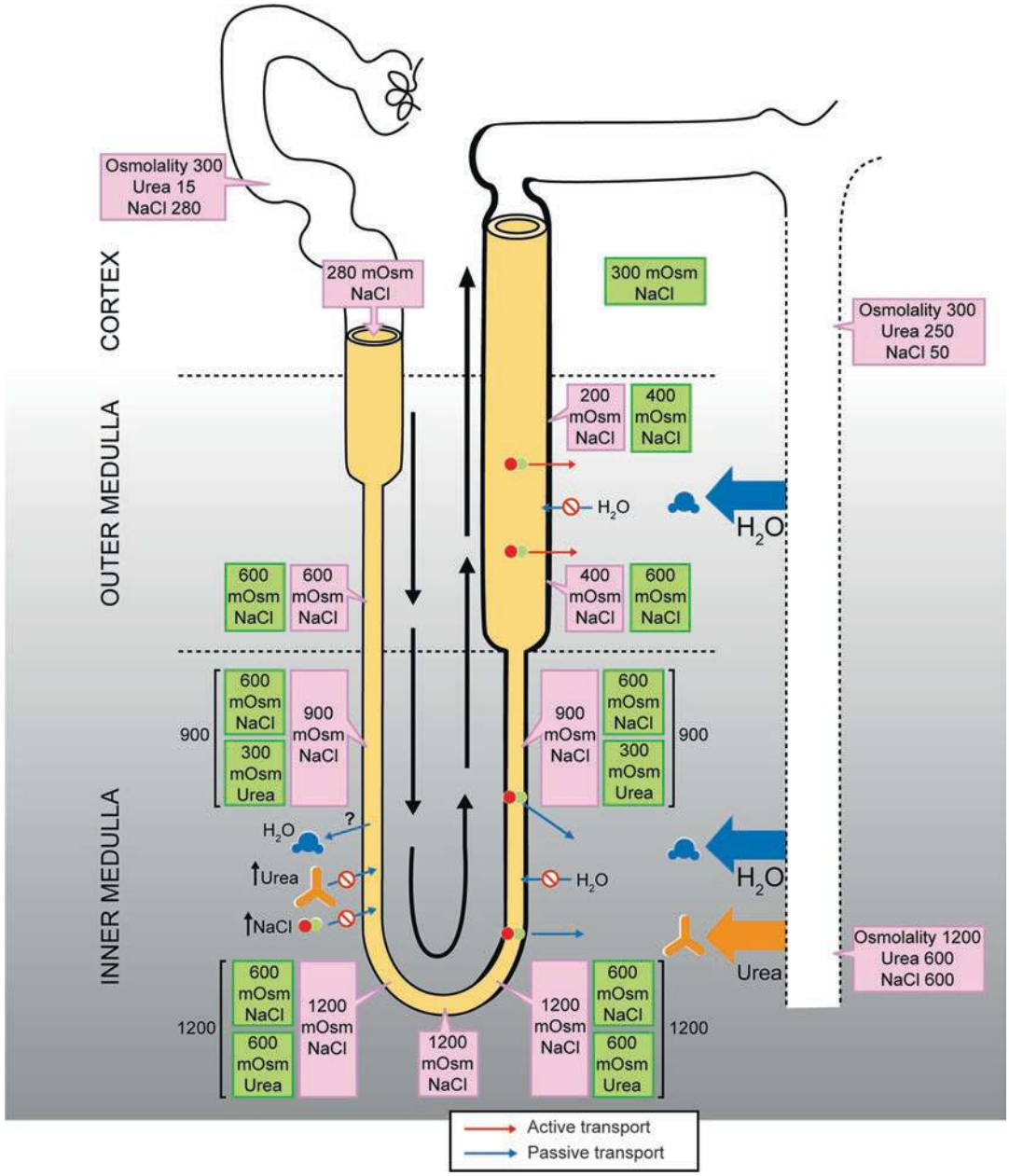

Fig. 40.1 Diagram of the renal concentration and dilution mechanism. The numbers indicate approximate osmolalities of the tubular and interstitial fluids. The names of the relevant transport proteins are indicated. The concentration gradient is mainly generated by the active reabsorption of solutes in the thick ascending limb by the transporter NKCC2. Note that urine exiting the loop of Henle is hypotonic. Final urine concentration is then dependent on the availability of AQP2 water channels in the collecting duct. For further details, see text

Consequently, concentration of the tubular fluid in TDL of these nephrons is assumed to occur via passive sodium influx [33]. Urine subsequently enters the thick ascending limb (TAL), which is impermeable for water, but actively removes sodium chloride, via the co-transporter NKCC2 [34]. Therefore, urine is diluted on its way up the TAL by active removal of solutes. The

accumulation of solutes in the interstitium in turn generates the driving force for the removal of water from the thin descending limb (in the long-looped nephrons) and the entry of sodium chloride (into the short-looped majority of nephrons), completing the countercurrent multiplier.

There is further removal of sodium chloride in the distal convoluted tubule via the thiazide-sensitive co-transporter NCC and at entry in the collecting duct, urinary osmolality is typically around 50–100 mOsm/kg. The final osmolality of the urine is now solely dependent on the water permeability of the collecting duct and thus the availability of water channels. If water channels are present, water will exit the tubule following the interstitial concentration gradient and the urine is concentrated. If no water channels are present, dilute urine will be excreted.

AVP Effects in the Kidney

The availability of water channels in the collecting duct is under the control of AVP. The final regulated step is the insertion of AQP2 into the apical (urine-facing) side of the membrane of principal cells in the collecting duct [35]. Figure 40.2 shows a model of a principal cell. AVP binds to the vasopressin receptor (AVPR2) on the basolateral (blood-facing) side. AVPR2 is a G-protein coupled receptor that upon activation stimulates adenylate cyclase, thus raising cAMP production [36–39]. Protein kinase A (PKA) is stimulated by cAMP and phosphorylates AQP2 at a consensus site in the cytoplasmic carboxy-terminal tail of the protein, serine 256 (S256) [12, 40, 41]. Unphosphorylated AQP2 is present in intracellular vesicles, which upon phosphorylation at S256 are fused in the apical membrane [42]. Of note, AQP2 water channels are homotetramers, i.e. consisting of four subunits. In vitro evidence suggests that minimally three subunits need to be phosphorylated for the channel to be fused in the plasma membrane [43].

After insertion of AQP2 in the apical membrane water can enter from the tubular lumen into the cell and exit via the basolateral water channels AQP3 and AQP4. While AQP4 appears to be constitutively expressed in collecting duct, there is some evidence that AVP may also regulate the expression of AQP3 [44–46].

Extrarenal Effects of AVP

The vasopressive and glycogenolytic effects of AVP are mediated through AVP1 receptors expressed in vasculature and liver [47], while the renal effects, especially the increase in water permeability of the CD are mediated by AVPR2 [48]. Interestingly, administration of the AVPR2-specific agonist 1-Desamino-8-D-Arginine Vasopressin (DDAVP) results not only in an increase in urine osmolality, but also has extrarenal effects, including:

1. A small depression of blood pressure with a concomitant increase in heart rate and increase in plasma renin activity [49, 50].
2. An increase in factor VIIIc and von Willebrand factor with a decrease in bleeding time [49, 51, 52].

These extrarenal effects are abolished in patients with X-linked NDI, suggesting that AVPR2 is expressed beyond the kidney. Clinically, this can be used to differentiate between X-linked and autosomal recessive NDI (see below).

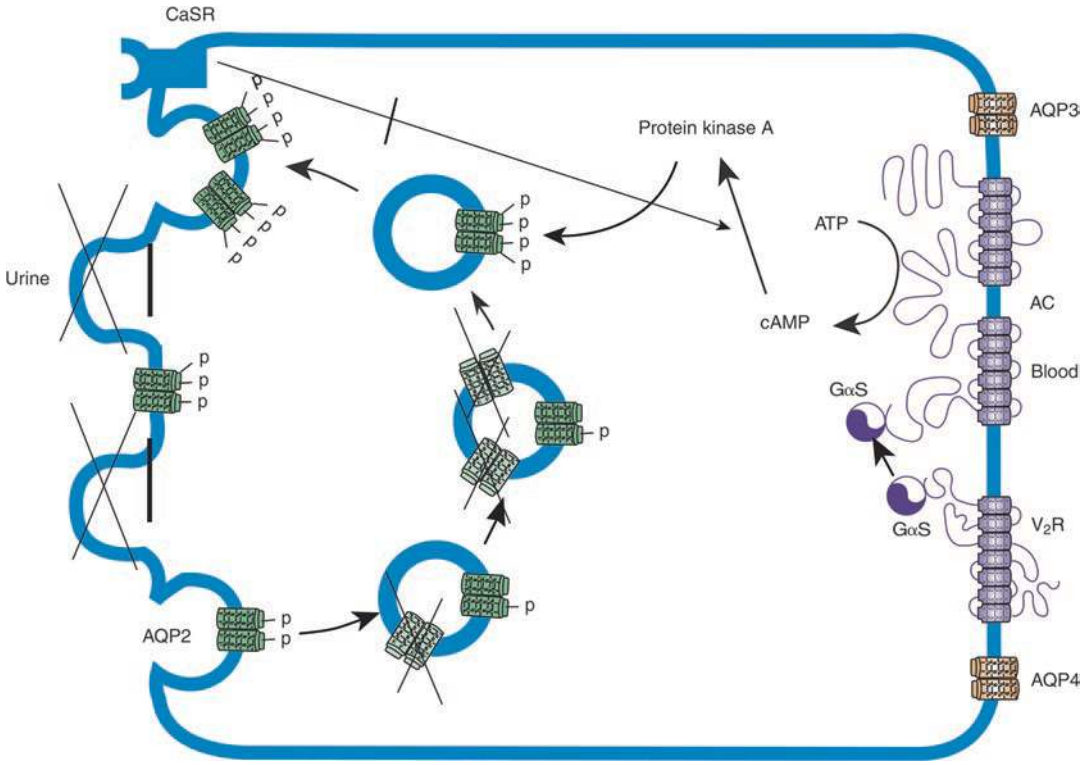

Fig. 40.2 Diagram of a principal cell. Depicted is a schematic of a principal cell with relevant proteins for water transport. AVP binds to the AVR2 receptor (depicted in green), expressed on the basolateral side of the cell. AVPR2 is a G-protein-coupled receptor and AVP binding releases the stimulatory G-protein GαS, which, in turn, stimulates adenylcylase. The increased production of cycline adenosine monophosphate (cAMP) stimulates protein kinase A (PKA), which phosphorylates the water channel AQP2, leading to insertion of these channels into the apical membrane. Water can then enter the cell from the tubular lumen and exit into the interstitium via the constitutively expressed water channels AQP3 and AQP4

Diagnosis

The presence of inappropriately dilute urine in the face of an elevated serum osmolality defines DI. Dehydration in a child with good urine output should always prompt consideration of a urinary concentrating defect. The diagnosis is easily made by obtaining serum and urine biochemistries. Maximal urinary concentrating ability increases with age, but a urine osmolality below plasma osmolality in a dehydrated child establishes a diagnosis of DI [53]. In classic NDI the urine osmolality is always below 200 mOsm/kg.

Diagnostic Procedures

Water Deprivation Test

The aim of the water-deprivation test is to induce mild dehydration and thus challenge the kidney to preserve water. Water is withheld until serum osmolality is just above the upper limit of normal (>295 mOsm/kg). Obviously, no child presenting with hypernatraemia and inappropriately dilute urine needs to undergo this test, as the challenge had already presented naturally. A water deprivation test carries the risk of severe hypernatraemic dehydration, especially in infants, as

there may be delays in obtaining and reacting to laboratory results. It is useful to distinguish habitual polydipsia from central DI in patients with a good response to DDAVP, and is usually reserved for those particular patients: those with habitual polydipsia will be able to increase urine osmolality with water deprivation, whilst those with central DI will not. For a first assessment, a simple and informal water deprivation test is to ask the parents to obtain the first morning urine on their child and note the last time the child has drunk (water should not be withheld from the child). This can be used as an initial screening test in polyuric patients, as a concentrated urine excludes a diagnosis of DI.

DDAVP Test

The kidney concentrates the urine in response to the pituitary hormone AVP. Failure to concentrate can therefore be due to a deficiency in AVP (central DI or CDI) or an inability of the kidney to respond to it (NDI). AVP effects are mediated via two different receptors: the vasoconstriction ("vasopressin") is mediated by AVP receptor 1 (AVPR1), while the antidiuretic response is mediated by receptor type 2 (AVPR2). DDAVP has a high specificity for AVPR2 and can therefore be used to assess the renal response while avoiding the systemic effects mediated by AVPR1. Different protocols exist in the literature with respect to dosage and route of administration of DDAVP. Some authors use intranasal DDAVP, others oral, subcutaneous (sc), intramuscular (im) or intravenous (iv) administration [49, 54, 55]. While oral or intranasal DDAVP is less invasive, absorption is less reliable. Thus, if the result of the test is inconclusive, it may need to be repeated using injected DDAVP. DDAVP given iv requires a shorter observation period (2 h) than other modes of administration (4–6 h), where absorption is more protracted. Moreover, DDAVP at the dose commonly used in von Willebrand disease (0.3 μg/kg iv) induces some systemic side effects in the form of a mild decrease in blood pressure and concomitant increase in heart rate via AVPR2 (see above). Consequently, patients with mutated AVPR2 (X-linked NDI) do not experience these haemodynamic changes, while patients with intact AVPR2, but mutated AQP2 (autosomal DI) do. The DDAVP test can therefore help differentiate between these two forms. A typical protocol, modified from [49] is given in Table 40.1. A commonly feared, but actually rare complication of

Table 40.1 Protocol of a DDAVP test

Time	-30	-15	0	10	15	20	30	40	50	60	75	90	110	130	150
Actual time (example: 09:00)	__:__	__:__	__:__	__:__	__:__	__:__	__:__	__:__	__:__	__:__	__:__	__:__	__:__	__:__	__:__
dDAVP infusion	■	■					■	■	■	■	■	■	■	■	■
Blood pressure (mmHg)					■							■			
Pulse (b/min)					■							■			
Fluid intake (ml)															
Urine: volume (ml)				■	■	■		■	■		■		■	■	
Osmolality				■	■	■		■	■		■		■	■	
Na				■	■	■		■	■		■		■	■	
Plasma: U&E		■	■	■	■	■		■	■	■	■	■	■	■	
Osmolality		■	■	■	■	■		■	■	■	■	■	■	■	

Patients should be observed for a minimum of 2 h (iv) to 6 h (oral and nasal) after DDAVP administration. Volume of fluid intake during the test must be limited to the volume of urine produced in order to avoid hyponatremia
See text for more information and interpretation of results

the DDAVP test is hyponatraemia. Patients with intact thirst mechanism who respond to DDAVP will stop drinking water, due to their stable serum osmolality. Yet patients with habitual polydipsia, who will keep on drinking despite lowered serum osmolality, and infants, who continue to be fed by their caregivers throughout the test are at risk for hyponatraemia. Thus, close observation and strict limitation of fluid intake to a volume equal to urine output during the test period is critical to prevent this complication.

A urine osmolality after DDAVP below 200 mOsm/kg is consistent with a diagnosis of NDI, while patients with intact urinary concentrating ability typically achieve urine osmolalities greater than 800 mOsm/kg (>300 in infants) [53]. Patients with intermediate values should be assessed for inaccurate test results (especially when DDAVP was administered intranasally) or intrinsic renal disease limiting the urinary concentrating capacity (including chronic renal failure and obstructive uropathy, see below).

Differential Diagnosis

Central DI

A urinary concentrating defect can be due to a lack of AVP (central DI) or the inability of the kidney to respond to it (NDI). A DDAVP test helps to differentiate between the two (see above). Central DI is most commonly the consequence of head trauma, or other diseases affecting the hypothalamus or pituitary, but there are some rare cases of hereditary DI due to mutations in the gene encoding *AVP* [56–58]. In addition, loss-of-function variants in the *PCSK1* gene, encoding Proprotein Convertase 1, an endopeptidase involved in cleaving of precursor proteins into active peptide hormones have a complex endocrine phenotype that can include polyuria-polydipsia [59].

X-Linked and Autosomal NDI

A careful family history and assessment of systemic effects in the DDAVP test can discriminate the more common X-linked (90% of patients with identified mutations) from the rare autosomal NDI (10%). In approximately 10% of patients with presumed primary NDI, no mutation in either AVPR2 or AQP2 is identified [60]. Thus, these patients either have mutations in genes not yet identified to cause primary NDI, or in regions of the two known genes not assayed (e.g. introns or promoter), or have been misdiagnosed and actually have a secondary form of NDI (see below).

Partial NDI

An intermediate urine osmolality, that is between 200 and 800 mOsm/kg after administration of DDAVP is referred to as partial NDI. As discussed above, children less than 3 years of age, and especially in the first months of life, may not be able to maximally concentrate their urine yet and a value below 800 can be physiologic [53]. Further, technical problems with the DDAVP test should be excluded, especially if administration was intranasally, before a diagnosis of partial NDI is considered.

Inherited forms of partial DI are typically due to mutations in the *AVPR2* gene, that allow proper expression of the receptor at the cell membrane, but decrease the affinity to AVP, thus shifting the dose-response curve and requiring higher amounts of AVP to increase urinary concentration [61, 62]. However, mutations in *AQP2* with some retained urinary concentrating ability have also been identified [63]. Obviously, since these patients have a partially retained ability to concentrate their urine, their clinical symptoms are milder.

Secondary NDI

The defining feature of NDI is a pathologic deficiency of AQP2 in the apical membrane of the collecting duct. This can be primary inherited, i.e. due to mutations in either *AQP2* itself or in *AVPR2*, or occur as a secondary phenomenon: as a side effect of medications, anatomical problems or in the context of other tubulopathies [64]. The distinction is important, as misclassification as

primary NDI may miss the opportunity to identify a remediable cause, such as urinary obstruction, or to make the correct diagnosis, which may result in potentially harmful treatment. Thus, in any patient with clinical NDI who displays unusual features, such as a history of polyhydramnios or hypercalciuria/nephrocalcinosis or hypokalaemia (before thiazide treatment), a secondary form of NDI should be considered and consequently a primary diagnosis sought.

Secondary Inherited NDI

A secondary form of NDI has been observed in other inherited tubulopathies, which can lead to misdiagnosis [65]. This seems to occur most commonly in Bartter syndrome types 1 and 2 [66–68]. Whilst isosthenuria (i.e. an impaired ability to either concentrate or dilute the urine with a urine osmolality similar to that of plasma) is an expected feature of Bartter syndrome (see section "Disorders Impairing the Generation of a Medullary Concentration Gradient" below), some of the affected patients clearly have hyposthenuria (i.e. a urine osmolality persistently below that of plasma). Indeed, in the laboratory in Montreal, the genes underlying type 1 and type 2 Bartter syndrome, *NKCC2* and *KCNJ1*, are tested next if no mutations were identified in *AVPR2* or *AQP2* in the DNA of patients referred with a clinical diagnosis of NDI [64].

Secondary NDI has also been described in many other inherited diseases affecting the kidney, including renal Fanconi syndromes, especially cystinosis, distal renal tubular acidosis (dRTA), apparent mineralocorticoid excess (AME) and ciliopathies [64, 65].

The precise etiology of this secondary NDI is unclear, but may be related to the electrolyte abnormalities inherent in these disorders, especially hypercalciuria and hypokalaemia (see below). Regardless of the etiology, establishing the correct diagnosis is obviously important: some of the primary disorders can be treated specifically (e.g. cystinosis, dRTA or AME). Moreover, the thiazide treatment commonly used in NDI could compound the defect in tubular salt reabsorption inherent to some of these disorders (e.g. renal Fanconi syndromes, Bartter syndrome) resulting potentially in serious hypovolaemia.

Obstructive Uropathy

Polyuria after release of urinary tract obstruction is a well-recognized phenomenon (post-obstructive diuresis). However, if obstruction is incomplete, it is often associated with polyuria, as well. Animal studies show a decreased level of AQP2 expression with bilateral ureteric obstruction [69]. Experiments with unilateral obstruction show a marked decrease in AQP2 in the obstructed kidney, consistent with the view that local factors, such as increased pressure, affect AQP2 expression [70]. Supporting this view is also the fact that other signs of distal tubular dysfunction are usually present in obstructive uropathies, like hyperkalaemia and acidosis. The downregulation of AQP2 persists up to 30 days after release of obstruction, explaining the post-obstructive diuresis [35].

Interstitial Renal Disease

Polyuria is frequently seen in renal failure, especially if the underlying aetiology primarily affects the interstitium, such as in renal dysplasia (see Chap. 9), nephronophthisis (see Chap. 12) or tubulointerstitial nephritis (see Chap. 37). It is also commonly seen after ischemic renal failure [71, 72]. However, these patients will typically have isosthenuria. This is in contrast to the hyposthenuria, i.e. a urine osmolality persistently below that of plasma that characterizes NDI.

Lithium

While rarely used in children, lithium therapy is a common treatment for manic-depressive disease in adults and roughly a fifth of patients develop polyuria [73]. Animal studies have shown decreased expression of AQP2 in principal cells, probably due to inhibition of cAMP formation in the collecting duct [38, 74–76].

Hypercalcaemia and Hypercalciuria

Hypercalcaemia can be associated with polyuria and two mechanisms have been proposed to explain the AVP-resistant concentrating defect; both likely involving the calcium-sensing receptor (CaSR). This receptor is expressed on the basolateral (blood) side of thick ascending limb cells and indirectly inhibits the NKCC2 co-transporter, thus impairing the generation of a

medullary concentration gradient [77–79]. Second, this receptor is also expressed on the luminal (urine) side of collecting duct cells and thought to affect AQP2 trafficking [80, 81]. The latter mechanism would thus be mediated by hypercalciuria and has been proposed to constitute a protective measure against the formation of calcium-containing stones [82]. It would also provide an explanation for the hypercalciuric forms of secondary NDI, such as in Bartter syndrome (see above). However, doubts have been raised about the clinical relevance of this mechanism, as the protection against stones would come at the risk of dehydration. Indeed, in healthy control subjects the highest urine calcium concentrations were found in the most concentrated urine samples, arguing against a clinically relevant effect of urine calcium on urine concentration [83].

Hypokalaemia

Hypokalaemia causes an AVP-resistant concentration defect. As in the other forms of acquired NDI, reduced expression of AQP2 has been demonstrated [84]. Thus, downregulation of AQP2 seems to be a common feature in acquired NDI [35]. However, the mechanism by which hypokalaemia affects this remains to be elucidated.

Disorders Impairing the Generation of a Medullary Concentration Gradient

Bartter Syndrome

As discussed above, the loop of Henle and active salt reabsorption in thick ascending limb are necessary for the generation of a medullary concentration gradient. Therefore, factors impairing salt reabsorption in the thick ascending limb will lead to a urinary concentration defect (hypo- or isosthenuria). Patients with Bartter syndrome have inherited defects in thick ascending limb salt transport and symptoms include polyuria and episodes of hypernatraemic dehydration (see Chap. 29), similar to patients with NDI. However, the presence of a hypokalaemic alkalosis and elevated urinary electrolytes, particularly chloride, help differentiate it from NDI, although the former may be absent in young infants [67]. A history of polyhydramnios further helps to exclude a diagnosis of NDI.

Urea Transporter

Urea is an important constituent of the medullary interstitial concentration gradient. Urea is a bipolar molecule and thus can only diffuse slowly through membranes [85]. Diffusion is facilitated by urea transporters and two genes encoding these transporters have been identified in humans [86]. A mild urinary concentrating defect has been described in patients not expressing the minor blood group antigen Kidd (Jk) [87]. Later, this antigen was identified to be identical with the urea transporter UT-1, encoded by *SLC14A1* and several mutations in this gene have been identified in Kidd-negative individuals [88–90]. Recent evidence suggests that UT-1 is also expressed in the endothelium of the vasa recta and that the combined defect in red cell and vascular urea diffusion impairs countercurrent concentration [91, 92]. Interestingly, no mutations have been found so far in the gene encoding the urea transporter expressed in renal tubule UT-2 (*SLC14A2*).

Genetics

AVPR2

The majority of cases of NDI (90%) are due to mutations in the *AVPR2* gene [93, 94]. The gene is located on chromosome region Xq28 and the mode of inheritance is X-linked recessive. Therefore, the majority of patients with NDI are male, but due to skewed X-inactivation (lionization), females can be affected with variable degrees of polyuria and polydipsia [20, 95–97]. Indeed, in some families, X-inactivation is strongly biased leading to a pseudo-dominant inheritance pattern [98]. X-inactivation may be strongly biased because of (a) chance, (b) a co-existent mutation on the affected X-chromosome affecting cell survival or (c) a co-existing mutation in a gene regulating X-inactivation [99].

So far, more than 211 distinct putative disease-causing mutations have been described in more than 326 families [93, 100]. When investigated in vitro, these mutations can be classified according to their effect [101–118]:

Class 1 mutations result in frame-shifts, premature stop-codons and aberrant splicing and prevent translation of the receptor protein.

Class 2 mutations are missense mutations that allow for translation of the protein, but lead to aberrant trafficking. Typically, these mutations induce improper folding with subsequent trapping in the endoplasmic reticulum (ER).

Class 3 mutations allow the mutated protein to reach the cell-surface, but impair the receptor's signaling, typically by affecting binding of AVP.

The majority of mutations identified in X-linked NDI belong to class 2 [119]. Conversely, mutations identified in inherited partial NDI fall into class 3: these mutated receptors reach the cell membrane, but have a decreased affinity for AVP [54, 61].

Interestingly, three distinct class 3 mutation have been identified in the *AVPR2* gene, leading to gain-of-function with constitutive activation of the receptor and thus to a "nephrogenic syndrome of inappropriate antidiuresis" [120, 121].

AQP2

The analysis of a pedigree with affected females and the presence of intact extrarenal responses to DDAVP in some patients with NDI lead to the postulation of an autosomal inherited "post-receptor" defect in these individuals [122–124]. The molecular basis for this distinct form of NDI was identified in 1994 to be the water channel aquaporin-2, which is expressed in collecting duct [14]. Approximately 10% of all patients with NDI carry mutations in the *AQP2*. As expected for a loss-of-function defect, inheritance is usually recessive and—similar to AVPR2—the majority of mutations fall into class 2 with retention in the endoplasmic reticulum [119, 125]. Interestingly, there are some families with autosomal dominant inheritance of NDI. Molecular analysis has shown that affected members carry mutations in the c-terminus of AQP2 [43, 126–128]. So why does this lead to a dominant inheritance? The final waterchannel is a homotetramer, meaning it consists of four AQP2 subunits (see Fig. 40.2). Dominant mutations in the C-terminus lead to aberrant trafficking (class 2), but are able to oligomerize with wild-type protein to form the tetramer. As tetramerization takes place before export to the plasma membrane, these mutations exert a dominant-negative effect on AQP2 function, by misguiding trafficking of the assembled tetramer. Interestingly, specific mutations direct AQP2 trafficking to distinct cellular compartments, such as the Golgi complex [43], late endosomes/lysosomes [126] or the basolateral membrane [128, 129].

Treatment

General Aspects of Treatment

The importance of prompt treatment of NDI is highlighted by the fact that mental retardation used to be an invariable feature, but can be completely prevented by proper treatment. Caring for a patient with NDI is most difficult during infancy, when the babies are dependent on their caregivers for access to fluids. Therefore fluids should be offered in 2-h intervals, placing a considerable burden on the caregivers, particularly at night. Feeding per nasogastric tube is often helpful in this period. A continuous overnight feed delivered by a pump will provide fluid and calories to the baby and much needed rest to the parents. Families also need to be instructed to bring the child to immediate medical attention, when there are increased extra-renal fluid losses, such as when diarrhoea, vomiting or fever are present. It is often helpful for the parents to have a letter detailing the condition of their child and the need for prompt physical and biochemical assessment, that they can present in these instances in order to avoid being sent home by medical personnel with no experience in this condition. There should be

a low threshold for admission and intravenous hydration in these instances to prevent dehydration. When in hospital, hypotonic fluids, such as 5% Dextrose or 0.22% saline are usually appropriate for intravenous hydration, because of the obligate water losses in the urine. Replacement fluids with a higher osmolality than urine osmolality will exacerbate hypernatraemia. For instance, 0.45% saline results in an osmotic load of 154 mOsm/L (77 mOsm Na and 77 mOsm Cl). A patient with a maximal urine osmolality of 100 mOsm/kg will need to excrete 1.54 L of urine for each litre of 0.45% saline received in order to excrete the osmotic load presented by the replacement fluid (see below). Thus, in patients with NDI, the administration of fluids that are hypertonic compared to urine can lead to hypernatraemic dehydration, even though the fluid may be hypotonic to plasma. However, if there are increased salt losses, as can occur with diarrhea, or if hypotonic fluids are administered at a rate higher than the urine losses, hyponatraemia could ensue. Close monitoring of the patient with respect to weight, fluid balance, clinical symptoms and biochemistries is therefore imperative to prevent complications.

Osmotic Load Reduction

The most important part in the treatment of patients with NDI is a reduction in their osmotic load, also called renal solute load, which determines urine volume. Therefore close involvement of a dietician with experience in the management of children with kidney problems is necessary. The osmotic load consists of osmotically active substances that need to be excreted in the urine, i.e. proteins, as they are metabolized to urea, and salts. A typical western diet contains about 800 mOsm per day. Thus, an individual with a urine osmolality of 800 mOsm/kg only needs 1 L of water to excrete that load. Yet a patient with NDI and a maximal urine osmolality of 100 mOsm/kg needs at least 8 L of water for excretion and if the urine osmolality is 50 mOsm/kg then 16 L of water are required. One gram of table salt is equivalent to about 18 mmol NaCl, providing an osmolar load of 36 mOsm (18 mOsm Na and 18 mOsm Cl). Consequently, for a patient with a urine osmolality of 100 mOsm/kg, each gram of salt ingested increases obligatory urine output by 360 mL. The osmolar load of a diet can be roughly estimated by the following formula: twice the millimolar amount of sodium and potassium (to account for the accompanying anions) plus protein [g] times 4 (as metabolisation of each g of protein yields approximately 4 mmol of urea) [130]. Since lipids and sugars are metabolized without byproducts requiring renal excretion, only protein intake needs to be limited, but should still meet the recommended daily allowance to enable normal growth and development. A reasonable goal is a diet containing about 15 mOsm/kg/day. A child with a urine osmolality of 100 mOsm will need a fluid intake of 150 mL/kg/day to be able to excrete that load, which is achievable. Enriching the fluid intake with carbohydrates will provide additional calories without increasing the osmolar load.

Diuretics

The use of a diuretic in a polyuric disorder appears at first glance counterintuitive, but does make physiologic sense. The successful use of thiazides in NDI with a subsequent increase in urine osmolality and concomitant decrease in urine output was first reported in 1959 [131, 132]. Thiazides inhibit reabsorption of sodium and chloride in the distal convoluted tubule (part of the section "Tubular Concentration/Dilution Mechanism (Countercurrent Mechanism with Figure)"—see above) and thus increase the salt concentration and osmolality of the urine. The increased salt losses decrease intravascular volume with a subsequent up-regulation of proximal tubular reabsorption of salt and water. Consequently, less volume is delivered to the collecting duct and lost in the urine. Typically used is hydrochlorothiazide at 2 mg/kg/day in two divided doses. The more long-acting Bendroflumethiazide (50–100 μg/kg/day) can be given as a single daily dose. Hypokalaemia is a common complication of thiazide administration, but supplemen-

tation with potassium salts increases the osmolar load. Therefore, combination of the thiazide with a potassium-sparing diuretic, such as amiloride (0.1–0.3 mg/kg/day) is advantageous, but the latter can cause gastrointestinal side effects, especially nausea.

Non-steroidal Anti-inflammatory Drugs (NSAID)

Like in many other tubular disorders NSAID (prostaglandin synthesis inhibitors) are used in NDI. Initially, it was thought that these worked by reducing GFR and thus a "partial chemical nephrectomy" to minimize losses. However, observations in animals and humans suggest that NSAID can increase urine osmolality without decreasing GFR. In fact, an improvement in GFR can be observed, presumably reflecting improved volume status [66]. The exact mechanism remains to be elucidated, but appears to be ADH-independent [133–135]. Some evidence suggests that activation of basolateral prostaglandin receptors by prostaglandin E2 inhibits Adenylcyclase and/or the shuttling of AQP2 to the apical membrane [136–138].

Typically used is Indomethacin (1–3 mg/kg/day in three to four divided doses). The long-term use of this drug is associated with deterioration of renal function and haematological, as well as gastro-intestinal side effects including life-threatening haemorrhage [139, 140]. The latter may be avoided by using a selective COX-2 inhibitor and the successful use of these in NDI has been reported [141, 142]. However, there are concerns about cardiotoxic side effects of these drugs, as evident by the removal of Rofecoxib from the marketplace [143]. In our experience, the combination of Hydrochlorothiazide with an NSAID is useful during the first years of life, with a subsequent switch to Bendroflumethiazide with or without Amiloride. Key is the close observation of the individual patient for side effects and for changes in urine output or growth percentiles.

The concurrent use of antacids can help with the vomiting often seen in infants with NDI and help prevent gastro-intestinal side effects of NSAID.

Future Perspectives

An increasing understanding of the molecular mechanisms of the (patho)physiology of urinary concentration opens up perspectives for novel treatments [144].

Molecular Chaperones

The vast majority of mutations identified in the *AVPR2* gene lead to improper folding of the resultant protein with entrapment in the endoplasmic reticulum (see section "AVPR2"). Retention is dependent on specialized endoplasmic reticulum proteins, many of which require calcium for optimal function. Therefore, depletion of endoplasmic reticulum calcium stores by inhibiting the sarcoplasmatic calcium pump may be useful to overcome entrapment [145]. Indeed, this approach has been successfully used in vitro to induce surface expression of an AVPR2 mutant [146]. More specific, is the idea to use small pharmacological chaperones that can enter the cell, bind to the mutant receptor and thus induce proper folding with subsequent release from the endoplasmic reticulum [147, 148]. With the development of small membrane-permeable AVPR2-receptor antagonists, designed to fit neatly into the binding fold of the receptor, this approach has become feasible and indeed successful in vitro [149–151]. More importantly, a recent trial of a AVP antagonist in five patients with NDI, bearing either the mutation del62-64, R137H or W164S (all of which lead to ER retention), has shown a significant decrease in urine output with a concomitant increase in urine osmolality [152]. Total 24-h urine volume decreased from a mean of 11.9–8.2 L and mean urine osmolality rose from 98 to 170 mOsm/kg and thus the observed effect was modest. Nevertheless, these results hold the promise of a targeted, mutation-specific therapy in patients with NDI. Increasingly, gene therapy is becoming a reality, either by provision of the wild-type gene or by correcting the specific genetic mutation through gene editing [153]. Once safe and efficacious methods to deliver such treatments to the kidney have been developed, these obviously would provide specific treatments for inherited NDI.

Prostaglandin Receptor Agonists

The identification of cAMP as a key messenger in urinary concentration has led to the search for compounds that could increase cAMP in the principal cell independent of AVPR2. Recently, it was shown that agonists for the prostaglandin receptor EP2 and EP4, such as prostaglandin E2 could provide such an alternative pathway [154]. Indeed, in a rat model of NDI these compounds were able to reduce urine output significantly. Yet, giving prostaglandins to treat NDI is in apparent contradiction to the clinically proven efficacy of prostaglandin synthesis inhibition (see above) and more data are needed to resolve this conundrum.

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Part VIII

Renal Neoplasia and Tubulointerstitial Disease

Pediatric Renal Tumors

41

Kathryn S. Sutton and Andrew L. Hong

Introduction

Pediatric renal tumors include a heterogeneous group of diagnoses that vary greatly in both required treatment and long-term outcome. This chapter will review both benign and malignant renal masses, with a particular emphasis on pediatric kidney cancers given their impact on morbidity and mortality. Approximately 600 children are diagnosed with renal cancer each year in the United States, accounting for 7% of all pediatric malignancies. The predominant renal tumor pathology in young children is Wilms tumor, although renal cell carcinoma overtakes Wilms tumor in older adolescents [1, 2]. Additional tumor types of varying malignant potential arising in the kidney include renal medullary carcinoma, malignant rhabdoid tumor, clear cell sarcoma of the kidney, and congenital mesoblastic nephroma (Table 41.1) [3]. Pediatric leukemia and lymphoma can also present as a renal mass, although often there are additional sites of disease [4–6]. Benign kidney masses include metanephric neoplasms and cystic nephroma [3]. Rounding out the differential diagnosis of a pediatric renal mass are tumors that are adjacent to, but not arising from the kidney, most commonly adrenal tumors such as neuroblastoma and adrenal cortical carcinoma, or kidney malformations such as multicystic dysplastic kidneys.

Typical presentations of each diagnosis will be discussed in detail below; however, in general presenting symptoms of pediatric renal tumors may include painless abdominal masses or swelling, abdominal pain, hypertension, and hematuria. The initial workup for pediatric abdominal masses includes ultrasonography and laboratory evaluation, with further imaging recommended based on preliminary findings. Laboratory testing can both help to rule in and classify a renal tumor and to rule out other common pediatric abdominal cancers (Table 41.2).

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Table 41.1 Pediatric renal tumors

Malignant	Wilms tumor (nephroblastoma) Nephroblastomatosis (pre-malignant)
	Clear cell sarcoma of the kidney
	Renal cell carcinoma
	Renal medullary carcinoma
	Malignant rhabdoid tumor
	Ewing sarcoma of the kidney
Low malignant potential	Angiomyolipoma/malignant epithelial angiomyolipoma
	Congenital mesoblastic nephroma
Benign	Cystic nephroma
	Ossifying renal tumor of infancy
	Metanephric adenoma/metanephric stromal tumor/metanephric adenofibroma
Secondary tumors	Leukemia/Lymphoma
	Neuroblastoma (suprarenal)

Table 41.2 Initial laboratory evaluation for pediatric renal masses

Lab	Possible findings/interpretation
Complete Blood Count with differential	Anemia due subcapsular hemorrhage Pancytopenia or blast cells due to leukemia/lymphoma Polycythemia in metanephric adenoma/adenofibroma or renal cell carcinoma
Complete Metabolic Panel	Renal dysfunction Hypercalcemia in congenital mesoblastic nephroma Liver dysfunction in the setting of metastatic disease
Urinalysis	Hematuria
Coagulation studies (PT/PTT)	Acquired Von Willebrand's Disease in Wilms tumor
Urine catecholamines	Elevated in neuroblastoma (arises in suprarenal region)
LDH/Uric acid	Elevated in tumor lysis syndrome, especially in leukemia/lymphoma

Wilms Tumor

Epidemiology

Wilms tumor (WT), also known as nephroblastoma, is named for Max Wilms, a German pathologist and surgeon, who described the tumor in 1899 [7]. Wilms tumor represents the vast majority of pediatric renal tumors, accounting for 85–90% of kidney tumors diagnosed in children younger than age 14 [1]. There are approximately 600 new cases of WT in the United States annually, and worldwide the incidence approaches 1:10,000 children. There is a very slight female predominance, and WT is more common in Blacks and Whites than Asians [8].

Wilms tumor presents most commonly in preschool aged children, with the majority of cases diagnosed before the age of 5 years and nearly all before 10 years. Boys tend to present at a younger age than girls. Approximately 5–10% of WTs are bilateral at diagnosis, and these patients present earlier, with a median age at diagnosis of 31 months as compared to 44 months for patients with unilateral tumors [8]. Ten to 15% of WT are associated with an underlying predisposition syndrome (Table 41.3) [9, 10].

Predisposition Syndromes

The majority of Wilms tumor predisposition syndromes are associated with two genes, *WT1* and *WT2*, both located on chromosome 11 [9]. Patients with predisposition syndromes are more likely to present at a younger age and with bilateral disease [8]. The treatment approach to WT arising in patients with underlying predisposition syndromes requires modification to preserve

Table 41.3 Wilms tumor predisposition syndromes [9–11, 20, 22, 23]

Syndrome	Gene (locus)	Wilms tumor risk
WAGR	*WT1* (11p13)	50%
Denys-Drash	*WT1* (11p13)	>90%
Frasier	*WT1* (11p13)	8%
Beckwith-Weidemann	WT2 locus: *CDKN1C*, *H19*, *LIT1*, *IGF2* (11p15.5)	5% (Can be higher depending on specific genetic finding)
Simpson-Golabi-Behmel	*GPC3* (Xq26), *GPC4* (Xq26), *OFD1* (Xp22)	8%
Sotos	*NSD1* (5q35.3)	<5%
Perlman	*DIS3L2* (2q37.1)	50–75%
Trisomy 13	Chromosome 13	Rare
Trisomy 18	Chromosome 18	>1%
Mulibrey nanism	*TRIM37* (17q22)	7%
Bohring-Optiz	*ASXL1* (20q11.21)	7%
Isolated hemihypertrophy	WT2 locus (11p15.5), among others	5%
Familial Wilms	*FWT1* (17q12-21), *FWT2* (19q13)	Variable, approximately 15–30%
Gorlin (9q22.3 Microdeletion)	*PTCH1* (9q22.3)	<5%
Mosaic Variegated Aneuploidy/ Premature Chromatid Separation	*BUB1* (2q13), *TRIP13* (5p15.33)	>20%
Bloom	*RECQL3* (15q26.1)	<5%
Fanconi Anemia with Bialleic BRCA2/PALB2 mutations	*BRCA2* (13q13.1), *PALB2* (16p12.2)	>20%
DICER1	*DICER1* (14q32.13)	<5%
Li Fraumeni	*TP53* (17p13.1)	<5%
Hyperparathyroidism-Jaw Tumor	*HPRT2* (1q31.2)	<5%
PIK3CA-related Segmental Overgrowth (CLOVE)	*PI3KCA* (3q26.32)	<5%
2q37 microdeletion	2q37.1	3%

normal kidney tissue and function and will be discussed later.

WT1, found at chromosome 11p13, encodes for a transcription factor associated with kidney and gonadal development [9]. Mutations of *WT1* are typically autosomal dominant and the majority arise *de novo* [11]. Deletion of *WT1* results in Wilms tumor-Aniridia-Genitourinary anomalies- Intellectual disability (formerly mental Retardation) (WAGR) Syndrome first described in 1964 [12]. Patients with WAGR Syndrome have an approximately 50% lifetime incidence of Wilms tumor [13]. Aniridia is nearly universal and is due to deletion of the neighboring *PAX6* gene, and developmental delay is present in the majority of patients. Renal failure can occur secondary to nephropathy and glomerulonephritis and occurs in 20–40% of patients [14, 15]. Variable screening recommendations for WT in patients with WAGR Syndrome have been proposed. All groups recommend renal ultrasounds every 3–4 months beginning at the time of diagnosis of the underlying syndrome; however, recommended duration varies from through age 5 to through age 8 years [11, 14, 16, 17]. Point mutations of *WT1* result in multiple syndromes, including Denys-Drash and Frasier Syndromes [10, 18, 19]. Denys-Drash Syndrome portends a greater than 90% incidence of WT [10]. On the contrary, patients with Frasier Syndrome have only an 8% likelihood of developing WT [20]. Both may present with nephropathy and genitourinary abnormalities. Males with Denys-Drash Syndrome may present with hypospadias, ambiguous genitalia, or streak gonads. The associated nephropathy (mesangial sclerosis) commonly progresses to end stage kidney disease (ESKD) by the second decade of life. Frasier Syndrome is associated with focal segmental glomerulosclerosis with later onset of renal failure. Patients with Frasier Syndrome have gonadal dysgenesis and a propensity for development of gonadoblastoma in addition to WT [20].

Syndromes associated with the *WT2* gene found at chromosome 11p15 are associated with imprinting or hypermethylation of *WT2* as well as neighboring genes, including *IGF2* and *CDKN1C*. Due to effects on *IGF2*, these syndromes are frequently associated with body and visceral overgrowth [11, 21]. Beckwith-Weidemann Syndrome (BWS) may present with hemihypertrophy, omphalocele or abdominal hernia, macroglossia, ear creases or pits, and neonatal hypoglycemia [20]. Patients with BWS are at increased risk of multiple embryonal tumors, including Wilms tumor, neuroblastoma, hepatoblastoma, and embryonal rhabdomyosarcoma, as well as adrenal cortical carcinoma. The overall malignancy rate approaches 10%, with WT making up approximately 50% of those cancers. Cancer screening recommendations in BWS may be further tailored depending on the specific genes affected; however, general recommendations include ultrasound every 3 months through age 7: abdominal ultrasound until the fourth birthday to also screen for hepatoblastoma followed by renal ultrasounds until the seventh birthday. In addition, alpha fetoprotein (AFP) and urine catecholamines are employed to screen for hepatoblastoma and neuroblastoma, respectively [11, 21]. Table 41.3 provides a complete list of syndromes predisposing to WT.

Pathology

Wilms tumor is an embryonal tumor, arising from pluripotent fetal metanephric tissue. WTs display triphasic histology, including epithelial, stromal, and blastemal components, though monophasic and biphasic tumors can occur (Fig. 41.1). The epithelial component often resembles rudimentary renal tubules; whereas, the blastemal component consists of undifferentiated small blue cells favoring embryonic renal mesenchyme [24]. The compilation of histologic subtypes is a key feature in defining prognosis and occurs along a continuum with epithelial-predominant tumors behaving less aggressively and blastemal-predominant portending increased risk [25–28].

Wilms tumor histology is further classified as favorable histology Wilms tumor (FHWT) versus anaplastic Wilms tumor (AWT) by the presence or absence of anaplasia, described as large, pleomorphic, hyperchromatic tumor cell nuclei with abnormal multipolar mitotic figures (Fig. 41.1) [25]. Anaplasia may be focal or diffuse, and is present in the tumors of 6–8% of patients. Anaplastic Wilms tumor is more common in older patients and in patients of African descent. Anaplasia, especially when diffuse, is associated with chemotherapy resistance and overall poor prognosis [25, 28–30]. Anaplastic Wilms tumors frequently express aberrant *TP53*, a prominent

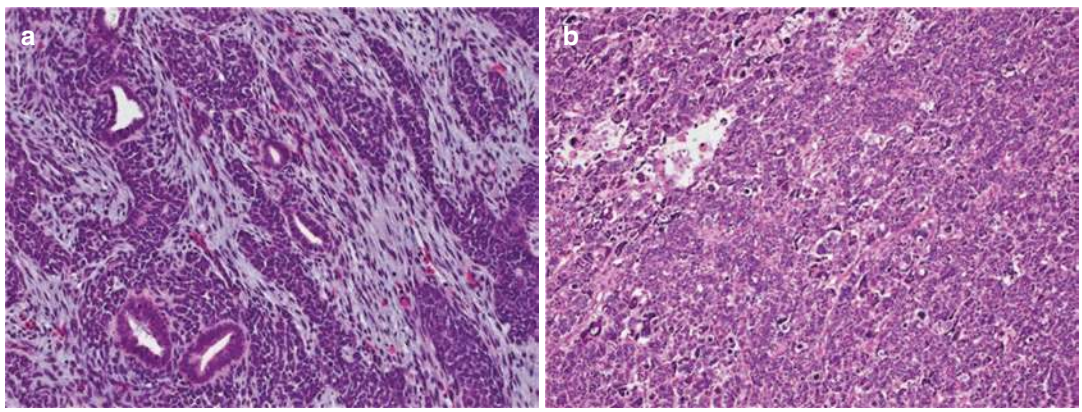

Fig. 41.1 Histology of Wilms tumor—(**a**) Triphasic histology of favorable histology Wilms tumor showing tubule formation by the epithelial component surrounded by blastemal cells separated by pale-colored stroma (200×), (**b**) diffuse anaplastic histology (100×). (Courtesy of Hong Yin, MD; Children's Healthcare of Atlanta)

tumor suppressor gene known to be somatically mutated in many aggressive cancers and associated with the cancer predisposition syndrome, Li Fraumeni Syndrome, when mutated in the germline [31].

Further pathologic characteristics critical for appropriate staging and, therefore, treatment of WT include tumor size and weight; the extent of tumor including involvement of the renal sinus, renal vein, or extension through the renal capsule; margin status following resection; pathologic status of resected peri-renal lymph nodes; and evaluation of additional somatic mutations.

In addition to germline syndromes, somatic mutations commonly found in WTs have both prognostic and therapeutic implications. The most commonly mutated genes in WT include *WT1*, Wnt-signaling pathway genes such as *CTNNB1*, and the oncogene *MYCN*. Therapies targeting several of these frequent mutations are in development [9]. In addition, specific genomic findings, including copy number variations and loss of heterozygosity (LOH), are prognostic in WT, allowing for therapeutic risk-stratification in clinical trials. Adverse biologic markers include LOH of chromosome 1p and 16q, LOH or loss of imprinting at 11p15, gain of 1q, and loss of 17p [9, 31–42].

Nephrogenic Rests and Nephroblastomatosis

Nephrogenic rests and nephroblastomatosis are pre-malignant lesions associated with Wilms tumor. Fetal metanephric cells typically disappear by 36 weeks gestation. Nephrogenic rests are clusters of pluripotent embryonal cells persisting past that developmental time point and into post-natal life [43]. They are common, being incidentally found in 1% of unselected pediatric kidneys at autopsy, 35–40% of kidneys with unilateral WT, and nearly 100% of kidneys with bilateral WTs. Nephroblastomatosis is the presence of multiple or diffuse nephrogenic rests and can be perilobar or intralobar. Nephrogenic rests and nephroblastomatosis may self-resolve, persist, or undergo malignant transformation into WT [44]. Diffuse hyperplastic perilobar nephroblastomatosis (DHPLN) is a specific form of nephroblastomatosis, strictly defined as "massive enlargement of the kidney with a rind-like expansion of the renal cortex of homogeneous signal intensity, and preservation of the renal shape." DHPLN carries an incredibly high likelihood of malignant transformation and treatment with chemotherapy should be strongly considered even before the development of frank WT [43].

Presentation

Wilms tumor most commonly presents as a painless abdominal mass in a young child, although a minority of patients may present with abdominal pain, gross hematuria, fever, or constipation. Rarely, patients will report anorexia or weight loss. Approximately 25–67% of patients are hypertensive at presentation, predominantly secondary to renin-angiotensin system activation in the setting of renal ischemia. These patients frequently require anti-hypertensive medications prior to nephrectomy which can often be weaned in the weeks following tumor removal [45–48]. Quite rarely, male patients with left-sided renal tumors can present with a left-sided varicocele secondary to compression of the left renal and testicular veins. (The right testicular vein does not arise from the right renal vein, so right-sided varicoceles do not occur) [49]. Although not common, patients can present acutely following subcapsular hemorrhage of their tumor. This can lead to rapid abdominal enlargement, acute anemia, blood pressure abnormalities (hyper- or hypotension), and fever and often requires urgent surgical intervention [50–52]. Patients with WT should be assessed for findings of an associated predisposition syndrome as described previously, including hemihypertrophy, macroglossia, aniridia, genitourinary anomalies, and developmental delay.

Metastatic disease is present in approximately 12% of patients at the time of diagnosis. The lungs are by far the most common site of spread (80%), followed by lymph nodes (15–20%) and the liver (15%), with other sites being extremely

rare at the time of presentation. The likelihood of metastatic disease increases with increasing age at diagnosis and with signs of aggressiveness at the primary tumor site [53]. It is rare for a child to present with symptoms related to metastases, which are often only discovered during a thorough staging evaluation.

Evaluation and Staging

Following initial history and physical, pediatric abdominal masses are typically evaluated via ultrasonography. On ultrasound, Wilms tumor can frequently be identified as a well-circumscribed mass of varying echogenicity arising from the kidney. The classic finding is a "claw sign" where the renal parenchyma is seen wrapping around the mass like an open hand or claw [54]. Recommended initial laboratory evaluation includes a complete blood count with differential, complete metabolic panel, urinalysis, coagulation studies, and tumor markers to rule out other common pediatric abdominal tumors (Table 41.2). The complete blood count is important for the evaluation of intra-tumoral hemorrhage, the urinalysis for the evaluation of hematuria, and coagulation studies to evaluate for paraneoplastic acquired Von Willebrand Disease which can accompany WT [55].

Once a renal tumor is suspected on ultrasound, further anatomic imaging of the chest, abdomen, and pelvis should be completed along with consultation of a pediatric oncologist. Abdominopelvic computed tomography (CT) and magnetic resonance imaging (MRI) are both appropriate for further evaluation of renal tumors, although CT is often more facile to obtain at the time of diagnosis (Fig. 41.2). MRI is preferred in cases of suspected bilateral Wilms tumor and in patients with known predisposition syndromes due to improved ability to detect small tumors and nephrogenic rests [54]. In comparison to neuroblastoma, the other most common pediatric abdominal tumor, WTs lack calcifications and push away rather than encase surrounding structures [56]. In addition to further description of the renal mass, including measurement of volume and extent of disease, CT/MRI serve to evaluate for tumor thrombus in the renal vein and inferior vena cava (IVC), assess for pathologic adenopathy, and screen the contralateral kidney and liver for additional sites of disease. Finally, a CT of the chest should be obtained to assess for pulmonary metastases [54].

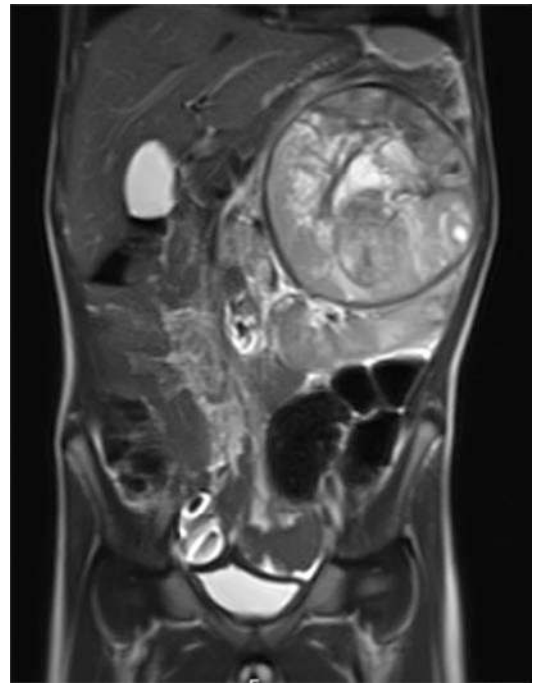

Fig. 41.2 Abdominopelvic T2 sequence magnetic resonance image displaying a left-sided Wilms tumor with left kidney displaced inferiorly and displaying a characteristic "claw sign"

Two different staging systems are employed for Wilms tumor. The varied approaches correspond with the opposing schools of thought regarding treatment approach between the North American and European pediatric oncology cooperative groups. While these differences will be discussed in more detail in the subsequent section "Treatment", it is imperative to understand that the North American (Children's Oncology Group [COG], formerly National Wilms Tumor Study Group [NWTS]) approach to WT includes up front nephrectomy when feasible. This lends itself to a pre-chemotherapy staging system based on extent of local and distant disease, as well as operative findings, including margin

Table 41.4 Renal tumors staging (Children's Oncology Group), applicable to Wilms tumor, clear cell sarcoma of the kidney, malignant rhabdoid tumor, and congenital mesoblastic nephroma; renal cell carcinoma staging discussed separately

Stage	Description
I	Completely resected primary tumor with negative margins Tumor limited to the kidney with renal capsule intact
II	Completely resected primary tumor with negative margins, but with extension outside of the kidney via penetration of the capsule, invasion of soft tissue or renal sinus, involvement of extra-renal blood vessels
III	Residual tumor confined to the abdomen • Incomplete resection/positive margins • Penetration through the peritoneal surface or peritoneal implants • Tumor rupture/spillage • Pre-operative chemotherapy • Pre-operative biopsy • Tumor removed in multiple pieces • Positive lymph nodes in the abdomen/pelvis Tumor excised from the thoracic vena cava or heart
IV	Distant metastases (lung, liver, bone, brain, other) Positive lymph nodes beyond the abdomen/pelvis
V	Bilateral renal tumors

status after nephrectomy and the presence of tumor spill into the peritoneal cavity. In addition, patients are up-staged in this system if pre-nephrectomy biopsy or neoadjuvant chemotherapy are undertaken (Table 41.4). On the contrary, the European equivalent to COG, the International Society for Pediatric Oncology (SIOP), uniformly recommends neoadjuvant chemotherapy and utilizes a staging system incorporating post-chemotherapy response. Additionally, pre-operative biopsy does not necessarily require up-staging of the patient in the SIOP system [57].

Treatment

The treatment of Wilms tumor has evolved over the past half century through the efforts of large collaborative groups. In North America this effort was undertaken initially by the NWTS, which has now become COG, while the European approach was developed by SIOP. The treatment paradigms now practiced by both groups espouse multimodal therapy (surgery, chemotherapy, and radiation) tailored by tumor risk classification. SIOP risk assignment is based upon stage, patient age, and post-chemotherapy histology, including extent of tumor necrosis, degree of blastemal tissue, and the presence of focal or diffuse anaplasia. In comparison, COG risk definitions include additional prognostic factors such as tumor weight and adverse biomarkers such as LOH of 1p and 16q, in addition to stage and histology (favorable vs. anaplastic) [57–59].

In general, the surgical approach for Wilms tumor is radical nephrectomy with sampling of the most proximal draining retroperitoneal lymph nodes. Ideally, the tumor is removed via a trans-abdominal incision with the capsule intact and tumor thrombus removed *en bloc* [58]. COG advocates for nephrectomy at the time of diagnosis whenever feasible. Reasons to delay nephrectomy in the setting of unilateral disease may include tumor thrombus extending above the hepatic veins, tumor invading contiguous organs, high risk of tumor spill or gross residual disease, or pulmonary compromise at the time of diagnosis. In such cases, biopsy is acceptable, although this does up-stage the patient. Nephrectomy should then be performed by weeks 6–12 of therapy. As discussed previously, the European standard is delayed nephrectomy regardless of initial tumor characteristics. This method allows for the incorporation of histologic chemotherapy response into further treatment decisions [57]. Despite their differences, both groups have achieved overall excellent response rates (see section "Prognosis and Outcomes" below).

In the setting of bilateral disease or a known Wilms tumor predisposition syndrome, focus

changes to preservation of normal renal parenchyma. This nephron-sparing approach includes neoadjuvant chemotherapy, which may proceed with or without upfront biopsy, followed by partial nephrectomy(ies) with a surgeon with such expertise depending upon tumor response [58, 60, 61].

A small subset of very low-risk patients may be cured by surgery alone, including patients younger than 2 years of age with small stage I tumors. This approach was initially established in the early NWTS trials and confirmed through a later COG trial for low-risk FHWT [40].

The vast majority of patients require chemotherapy in addition to surgery for cure of Wilms tumor. The goal of chemotherapy is to shrink known tumors and prevent the development of distant metastatic disease. Chemotherapy for FHWT builds upon a backbone of two drugs, vincristine and dactinomycin initially established in the first NWTS trial completed in 1975 [62]. Patients with low-stage disease have excellent cure rates with these two drugs alone [42]. Treatment for stage III–IV tumors or tumors with adverse prognostics markers such as combined LOH at 1p and 16q, includes at minimum the addition of the anthracycline doxorubicin and treatment for longer duration [39]. Additional agents such as cyclophosphamide and etoposide may be required for stage IV patients based on response of pulmonary metastatic disease and the presence of extra-pulmonary metastases [63]. Anaplastic Wilms tumor routinely requires aggressive chemotherapy, especially in the setting of diffuse anaplasia [30].

In patients with FHWT, radiation is reserved for patients with stage III–IV disease. Radiation to the primary renal tumor site is confined to the flank unless there is concern for gross tumor spill or peritoneal spread and is given at modest doses of 10.5–10.8 Gy. Radiation is also indicated for local control of extra-abdominal sites of metastatic disease, except that pulmonary radiation may be avoided in patients with a complete response of pulmonary disease to chemotherapy by week 6 [63]. Patients with stage II–IV AWT also require radiotherapy, while results from both COG and SIOP suggest omission of radiation for patients with stage I disease is possible in the setting of three-drug chemotherapy including doxorubicin [64, 65].

Prognosis and Outcomes

The overall prognosis for Wilms tumor has improved significantly since the adoption of multimodal therapy from 0% overall survival in the early twentieth century to 90% today; however, there remain a significant number of patients with more guarded outcomes [2, 58, 62, 66, 67]. In addition, the excellent outcomes for patients with low-risk disease require evaluation of when and where therapy can be further de-escalated.

Prognosis depends mostly upon stage and histology. Patients with low stage and favorable histology with epithelial predominance fare best even with limited therapy, while patients with higher stage and blastemal predominance or anaplasia fare the worst [26, 27, 30, 40, 63, 68]. Additional indicators of poor prognosis include older age at diagnosis, larger tumor size, and adverse chromosomal abnormalities including LOH at 1p and 16q, gain of 1q, loss of 11p15 (specific to very low-risk WT), and loss of 17p [9, 31–41, 57, 69–73]. Outcomes based on the most recently completed series of COG clinical trials are reviewed in Table 41.5. SIOP results have been similar. Salvage after relapse is possible, with a 5-year overall survival of 50%. Outcomes following relapse depend upon initial stage and treatment with patients initially receiving fewer chemotherapy agents faring better than those who required more aggressive treatment up front [74–76].

Late Effects

The multimodal therapy required to produce such excellent cure rates for Wilms tumor leads to significant late effects, with approximately 20% of long-term survivors reporting severe (grade 3–5) late effects at 20 years after diagnosis [78, 79]. In general, the adverse effects expected vary significantly based upon the presence of bilateral renal

Table 41.5 Results of COG renal tumors studies

Study	Stage/histology	Subgroup	Chemotherapy	Outcomes	Reference
AREN0532	Stage I FHWT	Very low-risk (age < 2 years, tumor < 550 g)	None	4-year EFS: 89.7% 4-year OS: 100%	[40]
	Stage I/II FHWT	LOH 1p + 16q	EE4A → DD4A	4-year EFS: 87.3% 4-year OS: 100%	[39]
	Stage III FHWT	No LOH 1p + 16q	DD4A	4-year EFS: 88% 4-year OS: 97%	[41]
		Negative LN and no LOH 1p or 16q	DD4A	4-year EFS: 96.7% 4-year OS: 99.4%	[41]
		Positive LN and either LOH 1p or 16q	DD4A	4-year EFS: 74% 4-year OS 92.4%	[41]
AREN0533	Stage III/IV FHWT	LOH 1p + 16q	DD4A → Regimen M	4-year EFS: 90.2% 4-year OS: 96.1%	[39]
	Stage IV FHWT	All	DD4A or DD4A→ Regimen M	4-year EFS: 85.4% 4-year OS: 95.6%	[63]
		LOH 1p + 16q	DD4A → Regimen M	4-year EFS: 100% 4-year OS: 100%	[63]
		No LOH, isolated lung mets, RCR	DD4A	4-year EFS: 79.5% 4-year OS: 96.1%	[63]
		No LOH, isolated lung mets, SIR	DD4A→ Regimen M	4-year EFS: 88.5% 4-year OS: 95.4%	[63]
		Isolated lung mets, RCR, gain of 1q	DD4A	4-year EFS: 57% 4-year OS: 89%	[63]
		Isolated lung mets, RCR, no gain of 1q	DD4A	4-year EFS: 86% 4-year OS: 97%	[63]
		Isolated lung mets, SIR, gain of 1q	DD4A → Regimen M	4-years EFS: 86% 4-years OS: 93%	[63]
		Isolated lung mets, SIR, no gain of 1q	DD4A → Regimen M	4-year EFS: 92% 4-year OS: 96%	[63]
AREN0534	Stage V (bilateral) FHWT	All	Most VAD → then per histology	4-year EFS: 82.1% 4-year OS: 94.9% 39% retained parts of both kidneys	[61]
	Variable	Unilateral tumors, multicentric or in predisposed patients	Variable	4-year EFS: 94% 4-year OS: 100%	[77]

(continued)

Table 41.5 (continued)

Study	Stage/histology	Subgroup	Chemotherapy	Outcomes	Reference
AREN0321	Stage I FAWT or DAWT	All	DD-4A	4-year EFS: 100% 4-year OS: 100%	[65]
	Stage II DAWT	All	Regimen UH-1	4-year EFS: 86.7% 4-year OS: 86.2%	[30]
	Stage III DAWT	All	Regimen UH-1	4-year EFS: 80.9% 4-year OS: 88.6%	[30]
	Stage IV DAWT	All	Regimen UH-1 or VI + Regimen UH-2	4-year EFS: 41.7% 4-year OS: 49.2%	[30]

FHWT favorable histology Wilms tumor, *DAWT* diffuse anaplastic Wilms tumor, *EFS* event free survival, *OS* overall survival, *Mets* metastases, *RCR* rapid complete response, *SIR* slow incomplete response, *EE4A* vincristine/actinomycin, *DD4A* vincristine/actinomycin/doxorubicin, *Regimen M* vincristine/actinomycin/doxorubicin/etoposide/cyclophosphamide, *VAD* vincristine/actinomycin/doxorubicin, *Regimen UH-1* vincristine/actinomycin/doxorubicin/carboplatin/etoposide/cyclophosphamide, *VI* vincristine/irinotecan, *Regimen UH-2* vincristine/actinomycin/doxorubicin/carboplatin/etoposide/cyclophosphamide/irinotecan

tumors, chemotherapy regimen, and use of radiation.

The cumulative incidence of ESKD in patients with unilateral WT at 20 years after diagnosis is less than 1%; whereas, the incidence increases to 12% in patients with bilateral WT [80]. The rate is increased in patients with metachronous presentation of bilateral disease and is as high as 19.3% [81]. The risk of renal disease is also impacted by underlying predisposition syndromes associated with nephropathy such as WAGR, Denys-Drash, and Frasier Syndromes, as well as the presence of intralobar nephrogenic rests [13, 80, 81]. Lower-stage chronic kidney disease occurs, however, in 23–55% of patients, and a significant proportion of patients have chronic hypertension by their mid-20s [82–84]. Patients requiring only unilateral nephrectomy without nephrotoxic chemotherapy or radiation have a much lower risk of chronic kidney disease and hypertension [85].

Renal toxicity can occur due to nephron loss during surgical resection and secondary to chemotherapy and ionizing radiation. The etiology of renal failure is more commonly surgical for patients with bilateral disease who require either bilateral nephrectomies or partial nephrectomies resulting in less than 25% residual renal parenchyma [80]. Patients requiring only unilateral nephrectomy are typically able to maintain renal function through compensatory hypertrophy of the remaining kidney [86]. The frequency of nephron-sparing surgery in patients with bilateral disease and those with WT arising in a solitary kidney is an on-going focus of research. On the COG study AREN0534, 39% of patients with bilateral WT retained parts of both kidneys and only 2.5% required bilateral nephrectomies following neoadjuvant chemotherapy [61].

The chemotherapy agents required for the majority of Wilms tumor patients (vincristine, dactinomycin, and doxorubicin) are rarely implicated in long-term renal toxicity; however, the alkylating agent ifosfamide and platinum agent carboplatin utilized for augmentation of therapy in more aggressive disease or in the setting of relapse may adversely affect renal function [87]. This risk is higher in younger patients and when combined with other nephrotoxic exposures, including additional nephrotoxic medications, renal radiation, and nephrectomy [88]. Radiation nephritis is the cause of renal failure in a minority of patients and is rare at the typical radiation doses used in the treatment of WT [88, 89]. Recommendations for screening for renal late effects is variable based on treatment received. Most patients treated for WT will require annual blood pressure monitoring, creatinine assessment, and urinalysis, with electrolyte monitoring recommended at the time of entry into long-term follow-up and then as needed. Counseling is required regarding risk of renal injury in the setting of a single kidney and use of nephrotoxic medication should be limited [90].

The most notable non-renal late effects for these patients include cardiac toxicity in the setting of doxorubicin exposure, secondary malignancies and poor growth due to both chemotherapy and radiation exposure, and infertility for patients requiring alkylating agents or carboplatin [91].

Clear Cell Sarcoma of the Kidney

Epidemiology

Clear cell sarcoma of the kidney (CCSK) is the second most common renal neoplasm in young children, accounting for 2–5% of primary kidney cancers in this demographic [92, 93]. It was first described by Kidd in 1970 and subsequently noted to be distinct from Wilms tumor due to its propensity to metastasize to bone [25, 92–95]. It presents more commonly in males than females at a 2:1 ratio. Clear cell sarcoma of the kidney is seen most commonly in toddlers with a median age at diagnosis of 36 months, and 50% arising in 2–3 year olds in the NTWS trials. It is incredibly rare in infants less than 6 months [92, 93, 96]. There are no known predisposition syndromes, although there is at least one case reported in a patient with Fanconi anemia [97].

Pathology

Clear cell sarcoma of the kidney typically presents as a large, unilateral, well-circumscribed mass. CCSK displays heterogeneous histology, with upwards of nine different histologic types described. Most tumors include the classic variant described as nests and cords of round or oval cells separated by fibrovascular septae. The clear cell appearance arises from extracellular mucopolysaccharide matrix located between the cord cells. Additional variants include: myxoid, sclerosing, cellular, epithelioid, palisading, spindle cell, storiform, and anaplastic patterns [92, 93, 98]. Most tumors contain a mixture of subtypes and, unlike WT, histologic subtypes are not used for risk stratification. There are two predominant known somatic genetic findings in CCSK which are mutually exclusive: a translocation involving *YWHAE* and *NUTM2B/E* (previously *FAM22*), t(10;17)(q22;p13), and internal tandem duplications of the BCL-6 co-receptor, *BCOR*. A third subset of patients displays neither event [92, 99, 100]. *TP53* is located at 17p13 and is overexpressed in the small subset of anaplastic CCSK, but does not seem to be a predominant driver in CCSK. A BCOR fusion has also been identified in a small case series of CCSK [99, 101]. Like histology, to date specific genetic findings have not been utilized for risk classification.

Presentation and Evaluation

Similar to WT, CCSK presents in young children as a painless abdominal mass or distension, although abdominal pain, hematuria, vomiting, anorexia, fever, constipation, and hypertension have all been reported. In addition, patients with metastatic disease may present with symptoms of bone pain or mass [92, 100, 102]. The initial workup mimics that of WT, including ultrasound followed by cross sectional imaging with CT or MRI. There are no defining imaging characteristics to differentiate CCSK from the much more common WT, and the diagnosis is often not made until postoperative pathologic review of the nephrectomy specimen.

The staging of CCSK is similar to that for WT. A minority (4–7%) of cases of CCSK are metastatic at diagnosis. The pattern of metastatic spread differs from WT, with CCSK having a strong propensity for bone and brain, although like WT can also affect regional lymph nodes and liver. Bilateral tumors are exceedingly rare [92, 93, 96]. As a result, in addition to a chest CT, the metastatic workup includes a brain MRI and systemic imaging for bony disease such as nuclear medicine bone scan or positron emission tomography scan.

Treatment

The treatment of CCSK is multimodal and includes surgery, chemotherapy, and radiation. The chemotherapy and radiation is more aggressive than that required for many patients with WT.

The surgical approach mimics that for WT, with COG advocating for upfront nephrectomy when feasible while SIOP recommends delayed nephrectomy following neoadjuvant chemotherapy [100, 103]. As in WT, sampling of lymph nodes is important for accurate staging [96, 104]. Given the rarity of bilateral disease, the focus on nephron-sparing surgery is less consequential in CCSK. Surgical metastasectomy is considered for feasible sites and level of disease burden.

Chemotherapy recommendations for CCSK are similar to those for high-risk WT. In the SIOP approach, patients with localized disease are treated initially with vincristine and dactinomycin, with doxorubicin added for patients with distant metastatic disease at diagnosis [103]. Both SIOP and NWTS/COG have demonstrated improved survival when doxorubicin is included in the treatment of CCSK, so the anthracycline is started or continued after nephrectomy once the diagnosis of CCSK is confirmed [93, 103]. In addition, the alkylating agents ifosfamide and cyclophosphamide along with etoposide are added for all stages [103]. In North America, patients with CCSK have been treated with a regimen of cyclophosphamide and etoposide alternating with vincristine, doxorubicin, and

cyclophosphamide since the study NWTS-5 in the mid-1990s [92, 104].

Most patients with CCSK require additional local control with radiation. In Europe, stage I CCSK patients have been spared radiation. A study in the United Kingdom omitted radiation for stage II patients, but revealed a high rate of local treatment failures, emphasizing the importance of radiotherapy in this group [92, 96]. Both collaborative groups recommend radiation for local control of metastatic disease [103, 104].

Prognosis and Outcomes

Outcomes for patients with CCSK are more guarded, leading to its label as a high-risk renal tumor. Prognosis depends most notably upon stage, with 5-year event-free survival (EFS) of 91% and overall survival (OS) of 98% for patients with stage I/II disease on the COG trial NWTS-5, compared with 79% and 90% for stage III/IV patients. The relatively poor prognosis is most pronounced in the metastatic patients, who when evaluated individually had a 5-year EFS of only 29% and OS of 36% [104]. Results by stage follow a similar trend in SIOP trials, with the 5-year EFS and OS for stage I patients reported as 79% and 87%, respectively, and that for stage IV being 59% and 73%, respectively [96]. Patients who fall outside of the typical age-range of 2–3 years at diagnosis also seem to fare more poorly [93, 96]. Clear cell sarcoma of the kidney differs from other pediatric renal tumors concerning the timing of relapse. Although most relapses occur by 3 years, a small subset of patients present with recurrent tumor 5–10 years after initial presentation, necessitating prolonged surveillance. The frequency of late relapses does, however, appear to be decreasing in the era of modern chemotherapy regimens [92, 100, 103, 104]. Most relapses are to metastatic sites, including brain, lung, and bone. Patients with relapsed disease are potentially salvageable with aggressive multimodal therapy; therefore, long-term follow-up to detect potentially treatable relapses is critical [105].

Late Effects

The late effects following treatment for CCSK are less well studied than that of WT due to the significantly smaller patient population. In general, the renal long-term complications of unilateral nephrectomy are the same as for WT, and the need for bilateral renal surgeries is essentially non-existent. However, the chemotherapy used up front, most notably ifosfamide and carboplatin, is more nephrotoxic than the therapy required for the majority of WT patients, and most patients require radiation. Nearly all patients will require routine monitoring of renal function and blood pressure. The risk for non-renal late effects is higher secondary to the augmented therapy required for all patients with CCSK and includes cardiac toxicity, secondary malignancies, poor growth, and fertility concerns.

Malignant Rhabdoid Tumor

Malignant rhabdoid tumor (MRT) of the kidney was identified in the late 1970s and early 1980s as part of the NWTS [106]. This tumor is an aggressive cancer with poor prognosis despite intensive chemotherapy, radiation therapy, and surgical management. These cancers share biology with renal medullary carcinomas (below), atypical teratoid rhabdoid tumors, and epithelioid sarcomas through the loss of *SMARCB1* and less commonly *SMARCA4*, both members of the SWI/SNF chromatin-remodeling complex. Rhabdoid tumor only causes 3% of pediatric renal tumors [107]. Studies have shown that children with rhabdoid tumors were demographically similar to case controls [108] and usually presented in the first year of life [109–111].

There are two known predisposition syndromes associated with MRT: rhabdoid tumor predisposition syndromes 1 and 2 (RTPS1/2). Patients have germline pathogenic variants in *SMARCB1* and *SMARCA4* [112]. These patients usually develop cancer at a median age of 4–7 months; whereas, those with sporadic MRT have a median age of presentation of 18 months. There are other germline variants of *SMARCB1*

and *SMARCA4* that lead to Coffin-Siris Syndrome, but these mutations are not known to lead to cancer. Recently, SIOP has put forth a set of recommendations for patients/probands with RTPS [112].

SMARCB1 is part of the SWI/SNF complex and serves as a tumor suppressor. This complex is preserved across species and affects many cancer related pathways such as the Hedgehog-Gli, Wnt/b-catenin, and retinoblastoma pathways along with pathways involving cell motility and differentiation [113–115].

Patients present similarly to others with abdominal masses, but in some cases disease progression is swift, prompting emergent chemotherapy. Staging of MRT is the same as for WT and CCSK. Currently, therapy is a combination of chemotherapy, surgery, and radiation therapy (depending on the age of the patient). There is suggestion that lower-stage MRTs are able to achieve cure with a 4-year overall survival in the 40% range. Yet, many patients have higher-stage disease, with 4-year overall survival <20% [109–111, 116, 117].

Pediatric Renal Cell Carcinoma

Renal cell carcinomas (RCC) are a group of heterogenous kidney tumors predominantly seen in adults. However, RCCs occur in children and young adults as well [118].

Pediatric and adolescent/young adult (AYA) renal cell carcinomas (pRCCs) account for 2–4% of childhood kidney cancers [119]. When further subtyped, MiT-RCC (or translocation RCC) are the most common RCC in childhood, causing 42% of RCCs. Other RCCs include papillary, chromophobe, clear-cell (the most common kidney cancer in adults), renal medullary carcinomas (RMC; further discussed below), fumarate hydratase-deficient, succinate dehydrogenase-deficient, TSC-associated (further discussed below), ALK-rearranged, thyroid-like, and myoepithelial carcinomas (see Fig. 41.3) [118]. Another 7–8% of RCCs are unclassified.

A number of predisposition genes have been implicated in this group of heterogenous cancers [120]. These include *VHL* (Von Hippel-Lindau disease and clear cell RCCs), *MET* (hereditary

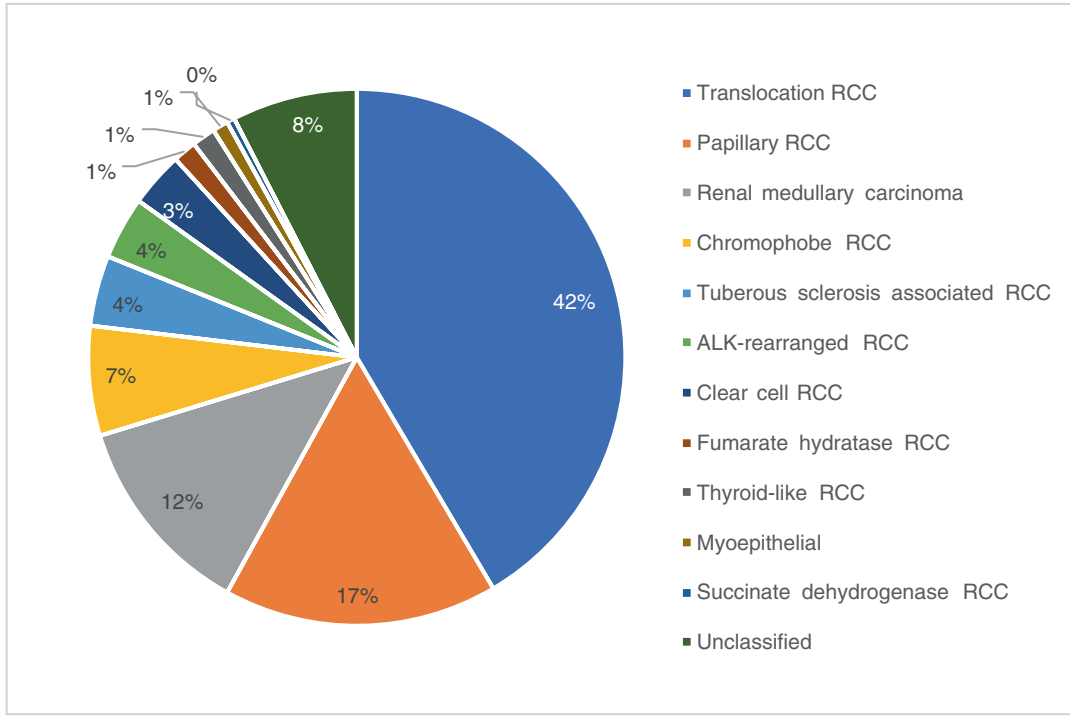

Fig. 41.3 Distribution of 212 pediatric and young adult RCCs from Cajaiba et al. [118]

papillary renal carcinoma; HPRC), *FLCN* (Birt-Hogg-Dube syndrome and various RCCs), *FH* (hereditary leiomyomatosis and renal cell carcinoma; HLRCC), *SDHB/C/D* (Succinate dehydrogenase-deficient renal cell carcinoma), *TSC1/2* (tuberous sclerosis complex; TSC), and mutations in *BAP1* and *MiTF*. Presentations of such tumors can occur in the second decade, but some are not seen until the sixth or seventh decade (e.g., HPRC). While the pathogenesis of cancer predisposition syndromes such as VHL have been well characterized [121], other genes of importance remain an area of study.

The clinical presentation of most pRCCs is similar to that of a child or young adult with any new renal tumor. Usually, the patient will have a painless but enlarging abdominal mass. Occasionally, there will be microscopic or gross hematuria depending on the location of the tumor, unexplained hypertension, or polycythemia. As described for WT, workup usually proceeds with imaging of the abdomen via ultrasound followed by a CT of the chest and CT or MRI of the abdomen and pelvis. Outside of a classical "claw sign," which usually is suggestive of a WT, the COG approach is to either request a pediatric surgical consultation to determine the feasibility of a nephrectomy or biopsy. In cases with bilateral tumors and depending on the patient's age, further discussions are needed to determine if upfront chemotherapy is preferred over biopsy.

Staging of pRCCs is based on the adult RCC staging system, the American Joint Committee on Cancer (AJCC)—TNM Staging System for Kidney Cancer [122]. The primary tumor is assessed by size in greatest dimension (T1 is 7.0 cm or less while T2 is greater than 7.0 cm) and whether the tumor has invaded or extended into the renal veins or above the diaphragm (T3a–c) or beyond Gerota's fascia (T4). Regional lymph nodes are then assessed as the lack of lymph node involvement (N0); involvement of a single lymph node (N1); or of more than one (N2). Metastasis is a binary categorization (M0 for no distant metastasis and M1 for distant metastasis). Based on the TNM status, stage grouping is assigned. Primary tumors at or less than 7.0 cm in dimension are classified as stage I, while stage II is larger than 7.0 cm in dimension. Single regional lymph node involvement (N1) or invasion of the renal veins (T3a–c) is considered stage III. Stage IV disease includes any T1–3 which has multiple regional lymph node involvement (N2) or distant metastasis (M1) and includes tumors that invade Gerota's fascia (T4).

While it is unclear whether the biology of pRCCs is similar to adult RCCs, there is evidence that outcomes are different. COG AREN0321, a phase II study, prospectively assessed outcomes in children and young adults with pRCCs. If complete resection was done, there was no further therapy. For those who had incomplete resections, given the generally poor outcomes and lack of definitive therapies, institutional preference dictated treatment options (e.g. immunotherapy or tyrosine kinase inhibitors). Those with stage I, II, and III disease had 96%, 100% and 88% 4-year overall survival, respectively [123]. However, those with stage IV disease had poor outcomes, with a 29% 4-year overall survival. Primary deaths occurred in patients with either translocation RCC or renal medullary carcinoma.

In summary, pRCCs are a heterogeneous group of kidney cancers for which some have an underlying cancer predisposition syndrome. This group of cancers make up the second most common kidney cancers in children, with the majority occurring in adolescent patients. Short-term outcomes for stage I–III pRCCs remain good with a 4-year overall survival in the 88–100% range. However, stage IV pRCCs (primarily translocation RCCs and renal medullary carcinomas) have poor outcomes.

Renal Medullary Carcinoma

Renal medullary carcinoma (RMC) is a subtype of renal cell carcinoma driven by loss of *SMARCB1*. Unlike malignant rhabdoid tumor, RMC occurs primarily in adolescents and young adults of African descent with sickle cell trait [124–128]. Estimated prevalence based on United States data is 5 in 100,000 patients with sickle cell trait [126, 129]. Although sickle cell trait is the primary hemoglobinopathy associated

with this cancer, there are case reports of other hemoglobinopathies such as sickle cell disease (HbSC and HbSS). There is no known predisposition syndrome other than the association with sickle cell trait. The gene implicated in RMC is *SMARCB1,* which has been discussed in reference to MRT above. Here, the primary mode of disruption is through a translocation event.

Currently, there is no standard of care treatment for RMC given the rarity of this disease and age of presentation (around 10–40 years). However, general consensus among physicians involved in the care of patients with RMC has been to treat with a platinum agent along with other chemotherapy, nephrectomy, and radiation therapy [130, 131]. Despite this aggressive approach, outcomes for RMC remain very poor as overall survival ranges from 4–5 months to 17–18 months for those with or without metastatic disease, respectively [129, 132].

Renal Angiomyolipoma and Epithelioid Angiomyolipoma

Renal angiomyolipomas (AML) are another rare kidney tumor which are considered benign tumors, but may lead to complications given the location [133]. First identified in 1900, AML describes the histopathological features of abnormal blood vessels with components of smooth muscle and adipose tissue [134]. Even more rare is epithelioid angiomyolipoma (EAML), a subtype of angiomyolipoma which is defined by having >10% epithelioid cells by histopathology. Renal AML, depending on the populations studied, affects women more than men [134, 135]. There is a bimodal distribution for the time of presentation. For those with an underlying genetic predisposition, they usually present during the first several decades, while sporadic renal AMLs tend to occur in the fourth or fifth decade [134–136].

Predisposition for renal AML has been associated with tuberous sclerosis complex (TSC) or lymphangioleiomyomatosis (LAM). Genes involved in TSC or LAM include *TSC1* and *TSC2*. TSC1 and TSC2 form a complex which inhibits downstream targets of mechanistic target of rapamycin (mTOR) [137]. For patients with TSC diagnosed in childhood, renal AML occur in over 50% of patients, typically arising in adolescents/young adults [138].

The clinical presentation for renal AMLs differs slightly from other renal tumors as hemorrhage is more commonly seen as compared to microscopic or gross hematuria [134]. Patients also present with abdominal pain or flank pain. AML is likely in a child with TSC presenting with a new renal mass.

Prior management was a complete or partial nephrectomy, but as the natural history of this tumor has been studied for the past century, more conservative approaches are being taken [139]. These lesions grow slowly so surveillance may be used as an initial approach in many patients. Medical therapy with an mTOR inhibitor, everolimus, is an option in select patients (sirolimus is also effective). Outstanding questions remain regarding the duration of therapy as lesions may regrow after a year of treatment. Recent studies have looked at extending therapy to 2 years with minimal regrowth during the second year of therapy. Overall response rate was 44% in patients with TSC or LAM [140]. These findings were further validated in the phase III EXIST-2 trial and the follow-up extension phase where patients received treatment for up to 4 years [141, 142]. Currently, surgery is not recommended, particularly for those with TSC given the potential growth of new renal AMLs in the remaining tissue, thereby compromising renal function further. Another approach is embolization of the vascular supply for the renal AMLs, but this is considered second-line therapy. For EAMLs, little is available to suggest a standard of care. Furthermore, for patients with a known history of TSC, abdominal imaging (ideally MRI) is recommended every 1–3 years throughout the patient's lifetime.

Ewing Sarcoma of the kidney

Ewing sarcoma of the kidney is a rare renal tumor with few cases in the literature. As the name implies, this renal tumor has a fusion oncoprotein between *EWS* and members of the

ETS family such as *FLI1* or *ERG*. Furthermore, these tumors express CD99, which can be detected by immunohistochemistry. The epidemiology of this cancer is not well described given the rarity and is limited to single institution experiences. Furthermore, there is no known predisposition syndrome related to this particular renal tumor.

Here, we will describe the M.D. Anderson experience as it is the most comprehensive institutional review to date of patients presenting with this renal tumor over 23 years [143]. Patients with Ewing sarcoma of the kidney presented with new flank or abdominal pain and/or hematuria. Age of presentation ranged from 8 to 69 years, with a median age at diagnosis of 30.5 years.

Staging was as follows: Group I had tumor confined to the kidney, Group II had local extension, and Group III had metastatic disease. Therapy included nephrectomy (Groups I and II primarily had upfront surgery, whereas Group III patients had biopsy initially and then subsequent nephrectomy with or without neoadjuvant chemotherapy), chemotherapy, and in some cases radiation therapy. Chemotherapy regimens included those utilized to treat extra-renal Ewing sarcomas, but a limitation noted by the authors was that this was inconsistent over the 23-year experience.

Overall, outcomes were poor. Four-year event free survival was 43% and overall survival was 63%. Those with metastatic disease had poorer outcomes. For those with Group I disease, nephrectomy followed by chemotherapy had a more favorable outcome, suggestive of potential for cure.

Congenital Mesoblastic Nephroma

Congenital mesoblastic nephroma (CMN) is a renal tumor that occurs in infancy. It has a low malignant potential and accounts for 2–3% of all pediatric renal tumors [144–146]. With respect to predisposition syndromes, there has been a rare association of Beckwith-Wiedemann syndrome in several case reports. Otherwise, congenital anomalies have been identified in several patients, but there have been no other constitutional syndromes identified. There are three subtypes of CMNs: classic, cellular and mixed (both classic and cellular) type. The genomics of the classic and mixed type of CMNs have not been well characterized. However in cellular, the most common genetic aberration is a translocation of t(12;15)(p13;q25) leading to a fusion of *ETV6* and *NTRK3* [147, 148]. Furthermore, trisomy 11 has been identified in this group.

CMN may present as either a mass detected during a prenatal ultrasound or in an infant found to have a new abdominal mass, hypertension, or hematuria. There may also be polyhydramnios, hypercalcemia, and hyperreninemia, with the latter two resolving following resection of the tumor [146]. Staging for CMNs follows that of WT, MRT, and CCSK. Please refer to staging in those sections. Treatment for these patients has primarily been surgical, with less consensus on postoperative therapies such as chemotherapy and radiation therapy.

The SIOP/German Society of Oncology and Hematology (GPOH) experience with CMNs showed that 5-year relapse free survival and overall survival were 93.8% and 96.1%, respectively [144]. Those with cellular or mixed MN had inferior relapse free survival as compared to classic MN, but overall survival was similar across these subtypes. This experience was similar to that of the United Kingdom Children's Cancer and Leukaemia Group [149]. Similar to that of other renal tumors, long-term issues relate to caring for a patient with a solitary kidney.

Cystic Tumors

Cystic tumors are rare and usually benign renal tumors which account for <1% of pediatric renal tumors [150]. These are further sub-categorized into cystic nephromas (CN) or cystic partially differentiated nephroblastoma (CPDN). In the case of CNs, there is an association with *DICER1* [151] and *DICER1* syndromes which may lead to tumors in the lungs, ovaries, thyroid and kidneys. Patients usually present with CNs in the first few years of life or during the AYA period. There is a

skew toward females being affected. For CPDN, there is no known genetic predisposition.

Children present with a new abdominal mass (may be an incidental finding unless there is a known personal or family history for *DICER1)*. Bosniak imaging scores (based on classifying adult renal cysts from a benign simple cyst to a malignant cystic mass) allows one to assess concerns for malignancy based on imaging [152]. Staging in CNs or CPDNs requires chest to pelvis imaging given risks of identifying an underlying *DICER1* syndrome. Pathology and treatment is primarily surgical in nature and, given the rarity, it is unclear if chemotherapy plays a role in these relatively benign tumors [150]. However, there is an association with *DICER1*-renal sarcomas in CNs suggestive that similar to pleuropulmonary blastoma in the lungs, there is a range of potential tumors that can develop in the kidney for those with *DICER1* syndrome [153].

Overall outcomes from small patient cohorts in national or international trials suggests that CNs or CPDNs are benign in nature. Given the potential risk of additional malignancies in patients with CNs in particular, referral to a cancer predisposition clinic is warranted.

Ossifying Renal Tumor of Infancy

Ossifying renal tumor of infancy (ORTI) was first described in 1980 [154]. It is extremely rare, with well under 50 cases reported in the literature [155]. Approximately 90% present in the first year of life and the oldest reported patient presented at 30 months. There is a strong male predominance [156, 157]. It most commonly presents with gross hematuria, although rarely a palpable abdominal mass is appreciated [156, 158]. Reported cases have been exclusively unilateral, and ORTI carries no known risk of metastasis [155, 156]. Imaging reveals an intact renal outline with a calcified intrapelvic mass which is hypointense on T2-weighted MRI [159]. Upon resection, ORTI is typically attached to a papilla within the calyceal lumen. On histopathologic examination, ORTI are composed of osteoid, osteoblastic cells, and spindle cells. The degree of ossification increases with older age at presentation. Interestingly, the spindle cell component may represent hyperplastic intralobar nephrogenic rests (see section "Nephrogenic Rests and Nephroblastomatosis" under Wilms Tumor) while the osseous component may be urothelial in origin [157]. No predisposition syndromes are known to be associated with ORTI. Clonal trisomy 4 has been detected in multiple tumor specimens [156, 160].

Although quite rare, knowledge of ORTI, including the typical presentation and imaging appearance, is important. Treatment for ORTI is surgical and may be via complete or partial nephrectomy as dictated by size and location within the kidney. There is no requirement for systemic therapy and to date no recurrences have been reported [155, 156]. The late effects of treatment are limited to the risk of nephron loss due to nephrectomy.

Metanephric Tumors

Metanephric adenoma (MA), metanephric adenofibroma (MAF), and metanephric stromal tumor (MST) are described together as metanephric tumors in the 2016 World Health Organization Classification of tumors of the kidney [161]. These three neoplasms share morphologic features with WT and are considered by some authors to represent differentiated tumors along the same spectrum [162]. Metanephric adenoma is purely epithelial and can closely resemble epithelial-predominant WT, MST is purely stromal, and MAF is biphasic, containing both epithelial and stromal elements. There are no reliable distinguishing imaging features allowing separation of these mostly benign tumors from WT, and as such, they may inadvertently be treated with neoadjuvant chemotherapy.

Metanephric adenoma was first described in detail in 1995. It can occur across the age spectrum, and when compared with MST and MAF, is the most common of the three in adult patients. There is a female predominance. In nearly 50% of cases, it is identified incidentally; however, it can present with polycythemia,

hypertension, hematuria, abdominal pain, or a palpable abdominal mass. It is routinely unilateral. Histologically, MA is described as small acinar cells within an acellular stroma, and the majority of cases express BRAF V600E mutations [163–165]. It is considered a benign lesion, although several reports of metastatic disease do exist [166]. In addition, there are overlap lesions between MA and epithelial WT [167]. Treatment is nephrectomy or partial nephrectomy followed by observation excepting in cases overlapping with WT or with metastases, and outcomes are typically excellent.

A purely stromal metanephric neoplasm coined metanephric stromal tumor was first noted by Beckwith in 1998 and further described in a review of 31 cases by Argani and Beckwith in 2000. In this initial review, the median age at diagnosis was 13 months, ranging from newborns to 11 years [168]. A more recent review reported a median age of 2 years and included three adult patients. There does not appear to be a strong gender predilection. Most patients present with an asymptomatic abdominal mass, although hematuria, hypertension, and abdominal pain have been reported. All cases are unilateral, although multifocal disease does occur [169]. Under the microscope these tumors develop a characteristic "onion skin" pattern of rings of cells surrounding renal tubules or blood vessels [168, 169]. A majority of MST have BRAF V600E mutations which can serve to differentiate them from other renal stromal tumors and provides further link to MA [170]. Despite one report of metastatic disease, most MST are cured with nephrectomy alone. As such, care must be taken to distinguish these tumors from more aggressive stromal renal tumors such as CCSK, which require much more aggressive intervention [168, 169].

The mixed lesion, metanephric adenofibroma, was originally reported in 1992 [171]. The stromal component of these tumors resembles, although is distinct from, mesoblastic nephroma, and is identical to MST, while the epithelial component favors nephroblastomatosis and epithelial WT and is identical to MA. Additionally, some tumors are described as MAF transitioning to epithelial-predominant WT. Finally, there may also be a component of low-grade papillary carcinoma or adenosarcoma present. Similar to MA and MST, patients presented with polycythemia, hypertension, or hematuria which resolved following nephrectomy [171, 172]. The median age at diagnosis in a series of 25 patients from the NWTS studies was 30 months, although MAF can present in adolescents and young adults [173]. This tumor is more common in males at a ratio of 2:1 [172]. Historically, all patients were treated similar to WT, with nephrectomy and adjuvant chemotherapy. More recently, patients with MAF without evidence of a malignant component are typically observed after nephrectomy with good outcomes [172].

Summary

Although overall uncommon, within the context of pediatric extra-cranial solid tumors pediatric renal tumors make up a large proportion of diagnoses. Accurate diagnosis is critical as the necessary treatment and prognosis varies greatly. Pediatric nephrologists may be involved at many points during the care of these patients, including initial diagnosis in a patient who presents with hematuria, early management in a patient with hypertension, and long-term in patients with significant nephron-loss or treatment-induced nephropathy.

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Tubulointerstitial Nephritis in Children

42

Priya S. Verghese, Kera E. Luckritz, and Allison A. Eddy

Introduction and Historical Perspective

An estimated 85% of the kidney consists of tubules and their surrounding interstitial space. Given their preeminence, it is crucial to understand the contribution of the tubulointerstitium in all renal disease processes. Despite its anatomical dominance, current understanding of the role of the interstitium in both primary and secondary disease processes remains incomplete.

The term acute interstitial nephritis (AIN) was coined by Councilman in 1898 when he provided the first and now classic description of the histopathologic changes following an investigation of autopsy specimens from patients with diphtheria, scarlet fever and other infectious diseases [1, 2]. Primary AIN is typically an immunologically-mediated disease characterized by tubular injury and interstitial inflammation, with relative sparing of the glomeruli and vessels, initiated by drugs, infections or other causes mentioned in detail in section "Etiology" [3]. Councilman's early description still has merit, though it may be more accurate to categorize the disease process as acute tubulointerstitial nephritis (TIN) since the renal tubules are also involved in all cases, both clinically and histopathologically.

In the pre-antibiotic era, systemic infections were the most common cause of tubulointerstitial disease. Today, a drug hypersensitivity reaction is a more common inciting event. Ironically many of these drugs were developed to treat the infectious disorders that had often been implicated as causes of AIN. In kidney transplant allografts, TIN can occur due to drugs, but also due to infections such as BK polyomavirus and adenovirus [4, 5] that often necessitate drastic reduction in immunosuppression to enable viral clearance.

Progressive chronic kidney disease (CKD), irrespective of the primary disease process, is characterized by significant chronic TIN indicating that TIN is a spectrum of pathologies, ranging from acute and reversible nephritis to chronic and

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irreversible disease with fibrosis. For each individual patient, it is critical to try to identify and discontinue the offending toxin or agent before acute injury progresses to the chronic stage.

Epidemiology

Acute injury to the interstitium and the surrounding tubules is an important cause of renal dysfunction, currently accounting for 5–27% of kidney biopsies performed for acute kidney injury [6–9]. Reliable data on the incidence and prevalence of TIN are lacking, especially in the pediatric population. Within available biopsy registries, TIN represents approximately 1–3% of all biopsy diagnoses [7, 10]. Often the diagnosis is made clinically without performing a renal biopsy to confirm the diagnosis. Furthermore, it is likely that many cases are self-limited and remain clinically silent. Thus, the estimated numbers are likely conservative and lower than the true incidence. The incidence of TIN in kidney transplant allografts is also unknown [4, 5].

Histology and Pathogenesis

By definition, TIN is characterized by interstitial cellular infiltrates, usually sparing the vessels and glomeruli (Fig. 42.1a), although it is noted that severe primary glomerular injury rarely occurs without concurrent tubulointerstitial injury. Tubular cell damage may be manifest as epithelial proliferation and/or tubular dilatation. Intratubular cast deposition is often present as well [11]. Interstitial fibrosis and tubular atrophy, often accompanied by the persistent mononuclear cell infiltrate [11], can be representative of chronic TIN. The infiltrate is composed predominantly of T cells with some macrophages and plasma cells [6, 11, 12]. An impressive number of eosinophils may be present and suggests a drug-induced etiology (Fig. 42.1d). These lymphohematopoietic cells are a rich source of cytokines that contribute to kidney injury. Granuloma formation is a feature of biopsies in 6% of the patients and can occur in any form of AIN; granulomas are considered common in drug-induced TIN, infection-associated TIN and renal vasculitis

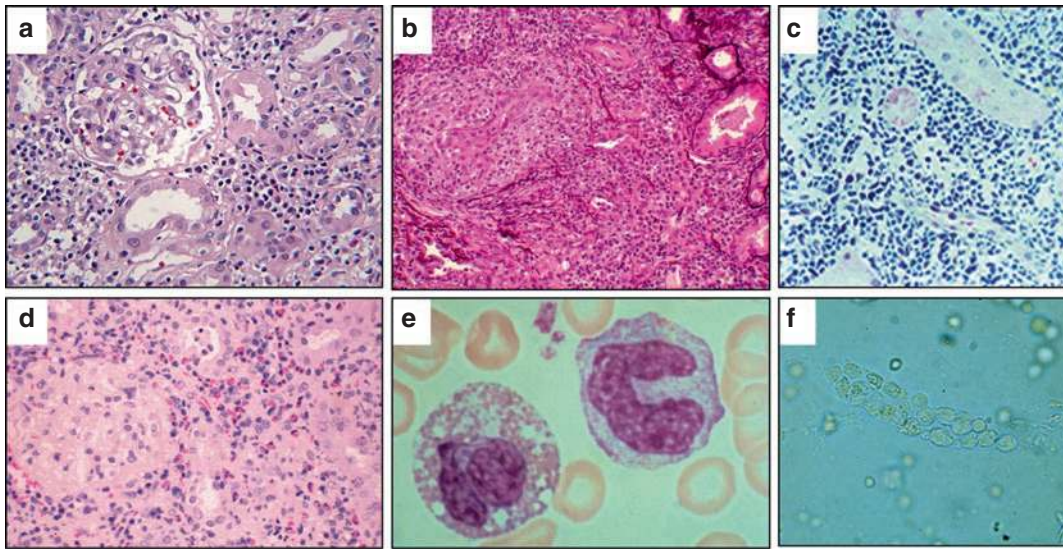

Fig. 42.1 Histological and urinary sediment features of acute tubulointerstitial nephritis (TIN). Histological photomicrographs illustrate an interstitial infiltrate of mononuclear cells, interstitial edema and tubular dilatation in acute TIN (**a**); acute TIN with granuloma formation (**b**); TIN characterized by an infiltrate of monomorphic interstitial mononuclear cell due to lymphoma (**c**); acute drug-induced TIN with numerous polymorphonuclear eosinophils (**d**). Examination of the urinary sediment may show eosinophils in drug-induced TIN (**e**), white blood cells and while blood cell casts (**f**)

[13] (Fig. 42.1b). Some studies have suggested that the degree of tubulointerstitial inflammation may be predictive of renal functional outcome, even in primary glomerular diseases [3, 8, 14, 15]. However, other studies have suggested the extent of chronic changes such as tubular atrophy on the initial biopsy are more predictive of long-term outcomes [8, 16]. Interestingly, in kidney transplant allografts, inflammation in areas of interstitial fibrosis is associated with decreased graft survival with or without concurrent evidence of rejection [17], once again supporting the prior theory that the degree of inflammation is more predictive of long-term outcomes.

In primary TIN, immunofluorescence staining for antibodies and complement proteins are typically negative. Occasionally, linear or granular deposits of IgG or IgM may be present along the tubular basement membranes [3]. Electron microscopy may reveal loss of continuity of basement membranes as well as thickened and multilaminated areas indicative of chronic damage [3].

These histopathologic findings, together with the apparent clinical response to corticosteroid therapy, supports a role for immune-mediated pathogenic mechanisms. Though the specific mechanisms remain unclear, an important role of chemokines and other inflammatory mediators is presumed [18]. A reliable animal model that faithfully mimics human acute drug or infection associated TIN is not available to elucidate specific pathways. Animal studies have shown that three endogenous kidney antigens (uromodulin, megalin, and a tubular basement membrane glycoprotein named TIN antigen) can elicit TIN, but the relevance of these findings to human acute TIN is unknown [9]. Isolated case reports describe autoantibodies to aquaporin 2 and HOXB7 [19], mitochondrial M2 protein [20] and two unidentified brush border antigens [21]. Current concepts suggest that an antigen, be it a hapten derived from a drug or microbe, can mimic a yet-to-be identified antigen normally present in renal tubules. When this antigen is presented to T-helper cells, an immune response is triggered. Macrophage and natural killer cell recruitment and activation follows. Evidence of a primary pathogenic role of T cells is supported by a study that demonstrated the presence of drug-specific sensitized T cells in the peripheral blood of patients with acute drug-induced TIN [22]. Four non-mutually exclusive theories of immune pathogenesis have been proposed [23]. (1) A component of the drug may be trapped along the tubular basement membrane (TBM) where it acts as a hapten, becoming the target of immune attack by sensitized T-cells, or less commonly, antibody producing B-cells. (2) A component of the circulating drug may be recognized as a foreign antigen that triggers an immune response. The antigen may be structurally similar (molecular "mimic") to a normal component of the tubulointerstitium (endogenous antigen) that becomes a target of the immune attack. (3) A drug-derived antigen may first be trapped "in situ" in the tubulointerstitium where immunologically reactive cells and/or antibodies are recruited. (4) Circulating antibodies generated against a drug-derived antigen may form immune complexes within the circulation that are subsequently trapped within the tubulointerstitium and initiate inflammation. Similar theories of pathogenesis have been proposed for "reactive" acute TIN triggered by an infectious agent.

A related drug-induced hypersensitivity syndrome is DRESS (drug reaction with eosinophilia and systemic symptoms), which is more common in adults than children. DRESS is characterized by a severe rash and visceral involvement that includes TIN in 10–30% of cases [24].

There is an animal model of anti-tubular basement membrane disease that has been well-characterized and thought to be mediated by an immune response to an endogenous TBM antigen [25]. However, human anti-TBM nephritis is distinctly rare; it is most commonly encountered in association with anti-GBM disease. Of interest, a patient harboring a deletion in the gene that encodes the human TIN antigen has been reported with CKD [26]. Three patients with autoimmune polyendocrine syndrome type 1 developed end-stage kidney disease (ESKD) due to TIN associated with autoantibodies to aquaporin 2 and HOXB7 [19]. Despite several studies, it is not clear that specific phenotyping studies of the infiltrating interstitial cells can differentiate the

antigenic trigger. The one exception is TIN due to lymphoma/lymphoproliferative disease where a single monomorphic cellular population invades the interstitium (Fig. 42.1c). The kidney is the most common solid organ to be infiltrated (60–90%) in patients with hematological malignancies [27].

Overlooked for many years, it is increasingly appreciated that many drug-induced nephrotoxic reactions triggered by tubular epithelial cell damage are associated with significant interstitial inflammation that also contributes to renal functional impairment. Recent studies have elucidated mechanisms that define "necroinflammation", which is distinct from the hypersensitivity-type responses that cause acute TIN (Fig. 42.2). Necroinflammation is defined pathologically as a pattern of injury associated with an auto-amplification loop that is triggered by a specific form of cell death called necroptosis—characterized by the release of intracellular debris into the interstitial space. This "debris" includes "danger-associated molecular patterns" or DAMPS that bind to unique pattern recognition receptors; an interstitial inflammatory response ensues. It is curious that a few drugs such as vancomycin [29], ciprofloxacin [30] and non-steroidal anti-inflammatory drugs are able to initiate either response. The nephrotoxic effects of some drugs are linked with intratubular crystal or cast formation that trigger necroinflammation (Fig. 42.2).

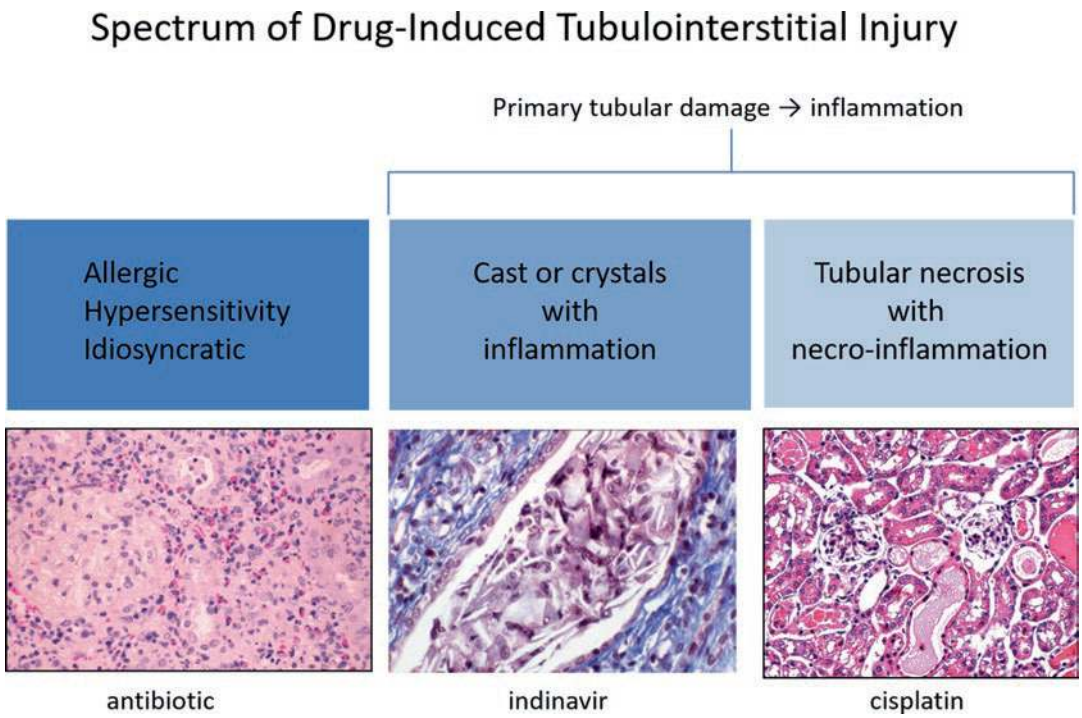

Fig. 42.2 Mechanisms of drug-induced TIN. Classical acute TIN results from an idiosyncratic allergic reaction with a primary interstitial inflammatory response (left). The nephrotoxic effects of some drugs such as indinavir are linked with intratubular crystal or cast formation, which causes tubular injury followed by interstitial inflammation (middle). Necroinflammation is a more recently recognized mechanism of tubular injury that can be initiated by drugs and is characterized by an auto-amplification loop of interstitial inflammation (right). Tubular death is caused by a specific mechanism called necroptosis, characterized by the release of intracellular debris into the interstitial space that activates unique pro-inflammatory signaling pathways. (The indinavir photomicrograph was reproduced from Fogo et al. [28] with copyright permission. The cisplatin photomicrograph was provided by Dr. Prasad Devarajan, University of Cincinnati College of Medicine)

In kidney transplant allografts, viral TIN secondary to BK polyomavirus or adenovirus is likely secondary to viral transmission via the donor organ [31].

Clinical Findings

When TIN was originally described, it was typically associated with systemic signs of inflammation. A "classic" triad of fever, eosinophilia and rash was observed in a third of the patients with methicillin-induced TIN. Recently, based on European studies of children with severe biopsy-confirmed TIN, clinical manifestations are frequently encountered but heterogenous and often non-specific [6, 8, 32–34]. When TIN occurs as a manifestation of a multi-system disease process, associated systemic symptoms may be present.

The classical clinical presentation of drug-induced TIN is acute kidney injury that begins after exposure to the offending drug. The kinetics of the onset of TIN varies depending upon the exposure history. Symptoms typically begin 3–5 days after re-exposure to the inciting drug, with a mean of 10 days until diagnosis, while it may take several weeks for the symptoms to develop with first-time drug exposure. The TIN risk is not dose-dependent, an observation that supports the theory that the pathogenesis of this disease is a 'hypersensitivity-type' immunological reaction. TIN recurrence following drug re-challenge also supports this hypothesis. Extra-renal symptoms and signs of hypersensitivity, including low-grade fever, a maculopapular rash and mild arthralgias, are more common in TIN associated with infectious and autoimmune diseases. Today, hypersensitivity symptoms are rare in drug-induced TIN and their presence does not exclude the possibility of drug-induced nephrotoxicity and/or acute tubular necrosis (ATN) rather than TIN as the primary kidney lesion. Nonspecific symptoms due to acute kidney injury including anorexia, nausea, vomiting and malaise were frequently reported in a significant number of the 106 children with a biopsy confirmed diagnoses of TIN [mean/median age ranged from 11.6–14 years; 22% males] published in five separate European studies (Table 42.1) [6, 8, 32–34]. Unlike limited studies in North America, however, a significant number of these children had tubulointerstitial nephritis and uveitis (TINU) syndrome. Kidney interstitial edema may cause renal enlargement and capsular swelling, thought to be the cause of flank pain that is present in some patients with AIN (33–79% of children as reported in the European studies) [6, 8, 32–34]. Adults (121 cases with 91% drug-induced TIN) [9, 35, 36] had similar clinical features as pediatric TIN patients, but with an increased reporting of skin rash and arthralgia.

Antimicrobials and non-steroidal anti-inflammatory drugs (NSAIDS) are most frequently suspected as the cause of drug-induced TIN, but the list of potential offending pharmacological agents is endless (Table 42.2). The risk of acute TIN is very low for an individual drug, despite a long list of single published case reports. The hallmark of TIN is an acute decline in renal function as evidenced by the rise in serum creatinine. This may be the only laboratory abnormality [3]. Acute TIN may also present as one of several more complex clinical scenarios:

1. Acute kidney injury. The absence of hypertension, significant albuminuria and red blood cell casts are clues to a diagnosis of TIN rather than glomerular or vascular disease, though in a given patient clinical manifestations may overlap considerably. Recent exposure to a potentially offending agent, significant pyuria in the absence of bacteriuria, a good urine output and evidence of tubular dysfunction suggest a diagnosis of TIN. Distinguishing between acute TIN and ATN may be challenging, although the presence of many renal tubular cells and muddy brown casts in the urine sediment is more suggestive of a diagnosis of ATN.
2. Chronic renal failure. When evaluating a new patient, the diagnostic challenge may be differentiating acute from chronic TIN. Small kidneys with increased echogenicity and anemia suggest a long-standing process. Many of the causes of chronic TIN in the pediatric population are associated with extra-renal

Table 42.1 Clinical features of TIN in pediatric patients

First author	Howell [32]	Jahnukainen [8]	Taktak [34]	Clavé [6]	Roy [33]	
Year	2016	2011	2015	2017	2020	
Country	England (GOSH)	Finland	Turkey	France	England (Liverpool)	
N	27	26	19	25	10	Extrapolated total
Years	1990–2012	1995–2007	1999–2014	2006–2016	2007–2014	
Anorexia	81%	54%		28%	Yes	43/78 = 55%
Vomiting	59%	35%	27%		70%	37/82 = 9%
Nausea	48%		21%			4/46 = 9%
Fever	41%	92%		28%	30%	45/88 = 51%
Loin/abdominal pain	33%	46%	79%	44%	Yes	47/97 = 48%
Uveitis	65%	46%	5%	28%	60%	43/107 = 41%
Initial creatinine[a] (μmol/L)	263	253	188	183	303	–
Follow up duration (months)	21	35	6	12	18.5	–
Abnormal renal function at last follow up (μmol/L)	56% have eGFR <80 mL/min/1.73 m^2	15% have eGFR <80 mL/min/1.73 m^2	Mean creatinine 49 μmol/L (35–73)	40% have eGFR <80 mL/min/1.73 m^2	50% have eGFR <80 mL/min/1.73 m^2	34/88 = 39%[b]
Proportion treated with steroids	96%	88%	32%	72%	80%	82/107 = 77%

[a] Median values reported (except for Taktak study in which mean value is reported)

[b] Taktak study excluded (follow-up renal functional data not provided)

Table 42.2 Drugs most commonly reported to cause acute TIN

Antimicrobials	Analgesics and narcotics	Diuretics	Others
Beta-lactams Methicillin Ampicillin Penicillin Oxacillin Nafcillin Amoxicillin Cephalosporins Sulfonamides Macrolides Erythromycin Clarithromycin Other antibiotics Colistin Rifampin Polymyxin Ethambutol Tetracycline Vancomycin [37] Linezolid [38] Ciprofloxacin Isoniazid Piperacillin-Tazobactam Clindamycin [39] Fluoroquinolone Anti-virals Acyclovir Indinavir Tenofovir Alpha-interferon Direct-acting antiviral agents for hepatitis C [40]	NSAIDs 5-Amino-salicylic acid [41] Mesalazine [42, 43] COX-2 inhibitors Acetaminophen *Drugs of Abuse* Cocaine [54–57] Synthetic cannabinoids [58] Anabolic steroids [59] Inhaled solvents/toluene [60–63]	Furosemide Thiazides Triamterene *Biologics* Nivolumab (anti-PD1) Vedolizumab [64] Pembrolixumab (anti-PD1) [65] Infliximab Adalimumab (anti-TNF) Atezolizumab (anti-PD-L1) Bortezomib [66]	Allopurinol Azathioprine [40] Ifosfamide H2 blocker Ranitidine PPIs Omeprazole Lansoprazole Pantoprazole [44] Antihypertensives Amlodipine Diltiazem Captopril Valsartan [45] Nifedipine [46] Anti-epileptics Carbamazepine Phenytoin Levetiracetam Clozapine [47] Miscellaneous Apixaban [48] Cetirizine Clozapine Ergotamine Etanercept Glucosamine [49] Immune checkpoint inhibitors [50, 51] Isotretinoin [52] IVIG [53] Lenalidomide Exenatide Mercury Rosuvastatin Warfarin Zoledronate

Citations are selected from recent publications. Unreferenced medications were cited in prior editions of this chapter
Abbreviations: *NSAID* non-steroidal anti-inflammatory drug, *COX-2* cyclo-oxygenase-2, *PPI* proton pump inhibitor

manifestations such as cystinosis, certain inborn errors of metabolism, and inflammatory bowel disease (IBD). More recently, chronic TIN has become a major cause of CKD in agricultural communities and is thought to be associated with heavy metal and/or pesticide exposure.

3. Tubulopathy. Patients may come to medical attention due to signs and symptoms of tubular dysfunction. The specific manifestations of tubular cell injury/dysfunction vary depending on the primary site of injury. Proximal tubular injury may cause Fanconi's syndrome with glucosuria, proteinuria, and phosphaturia, or it may present as a proximal renal tubular acidosis (RTA). Distal tubular cell injury may manifest as acidosis and hyperkalemia (type 4 RTA) while collecting duct damage typically results in a urinary concentrating defect (nephrogenic diabetes insipidus) (Fig. 42.3). Tubular cell injury may also manifest as potassium wasting. The pediatric case series by Howell et al. reported 7/10 with potassium wasting, 8/13 with reduced phosphate reabsorption and 7/16 with metabolic acidosis [32].

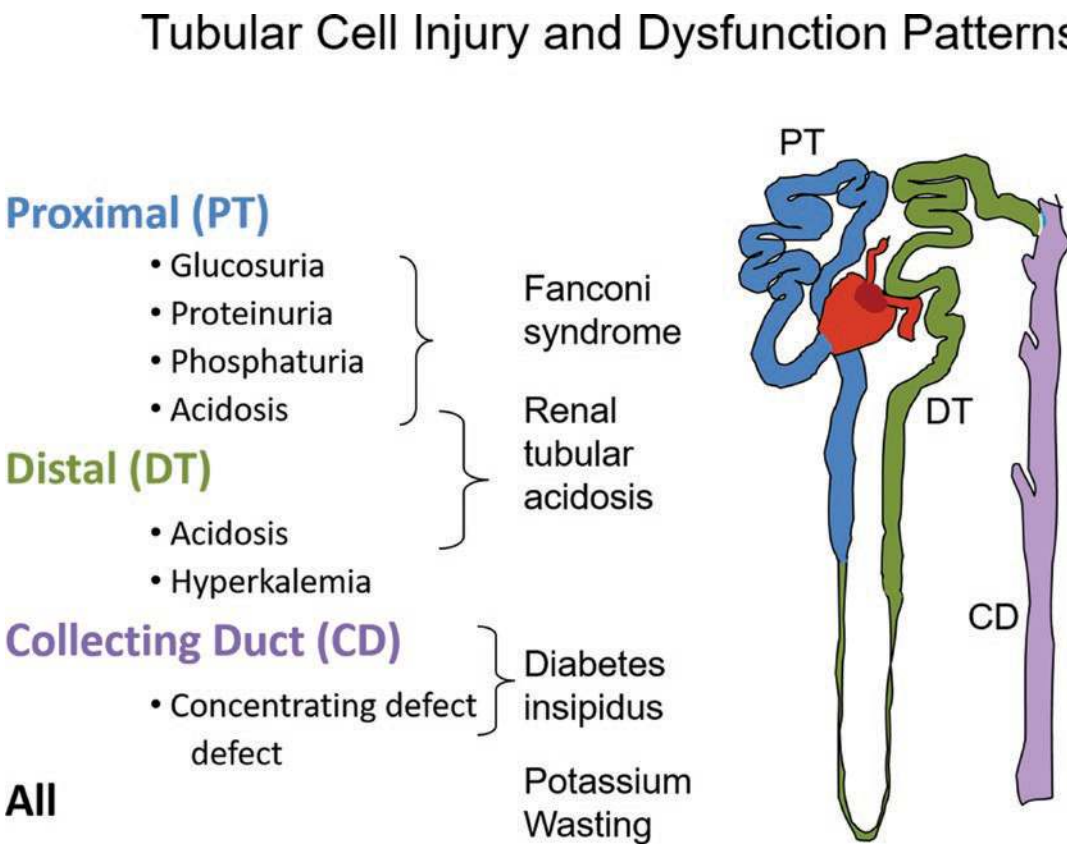

Fig. 42.3 Variable patterns of renal tubular functional defects present in patients with TIN, depending on which nephron segments (proximal, distal or collecting ducts) are injured because of the primary disease process and/or the associated interstitial inflammation

Diagnosis

Often diagnosed clinically, the sensitivity and specificity of the non-invasive diagnostic studies that are performed to diagnose TIN are poor.

Urinary Sediment

The urinary sediment often shows red cells, white cells and white cell casts (Fig. 42.1f) [3]. Sterile pyuria may or may not be associated with eosinophiluria. Urinary eosinophils (Fig. 42.1e) were once considered helpful, but more recent studies and a review of published data by Lusica et al. [67] conclude that urinary eosinophil counts lack adequate sensitivity or specificity. It is also important to remember that a bland urinary sediment does not exclude the diagnosis of acute TIN [15]. Proteinuria may be present, but is typically less than 1 g/24 h. Nephrotic range proteinuria is rare except in NSAID-induced TIN, where it is thought to be mediated by cytokine-induced glomerular injury.

Low Molecular Weight Proteinuria

Beta 2-microglobulin is a low molecular weight (~12 kDa) protein used in the evaluation of the

re-absorptive capacity of the proximal kidney tubule. Its daily production is constant and its clearance is almost exclusively by glomerular filtration followed by 99.9% reabsorption by the proximal tubule. Therefore, urinary beta 2-microglobulin levels are often elevated in patients with proximal tubular dysfunction, as frequently observed in TIN; an elevated urinary beta 2-microglobulin/creatinine ratio can help support a diagnosis of TIN [8]. Recent studies suggest that quantitative assessment of other low molecular weight urinary proteins such as α1 microglobulin, retinol binding globulin and vitamin D binding protein may also be informative [68, 69].

Blood Work

In the five most recent pediatric retrospective case series of biopsy-confirmed acute kidney injury due to TIN, the initial serum creatine (median in 4 series, mean in 1 series) ranged from 183 to 303 μmol/L (2.1–3.4 mg/dL). Anemia and elevated serum inflammatory markers (C-reactive protein and erythrocyte sedimentation rate) are common in patients with severe disease [6, 8, 32–34] (Table 42.1). Peripheral eosinophilia may be present in patients with TIN; and was described frequently in the era of methicillin-induced TIN.

Urinary TIN Biomarkers

There is considerable interest in the use of biomarkers to both differentiate causes of kidney disease non-invasively and to follow the disease course. A recent study of adult patients undergoing a kidney biopsy reported that patients with AIN have significantly higher urinary TNF-alpha and IL-9 levels than those with other causes of acute kidney injury [70]. Other promising urinary biomarkers include kidney injury molecule-1 (KIM-1) [71], N-acetyl-beta-D-glucosaminidase (NAG), complement C5b-9 [72] and increased urinary magnesium excretion [73].

Radiology

The renal ultrasound usually demonstrates increased echogenicity, often associated with an increased renal bipolar length, but these findings are non-specific [2, 3]. Gallium scanning has been proposed to differentiate between acute TIN and ATN, but the findings are often non-conclusive and the study is rarely performed now [2].

Biopsy

Since none of the non-invasive studies are both specific and sensitive for TIN, kidney biopsy remains the only definitive diagnostic study. For details on biopsy findings, see the section "Histology".

Causes, Treatment and Outcomes

The causes of TIN are numerous, but can be broadly divided into acute and chronic disorders, though there may be considerable overlap for any single etiology. Most of the larger case series have been conducted in adults and conclude that the majority (approximately 70%) are drug-related, followed by infections (16%) [12, 74]. In the five pediatric case series published since 2010 ($n = 106$ cases), 39% were due to drugs, followed by 36% due to TINU (5% in the adult series) and 17% associated with infections [6, 8, 32–34]. In a systematic review of 592 published TINU cases, it was reported that the median age was 17 years (interquartile range 13–46); 51% were under the age of 18 years [75]. The most common causes of acute TIN in pediatric patients are summarized in Fig. 42.4 and discussed in more detail in the following sections and Tables 42.2, 42.3 and 42.4.

Drugs

In the current era, drugs clearly surpass infections as the most commonly implicated cause of

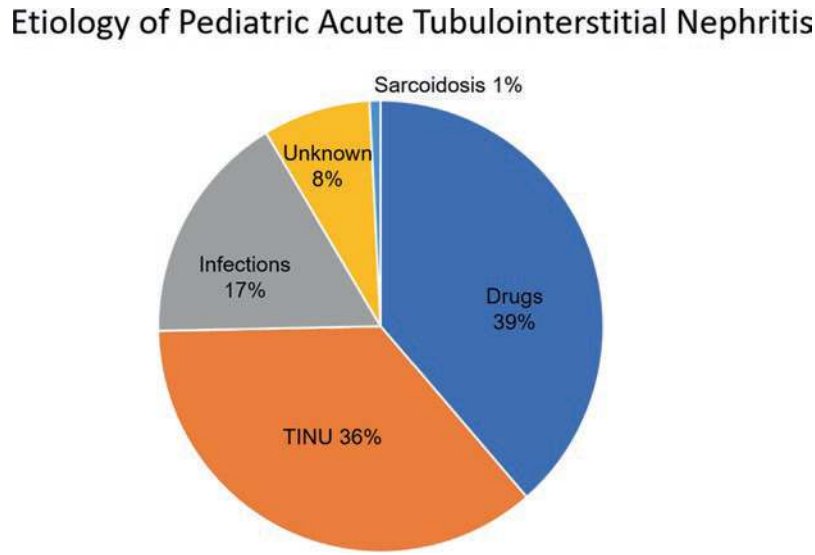

Fig. 42.4 Causes of pediatric acute TIN. Data are based on five European pediatric case series published since 2010, that also from the basis for Table 42.1. (n = 106 individual patients; etiology counted twice for 8 patients in the series by Howell et al. who had TINU plus a secondary etiology identified) [6, 8, 32–34]

acute TIN. In the adult literature, where there are more data, 35% are caused by PPIs, 35% by antibiotics and 20% by NSAIDS [78]. However, the list of potentially causative agents is extensive, expanding, and variable over time as drug prescribing practices change (Table 42.2).

Methicillin was long considered the prototypical cause of drug-induced TIN, as first reported in 1968 [79]. In fact, due to its infamy, its use has declined worldwide and it is no longer available in most countries. Methicillin and other beta-lactam antibiotics (penicillins and cephalosporins) are still more commonly associated with systemic signs of hypersensitivity, including the classic triad of rash, fever, and eosinophilia than any other group of drugs.

Rifampin has frequently been implicated as a cause of acute TIN. Affected patients fall into two groups: (1) Patients who receive short duration therapy with rifampin and (2) patients who have had prior or intermittent exposure to the drug. The first group typically lacks anti-rifampin antibodies and the onset of clinical symptoms is insidious. The second group may develop antibodies and clinical symptoms often begin abruptly [23]. Associated with certain agents such as rifampin and allopurinol, hemolysis or hepatitis may also be present [23].

NSAID-induced TIN may be associated with nephrotic syndrome in as many as 70% of the cases [23]. It is reported to occur more frequently in older patients, but it is unclear whether this is due to under-reporting in pediatrics, lower exposure rates or other factors. In NSAID-induced TIN, hematuria is almost always microscopic and extra-renal symptoms such as fever and rash occur in less than 10% of the patients [23]. The degree of interstitial inflammation is often less with NSAID-induced TIN. In addition to the classic TIN accompanied by "minimal change" glomerular disease, NSAIDs can also cause membranous nephropathy. Therefore, all patients with nephrotic syndrome associated with NSAID use should undergo a diagnostic renal biopsy [80].

The epidemiology of drug-induced TIN has changed significantly in the past two decades, especially following the first published report of PPI-induced TIN in 1992 [81]. In adults taking PPIs, a three times increased risk of developing TIN [82], a four times increased risk of acute kidney injury and a 20% increased risk for CKD [83] were reported. A meta-analysis also identified a 1.2 increased risk of CKD among PPI users, but no increased risk among H2 receptor antagonists [84]. Additionally, there is evidence that the duration of exposure to PPI is associated with increased risk and progression of CKD [85]. As newer therapies and drugs are introduced, one must maintain a high index of suspicion for drugs

Table 42.3 Infectious causes of acute TIN

Bacteria
Brucella
Campylobacter
Corynebacterium diphtheria
E. coli
Enterococcus
Legionella
Leptospira
Mycobacteria
Mycoplasma
Salmonella
Staphylococci
Streptococci
Syphilis
Yersinia
Viruses
Adenovirus [76]
BK polyoma
Cytomegalovirus
Epstein Barr virus
Hantavirus
Hepatitis A
Herpes simplex
Human immunodeficiency virus [77]
Influenza H1N1
Mumps
Rubeola
SARS-COV-2
Fungi
Cryptococcus
Histoplasmosis
Parasites
Babesiosis
Encephalitozoon cuniculi
Hydatid Disease
Leishmaniasis
Toxoplasmosis
Rickettsia
R. diaporica
R. rickettsii

Table 42.4 Causes of acute tubulointerstitial nephritis in the pediatric age-group (drugs and infections excluded)

Autoimmune disorders with TIN as typical renal manifestation
Tubulointerstitial nephritis and uveitis syndrome (TINU)
Sjögren's syndrome
Sarcoidosis
Anti-TBM nephritis (rare)
Autoimmune disorders with TIN usually associated with glomerular disease
Systemic lupus erythematosus
ANCA+ vasculitis
Many types of primary glomerulonephritis
Autoimmune disorders with TIN as rare manifestation
Inflammatory bowel disease
Ankylosing spondylitis
Malignant infiltration
Lymphoma
Leukemia
Other
Amanita mushrooms
Sickle cell nephropathy
Snake bites
Wasp and hornet stings (usually multiple)
Radiation nephritis
Renal allograft rejection
Xanthogranulomatous pyelonephritis
Idiopathic

as a cause of acute renal dysfunction without relying on the presence of the historically "classical" clinical features [86]. Future pharmacogenomic studies may identify patients at higher risk of TIN in association with the use of specific medications. One study failed to show that individuals with the CYP2C19 slower metabolizer genotype were at increased risk of omeprazole-induced AIN [87].

The primary treatment of drug-induced TIN is to identify and stop the offending agent. The immunologic trigger must be removed, particularly since persistent tubulointerstitial injury can progress to chronic irreversible damage. Early removal of the offending agent alone frequently leads to complete reversal of renal injury. Kidney biopsies are not performed and additional therapy is not required, if the drug exposure time was short and renal function improves quickly. After drug-induced acute TIN, the mean recovery time to the nadir creatinine is 1.5 months [23]. There are an increasing number of long-term follow-up studies in adults reporting an increased risk of CKD after PPI-induced TIN [85, 88].

Therapy with corticosteroids has been used for several decades to treat severe acute drug-induced TIN, but indications for treatment and evidence of efficacy are problematic due to the lack of prospective randomized controlled clinical trials. Earlier case series have suggested faster

rates of renal recovery with steroids, but their benefit to long-term kidney function is still debated. A systematic review of all studies published between 1975 and 2016 concluded: (1) Findings suggest that the evidence for the use of corticosteroids in the treatment of drug-induced AIN remains uncertain, (2) Given the shortage of proven treatments for drug-induced AIN to ameliorate the burden and consequences of acute kidney injury, suitably designed studies should be prioritized [89]. In the interim, there is a growing consensus of expert opinions that a course of corticosteroids is reasonable to treat acute kidney injury secondary to biopsy-proven acute TIN (in the absence of significant tubular atrophy and interstitial fibrosis) when kidney function does not improve within 3–6 days after the offending drug is withdrawn [90]. Studies by Gonzales et al. [36] and Fernandez-Juarez et al. [91] report worse renal functional outcome if treatment is delayed for more than a few weeks after the diagnosis is made (Fig. 42.5).

While older studies may have argued against the use of routine corticosteroids for severe drug-induced TIN, there are several possible explanations for a lack of glucocorticoid efficacy in

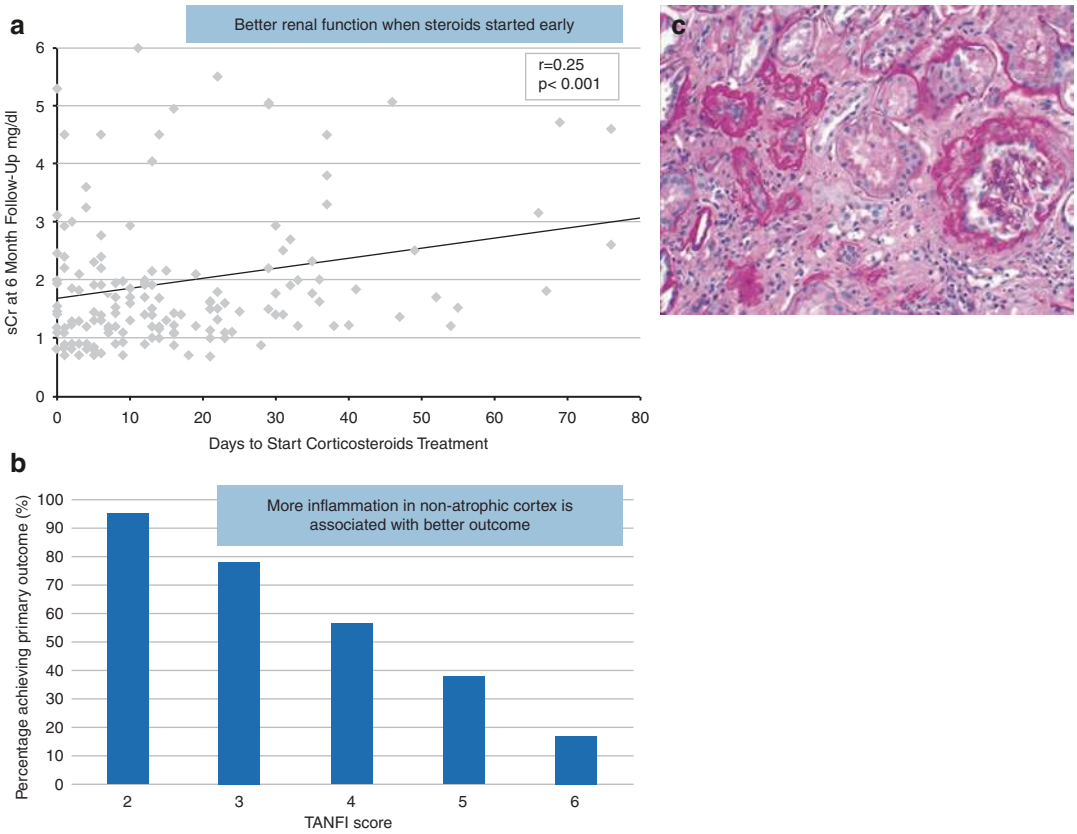

Fig. 42.5 Prognostic impact of early steroid initiation, and degree of tubulointerstitial inflammation and fibrosis/tubular atrophy on TIN outcomes. In a study of 182 adults with severe drug-induced TIN treated in Spain (mean peak creatinine 504 ± 309 μmol/L), the serum creatinine level 6 months after diagnosis was better when corticosteroid treatment was started early [91] (**a**). A kidney injury chronicity score applied to 120 adult kidney biopsies with primary acute TIN reported better clinical outcomes (50% reduction in serum creatinine or eGFR greater than 60 mL/min/1.73 m^2 at 1 year) in patients with low cortical tubular atrophy and with higher interstitial inflammation in non-fibrotic cortex scores. These data were combined into a single score called TANFI, calculated as the tubular atrophy score pulse the inverse of the non-fibrotic cortex with inflammation [92] (**b**). Representative photomicrograph of renal biopsy illustrating the features of chronic TIN—tubular atrophy, thickened tubular basement membranes and an expanded interstitial space occupied by scar tissue (**c**). (**a**, **b** were originally published in *Histopathology* and *CJASN*, respectively, and are reproduced with copyright permission)

earlier studies, including a bias towards treating the patients with worse disease, the possibility that a significant proportion of the patients had NSAID-associated TIN, which appears to be less likely to respond to glucocorticoid therapy, and the negative impact of delayed therapy onset [9, 23, 35]. Future studies will also need to control for the degree of chronic damage as quantified on the kidney biopsy (tubular atrophy and interstitial fibrosis), which negatively impacts reversibility and long-term prognosis [92]. When indicated, the leading experts recommend treating acute drug-induced TIN with prednisone 1 mg/kg/day for 2–4 weeks, followed by tapering to discontinuation over 3–4 weeks. The retrospective study by Fernandez-Juraz et al. with 182 adults from 13 centers in Spain reported that using high dose corticosteroids for longer than 3 weeks and total therapy duration longer than 8 weeks has a non-significant effect on renal recovery and may increase the risk for adverse steroid therapy effects [91]. Data on the use of immunosuppressive agents such as mycophenolate mofetil are too sparse to draw any conclusions.

While the serum creatinine at the time of biopsy is a poor prognostic indicator [36], evidence is emerging to suggest that patients with acute systemic inflammation (elevated ESR and CRP) [93] and low chronicity scores on biopsy [92] have better renal function outcome (Fig. 42.5).

Drugs have also been implicated in unusual cases of TIN. For example, anti-carbonic anhydrase II antibodies were detected in a patient with TIN associated with the use of famotidine [94].

Tubulointerstitial Nephritis with Uveitis

An association between TIN and anterior uveitis, occasionally associated with bone marrow granulomas, was first reported in 1975 and called TINU [95]. While anterior uveitis is more common, posterior uveitis can also occur. When first described, there was a female predominance; recent studies also indicate that 65% are female. The median age of onset is 17 years (55% are under 18 years of age) [75]. TINU is a syndrome of multiple etiologies. Though often idiopathic and presumed to be autoimmune in pathogenesis, it is important to search for evidence of the known causes of TIN with uveitis that are summarized in Fig. 42.6. It is speculated that disease pathogenesis involves an immunological response triggered by a recent drug exposure (often an

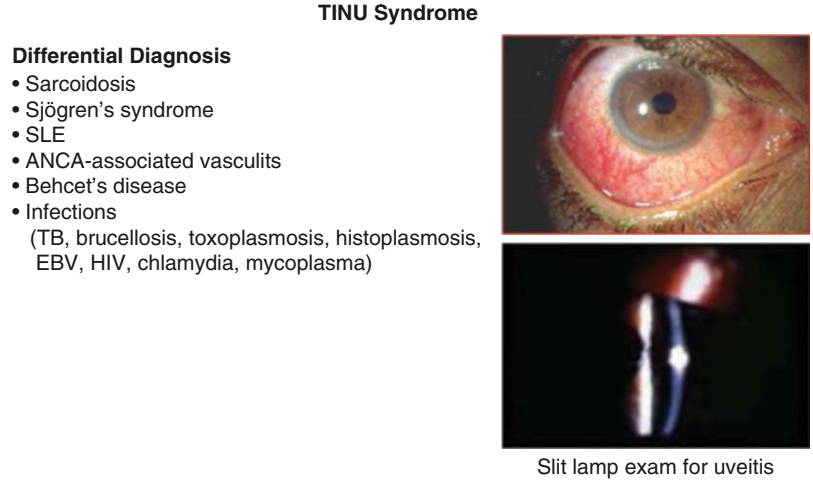

Fig. 42.6 Tubulointerstitial nephritis with uveitis syndrome (TINU). While often idiopathic, the known secondary causes of TINU listed in this figure should be considered in the differential diagnosis as specific therapy is available for many of them. The ocular photomicrographs, taken from patients with acute uveitis, serve as a reminder of the importance of performing a slit lamp examination as part of the evaluation of a patient with suspected acute TIN of unknown etiology

antimicrobial agent), an infection or an unknown agent. The systematic review of 592 cases, reported an association with drugs in 21%, infections in 6% and no identified trigger in 63% [75]. Some patients have serum auto-antibodies such as antinuclear antibodies, rheumatoid factor, antineutrophil cytoplasmic antibodies and/or anticardiolipin antibodies. One study posits that modified C-reactive protein might be a target antigen [96]. Some patients have associated autoimmune diseases such as hyperthyroidism [97], hyperparathyroidism, or rheumatoid arthritis. Patients may also have a history of recent insect bites [98].

The report of TINU in monozygotic male twins separated in onset by 2 years suggests the possibility of a genetic predisposition to the syndrome [99], as does a report in 1994 of identical female twins with the onset of TINU syndrome 1 year apart [100]. However, the lack of reports of multiple affected family members and the lack of geographic clustering questions the influence of environment and genetic factors. Reviewed by Cline and Vanguri [101], several small studies (2–20 patients from several countries), have suggested different HLA associations (especially some DRB1* alleles), but ethnic diversity among the cohorts prevents broader extrapolation of the findings.

Several of the non-specific symptoms associated with TIN may be present in patients with TINU syndrome. These include fever, weight loss, fatigue, malaise, loss of appetite, weakness, asthenia, abdominal or flank pain, arthralgias and myalgias. Less commonly, headache, polyuria, lymphadenopathy, edema, pharyngitis or rash may occur. The ocular manifestations commonly include eye pain and redness (77%), decreased visual acuity (20%) and photophobia (14%) [98], but recent studies have suggested that up to 58% of patients with TINU have reported no ocular symptoms despite slit-lamp confirmation of uveitis, making it critical that patients with acute TIN undergo regular eye examinations [8]. Onset of uveitis varies from several weeks before the onset of renal involvement, concurrent with the TIN, or up to 15 months after the onset of TIN. The systematic review by Regusci et al. reported the onset of uveitis after renal involvement in 52% of the patients [75]. Both recurrent acute and chronic uveitis are commonly described. The timing of uveitis recurrence has varied from 3 months after steroid tapering to 2 years after the first episode. The renal and ocular manifestations of TINU have also been reported to recur years after initial presentation, even after transplantation, further supporting a role for systemic immunological factors in the disease pathogenesis [102].

Laboratory findings may include elevated serum creatinine, evidence of tubular dysfunction, anemia, slightly abnormal liver function tests, eosinophilia, hypergammaglobulinemia and elevated erythrocyte sedimentation rate. A variety of serological markers have been reported without evidence of the associated diseases in 15% of patients (such as SLE, ANCA-associated vasculitis, anti-phospholipid syndrome, rheumatoid arthritis). However, definitive diagnosis requires a renal biopsy and a formal ophthalmologic slit-lamp examination to diagnose uveitis. Bone marrow and lymph node granulomas have been reported, but these studies are rarely performed now that TINU syndrome has become recognized as a distinct clinical entity.

The tubulointerstitial disease is self-limited in most patients, but there are reports of individuals progressing to ESKD [8, 102]. In the 2021 systematic review, 11% of the patients under 18 years had CKD at a median follow-up of 18 months [75]. The ocular disease usually requires treatment, with both topical and systemic steroids. There are anecdotal reports of utilizing other immunomodulatory therapies in the treatment of recalcitrant eye disease. While the acute eye disease usually improves, recurrences, complications, and chronic ocular disease are not uncommon. Most of the long-term complications of TINU have been ocular, estimated to occur in 20% of patients. These include posterior synechiae, optic disc swelling, cystoid macular edema, chorio-retinal scar formation, cataracts and glaucoma [95, 98]. Fortunately, the risk of visual loss appears low. Due to the morbidity associated with the ocular manifestations, early detection by slit lamp examination is essential.

Infections

Numerous infectious agents have been implicated in the pathogenesis of TIN, both acute and chronic. TIN was first recognized as a unique clinical entity in 1860 in a patient with scarlet fever. However, it was several decades later before Councilman introduced the term "interstitial nephritis" and described the histologic lesions. To quote his landmark paper "Acute interstitial lesions of the kidneys have been considered as common in scarlet fever, and are regarded by some authors as constituting the most frequent pathological alteration of the kidney in this disease. This has also been described in diphtheria and in other infectious diseases" [1]. The 1939–1945 era saw the eradication of serious and fatal streptococcal infections due to the introduction of antibiotics. In the current era, the infections implicated as causes of TIN vary from Councilman's time due to childhood immunizations and the use of effective antibiotics. In fact, since 1960 antibiotics rather than infections are a more common cause of acute TIN (Table 42.3, Fig. 42.4).

The infectious microorganisms may directly invade the renal parenchyma to cause a specific form of TIN (pyelonephritis). TIN is the most frequent renal biopsy finding in patients with renal tuberculosis [103]. Rare infectious processes may induce emphysematous or necrotizing interstitial parenchymal lesions [104, 105]. However, the traditional form of acute TIN is associated with infection at an extrarenal site and the tubulointerstitial inflammation is thought to represent a secondary or "reactive" immunological response to the infection. In the latter, the infectious agent is not cultured from the kidney or urine and cytokines derived from inflammatory cells are key kidney disease mediators.

When a renal biopsy is performed in a patient with pyelonephritis (not typically required or recommended), the interstitial lesion is often localized to a single pyramid and characterized by neutrophil predominance. In contrast, "reactive" TIN associated with a systemic infection is characterized histologically as either patchy or diffuse lesions that are associated with interstitial edema and a predominance of mononuclear cells. The pathogenic microbial antigens that initiate the immune response to cause TIN are largely unknown. One exception is leptospirosis, where an isolated outer membrane protein has been shown to interact in vitro with Toll-like receptor 2 to stimulate synthesis of inflammatory cytokines, chemokines and collagen by renal tubules [106]. The primary therapeutic measure is to treat the infection, preferably with non-nephrotoxic antimicrobials.

Viral infections are an important cause of TIN. Epstein-Barr virus (EBV) may have a pathogenetic role in certain forms of "idiopathic" TIN based on the detection of EBV genome in the proximal tubules in one case series [107]. HIV-1-assocated nephropathy is typically characterized by significant glomerular pathology, but coexistent TIN is common and more severe than observed in other primary glomerular disorders. This is likely related to the ability of HIV-1 to infect and damage tubular cells [108]. In a kidney biopsy series of 222 HIV-infected patients, 27% had TIN as the predominant lesion [77]. However, in at least half of the latter patient group, the concurrent use of nephrotoxic agents such as antiretroviral drugs may have contributed to the TIN. TIN has also been reported as a feature of the immune reconstitution syndrome in the HIV-infected population [109].

BK polyomavirus is an important cause of TIN in immunocompromised patients, especially following kidney transplantation, though it has been reported in other transplants recipients as well and rarely in children with a lymphoid malignancy [4, 110–112]. Adenovirus DNA has also been identified in a few kidney allografts with granulomatous TIN [5, 76]. Many other viruses listed in Table 42.3 have been associated with reported cases of TIN.

Granulomatous Interstitial Nephritis

TIN may be associated with the presence of granulomas on renal biopsy (Fig. 42.1b). Granulomatous TIN is found in approximately 6% of renal biopsies with TIN [13]. The differential

diagnosis of this histologically distinct variant includes drug-induced TIN (~25% of cases), infectious causes (tuberculosis, brucellosis, histoplasmosis, adenovirus, fungal), ANCA-associated vasculitis, sarcoidosis, TINU syndrome, multiple myeloma, IBD [113] and other dysproteinemias; it may be idiopathic in as many as 50% of the patients [114]. While TIN has been reported in up to 20% of renal biopsies performed in patients with IBD [41], granulomatous TIN is exceptionally rare and limited to a few IBD case reports [115].

Additionally, there is the frequent challenge of distinguishing between drug-induced AIN associated with IBD treatment and extra-intestinal manifestations of IBD primary disease. There are reports of biopsy proven AIN in IBD patients before they have started IBD treatment, suggesting a pathogenesis distinct from drug-induced AIN [116].

There are several systemic diseases that may cause acute TIN even in the pediatric age group (Table 42.4). A few of the more common examples are discussed briefly in the next sections.

Sarcoidosis

Sarcoidosis is a multisystem disease characterized by non-caseating granuloma formation in various organs, including the kidney. While the exact incidence of granulomatous TIN among patients with sarcoidosis is unknown, studies have cited an incidence up to 30% [114]. Although histologic evidence of renal involvement is said to be common in sarcoidosis, isolated renal sarcoidosis is rare [117, 118].

Patients with sarcoidosis tend to avidly absorb dietary calcium, leading to hypercalciuria and, less commonly, hypercalcemia. The clinical manifestations of calcium hyperabsorption may be silent or may cause nephrolithiasis, nephrocalcinosis, renal insufficiency or polyuria. Nephrocalcinosis is the most common cause of chronic renal failure in sarcoidosis [119]. Polyuria may be the result of hypercalcemia and hypercalciuria that decreases tubular responsiveness to antidiuretic hormone, or it may be a manifestation of diabetes insipidus or primary polydipsia as a consequence of granulomatous infiltration of the hypothalamus. It is important to recognize that the abnormalities in calcium metabolism can occur in other chronic granulomatous diseases due to increased calcitriol produced by activated mononuclear cells [120].

The urinary manifestations of sarcoid granulomatous TIN are similar to other forms of chronic TIN, often associated with a bland urine sediment, sterile pyuria and/or mild proteinuria. The serum creatinine is usually normal and CKD is rare. The renal biopsy findings may include TIN with mononuclear cell infiltration and non-caseating granulomas in the interstitium [121]. When glomerular disease is present, it is most frequently membranous nephropathy; however, granulomatous TIN is present in 2/3 of the cases with glomerular disease [122]. Chronic injury, manifest as interstitial fibrosis and tubular damage, is common in the primary sarcoidosis-associated glomerulopathies.

Corticosteroids remain the treatment of choice, with slowly tapered protocols to prevent disease recurrence [122–125]. While there are currently no large trials of therapeutic protocols for renal sarcoidosis, there are reports of tumor necrosis factor-α blocking agents improving renal function, supporting the theory that TNF-α may play a pathogenetic role [119, 126]. In the rare patient who develops ESKD, it is usually due to hypercalcemia and hypercalciuria rather than TIN [119]. Renal sarcoidosis has been reported to recur in approximately 15% of renal allografts [127, 128].

Sjögren's Syndrome

Sjögren's syndrome is classically described as a sicca syndrome that occurs as a consequence of lymphocytic (mainly activated CD4+ cells and B cells) and plasmacytic infiltrates in the exocrine glands, especially the salivary, parotid and lacrimal glands. This causes dry mouth and dry eyes. These sicca symptoms are less common in children; recurrent parotitis is a common presenting symptom [129]. The pathogenic immune process may also affect non-exocrine organs, including the skin, lung, gastrointestinal tract, central and peripheral nervous systems, musculoskeletal sys-

tem and the kidney. The most common renal manifestation is TIN with associated tubular dysfunction (Fanconi syndrome); glomerular disease has also been reported [130–132]. Though the presence of renal disease in patients with Sjögren's syndrome was first reported in the 1960s, its prevalence and primary pathogenesis remain ill-defined. In the literature, the frequency of renal abnormalities varies widely from 16% to 67% [130, 133]. The diagnosis of idiopathic Sjögren's syndrome is based on clinical and/or histopathological evidence of ocular, oral or salivary involvement and the presence of anti-Ro/SSA and/or anti-La/SSB auto-antibodies. Symptoms due to renal disease, such as polyuria and renal tubular acidosis, may precede sicca syndrome-related symptoms [133]. TIN is the most common renal finding in Sjögren's syndrome and carries the best prognosis [134], although one case series reported four patients with isolated TIN and primary Sjogren's disease that progressed to ESKD [131]. While steroids remain the mainstay of therapy for the renal manifestations, other medications such as rituximab have shown improvement in the extrarenal manifestations [131, 132, 135, 136].

Other Systemic Autoimmune Diseases

Renal involvement is common when systemic lupus erythematosus begins in the pediatric age group: 20–80% within a year of diagnosis and 48–100% at some point during the course of the disease [137]. Isolated TIN associated with tubular basement membrane (TBM) immune deposits can occur, but is extremely rare. Conversely, focal or diffuse interstitial inflammation in association with glomerular disease is relatively common and does not typically show a clear association with TBM immune deposits and the presence of TIN is not considered in the primary classification of lupus nephritis (Classes 1–VI) [138]. The severity of interstitial inflammation and, in particular, its association with tubular atrophy and interstitial fibrosis, are strong predictors of renal outcome [139].

The majority of pediatric patients with ANCA-associated systemic vasculitis have renal involvement (75–88%) [140]. TIN is typically present in association with focal necrotizing glomerulonephritis, though isolated cases of TIN have been reported [141]. The "signature" interstitial granuloma is only present in 6–12% of renal biopsies performed in patients with granulomatosis with polyangiitis [142].

Over the past decade, an increasing number of adults have been diagnosed with TIN due to an autoimmune multisystem disease referred to as IgG4-related disease [143], but there are very few published pediatric cases [144]. It is noted that IgG4+ cells can be detected in other forms of TIN [145]. Other causes of primary TIN include IBD [115, 116] and ankylosing spondylitis [113, 146]. In a study of native kidney biopsies performed in Finland, 13.3% of the patients with TIN had IBD [147]. Both IBD and some of the drug treatments (5-aminosalicylic acid, infliximab, vedolizumab) have been implicated as TIN triggers. Since many patients with systemic autoimmune disorders have complicated medical courses, it is always important to consider alternative causes for their TIN such as drugs and infection.

TIN has been reported in young boys with immunodysregulation, polyendocrinopathy. Enteropathy X-linked (IPEX), a rare genetic disease caused by an inherited mutation in the gene encoding forkhead box P3 (FOXP3) [148].

Xanthogranulomatous TIN

Xanthogranulomatous pyelonephritis (XGP) is a rare entity usually occurring in the fifth or sixth decade of life, though neonatal and childhood cases have been reported [149–151]. In a review of 66 children who underwent nephrectomy in Ireland between 1963 and 2016 for XPN, the median age was 4.84 years (range 1.1–14.8 years) [151]. It is a chronic destructive granulomatous inflammation of the renal parenchyma first described in 1916 by Schlagenhaufer in association with *Escherichia coli* and *Proteus mirabilis* urinary tract infections [152]. The exact etiology

remains unknown, but it is thought to be a result of chronic obstruction with persistent urinary infection [150, 153]. The disease may be mistaken for malignancy, with the consequence that diagnosis is often made on histology after nephrectomy. Medical management of the suppurative infection is possible when an early diagnosis is made, but this is unusual. Nephrectomy is not uncommon due to irreversible parenchymal destruction [154, 155].

Idiopathic TIN

Approximately 8–10% of cases of acute TIN remain idiopathic [6–8, 12, 32–34]. This diagnosis can only be made after all other possible causes have been eliminated by a thorough history, clinical examination, and relevant laboratory investigations.

Chronic Interstitial Nephritis

Epidemiology

The exact incidence and prevalence of primary chronic TIN is poorly documented. This topic is complicated by the fact that chronic interstitial changes typify virtually all chronic renal disorders that eventually progress to CKD stage 5.

Pathology

The early phase of chronic TIN shares histopathologic features with acute TIN, including interstitial inflammation and tubular cell activation. However, as the disease progresses, interstitial fibrosis and chronic tubular injury (dilated tubules with/without cast formation, atrophied tubules and thickened tubular basement membrane) appear (Fig. 42.5) [156, 157]. In the advanced stages, glomerulosclerosis may occur as a secondary consequence of the tubular damage or periglomerular interstitial fibrosis. For all chronic kidneys diseases, whether initially a glomerular or tubulointerstitial disorder, interstitial fibrosis severity is a strong predictor of renal functional loss and risk of progressive renal disease, as illustrated by a recent study of 1022 patients with IgA nephropathy [158].

Clinical Findings

The clinical findings in chronic TIN are similar to those in acute TIN, but tend to be more subtle and often go undetected until the patient develops signs and symptoms due to chronic renal insufficiency. Compared to chronic glomerular disease, in patients with chronic TIN hypertension is less common, daily protein excretion rates rarely exceeds 1.5 g/day and anemia may be disproportionately worse than the degree of renal functional impairment due to the loss of erythropoietin-producing cells in the peritubular interstitium. Bone disease may also be more prominent as a result of chronic phosphate wasting due to proximal tubular dysfunction.

Etiology

As in acute TIN, there are numerous causes of chronic TIN. In addition to diseases that may progress from acute TIN to chronic TIN, several diseases more typically present as chronic TIN. In the pediatric population, the causes of chronic TIN that are not sequelae of acute TIN can be broadly grouped into the following categories that are also summarized in Table 42.5; many are reviewed in greater detail in other chapters.

Genetic Kidney Diseases, especially the ciliopathies (nephronophthisis) and polycystic kidney disease, are associated with significant tubular damage and interstitial inflammation and fibrosis. Another group of inherited diseases that are increasingly recognized since first reported in 2002 are now classified as autosomal dominant tubulointerstitial kidney disease (ADTKD) [159]. They are rare diseases that are largely undetected in childhood, as the kidney disease is typically silent until clinical manifestations of chronic renal failure develop. Some patients provide a history of polyuria and/or enuresis due to a uri-

Table 42.5 Common causes of chronic TIN in childhood and adolescence

Category	Specific entity
Persistent TIN	All categories (late diagnosis)
Inherited kidney disease	Autosomal Dominant Tubulointerstitial Kidney Diseases (ADTKD) (*UMOD*, *MUC1*, *REN* and other less common gene mutations)
	Karyomegalic TIN (*FAN1* mutation)
	Nephronophthisis (ciliopathies)
	Polycystic kidney diseases
Inherited metabolic disease	Cystinosis
	Oxalosis
	Methylmalonic acidemia
	Mitochondrial cytopathies
	Adenine phosphoribosyltransferase (APRT) deficiency
Acquired metabolic disease	Nephrocalcinosis
	Uric acid-induced injury
	Potassium deficiency (anorexia nervosa)
Chronic nephrotoxicity	Chronic interstitial nephritis in agricultural communities (CINAC)
	Heavy metals (lead, cadmium)
	Calcineurin inhibitors
	Analgesic nephropathy
	Chinese herbs (Aristolochia fangchi)
	Chemotherapy (cisplatinum, isophosphamide)
Structural renal disease	Dysplasia
	Obstruction
	Reflux

nary concentrating defect. The first cases reported were caused by autosomal dominant mutations in *UMOD*, the gene that encodes the kidney-specific protein uromodulin (also known as Tamm-Horsfall protein). Patients often present with symptoms of gout between 15 and 40 years of age due to hyperuricemia (present in ~70% of affected patients) [160]. Stage 5 CKD due to chronic TIN develops between ages 30–60 years; the rate of progression may be decreased in the hyperuricemic patients with the use of allopurinol. Mutations in the *MUC1* gene, which encodes the glycoprotein mucin-1, were first reported in 2013. Mutations in *UMOD* and *MUCI* are the most prevalent etiologies of ADTKD [161]. It is estimated that ~50% of the ADTKD patients currently lack a genetic diagnosis [160]. Eight mutations have been reported in the *REN* gene that encodes prorenin [162, 163]. These patients may develop transient childhood anemia, have a tendency for hyperkalemia, defective urinary concentration and gout. Additional diseases have been classified as ADTKD, although the kidney phenotype may include features in addition to chronic TIN. These include patients with mutations in the genes *HNF1B* encoding hepatocyte nuclear factor 1 beta, and *SEC61A1* that encodes the alpha 1 subunit of SEC61 [161].

There is another rare genetic disease that resembles nephronophthisis histologically except for the presence of hyperchromatic and abnormally enlarged tubular epithelial cell nuclei that causes ESKD in the third or fourth decade of life. It was first recognized as a distinct entity and named karyomegalic interstitial nephritis (KIN) in 1979 [164]. In 2012 Zhou et al. [165] identified an autosomal recessive mutation in *FAN1* as a cause of TIN.

Other causes of chronic TIN include:

1. ***Congenital anomalies of the kidney and urinary tract.***
2. ***Inborn error of metabolism***, including cystinosis, oxalosis, methylmalonic acidemia, the mitochondrial cytopathies, adenine phosphoribosyltransferase (APRT) deficiency.
3. ***Chronic nephrotoxin exposure***, especially the calcineurin inhibitors, lithium, heavy met-

als (cadmium, mercury, and lead), chemotherapeutic agents (cisplatinum, ifosfamide), antimicrobials (amphotericin B, antiretroviral drugs), NSAIDs and certain Chinese herbs.

4. ***Chronic interstitial nephritis in agricultural communities (CINAC)***. This disease entity is increasing in endemic areas of the world, characterized as agricultural communities in hot tropical communities (patients in Sri Lanka and Central America are best studied) [166]. While male agricultural workers in the 20s to 40s age group are most frequently reported, it has also been reported in women, and markers of kidney damage can be found in children [167]. Histopathologically CINAC is a chronic TIN. The etiology is unclear and likely multifactorial. A recent kidney biopsy study by Vervaet et al. [168] reported abnormal proximal tubular lysosomes; by electron microscopy they contained electron-dense aggregates suggestive of a toxin-associate proximal tubulopathy. Several toxic agrochemicals and pesticides have been identified as candidates, but definitive proof of their role is still lacking. Exposure might be direct or may occur from contaminated water consumption or by inhalation. Heat stress and recurrent dehydration may be a contributing factor.
5. *Chronic allograft nephropathy.*

Treatment and Prognosis

Treatment of chronic TIN is based on the treatment of the primary disease process. In addition, there is increasing evidence that correction of anemia, reduction of proteinuria and suppression of inflammation may also slow the rate of the kidney disease progression [156]. Angiotensin converting-enzyme inhibitors and angiotensin receptor type 1 blockers are being used with increasing frequency for a variety of chronic renal diseases, especially when associated with hypertension and/or proteinuria. It is believed that in addition to decreasing intraglomerular pressure, these drugs reduce proteinuria and may also have an anti-fibrotic role related to angiotensin II blockade [169].

Outcomes

Patients with chronic TIN and CKD stage III (GFR 30–59 mL/min/1.73 m^2) or stage IV (GFR between 15 and 29 mL/min/1.73 m^2) are destined to progress to ESKD (GFR < 15 mL/min/1.73 m^2). Numerous comorbid factors correlate with a faster rate of renal functional decline, including hypertension, high-grade proteinuria, diabetes, smoking, obesity, dyslipidemia and anemia [12, 96].

While definitive therapy may not be available for the primary disease process that is responsible for chronic TIN, many of these comorbidities can be addressed therapeutically to preserve residual nephrons and slow the rate of CKD progression. Landmark studies by Risdon and Shainuck, more than half a century ago, highlighted the central importance of chronic TIN, assessed as the degree of tubular atrophy and interstitial fibrosis, to renal functional outcomes irrespective of the primary etiology of the CKD. Since then, advances in the field of cellular and molecular biology and genome sciences have utilized animal models and human kidney tissue biorepositories to decipher fundamental mechanisms that cause the chronic TIN component in human CKD. A major priority for ongoing and future studies is the identification of new therapeutic targets, development of safe therapeutic agents based on these "candidate" targets and subsequent randomized prospective clinical trials to establish their efficacy in patients with CKD. Analogous to current cancer treatment protocols, a multi-agent approach will almost certainly be necessary, taking into consideration specific genetic and molecular disease markers that get us closer to personalized medicine. Based on the current state of knowledge, several interrelated pathogenic pathways are potentially amenable to drug therapies [157]:

1. Preserving tubular epithelial cell integrity (and thus intact nephrons) by minimizing tubular injury/death/senescence and enhancing the repair of damaged tubules. Reactivating key kidney developmental pathways has been promising in experimental models. Permanent

tubular loss is a key predictor of irreversible CKD.
2. Blocking the numbers and/or function of a unique population of interstitial myofibroblasts that are the primary source of the scar-forming extracellular matrix proteins.
3. Regulating the interstitial cell inflammatory response that has multiple consequences—some harmful and others healing. The role of macrophages appears to be particularly important.
4. Disrupting the vicious circle of hypoxia and oxidant stress that develops at least in part because of the lack of preservation of a healthy interstitial capillary network vital for adequate kidney oxygenation.
5. Reducing the progressive accumulation of extracellular matrix proteins in the interstitium. Despite the identification of several matrix-degrading proteases in kidneys, there is still no convincing evidence that renal fibrosis can be reversed in humans. Effective therapies will need to target the extracellular matrix production pathways.

Further laboratory and clinical studies are needed to identify new evidence-based therapeutic options to improve long-term outcomes for patients with chronic TIN.

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Part IX

Urinary Tract Disorders

43 Diagnosis and Management of Urinary Tract Infections

Ian K. Hewitt and Giovanni Montini

Introduction

Urinary tract infection (UTI) represents colonisation and invasion of the urinary tract by bacteria. The term cystitis is used when the organisms are confined to the bladder and urethra, and usually accompanied by localized symptoms such as dysuria, frequency, malodorous urine, day time and night time urinary incontinence. They occur most predominantly in girls older than 3 years of age and are easily treated. Acute pyelonephritis, considered by many to be the most common serious bacterial illness in childhood, is an infection that involves the renal parenchyma. Most affected children, particularly infants, present systemically unwell with symptoms including high fever, which may be the only indicative feature, lethargy, abdominal pain, nausea, vomiting and irritability. These infections have been considered of most concern for their potential to result in renal scarring, with the potential for long-term morbidity. Asymptomatic bacteriuria is a further type of urinary tract infection whereby a significant growth of organisms from the urine occurs in the absence of any illness or symptoms. It has been found in approximately 1% of school-aged girls on screening, and does not warrant treatment or investigation, as trials have demonstrated a good outcome, uninfluenced by any intervention.

Approximately 2% of boys and 7–8% of girls will experience a urinary tract infection in the first 8 years of life [1]. Febrile urinary tract infections suggestive of acute pyelonephritis are most common in the first year of life with a male predominance in the first 6 months, after which they are more often seen in girls (Fig. 43.1). Non-febrile UTI, typically cystitis, is seen more commonly in girls as they get older.

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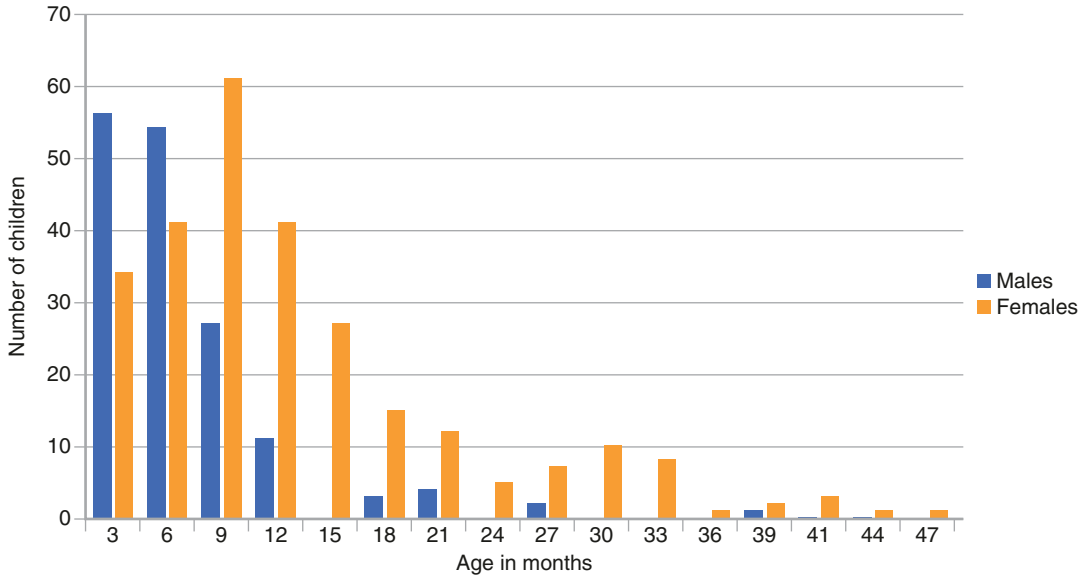

Fig. 43.1 Distribution by age (months) and sex of a cohort of 427 children (269 F, 158 M), aged 1 month to 4 years, at the first febrile urinary tract infection

Background

In recent years there has been a re-evaluation of the potential for UTI to culminate in severe long term renal parenchymal damage. In the past children usually came to attention only after experiencing UTIs, and were investigated with invasive imaging modalities, such as intravenous pyelography and voiding cystourethrograms. Small hypodysplastic and scarred kidneys, often in association with vesicoureteral reflux (VUR), were occasionally found, and were thought to be the consequence of previous episodes of unrecognized pyelonephritis. This led to the concept of reflux nephropathy, linking VUR to ascending infection and pyelonephritis, with subsequent renal scarring [2]. Consequent upon this, children with reflux were often placed on long term antibiotic prophylaxis or underwent surgical correction (Fig. 43.2a). Already at this time, not all agreed with this view. A French paper demonstrated that 11 of 12 nephrectomies performed because of severe reflux nephropathy were congenital small kidneys, with the remaining kidney showing isolated and specific histologic signs of chronic pyelonephritis [3]. Two early studies randomized children with VUR detected following a UTI to either antibiotic prophylaxis with surgical correction alone or combined with adjuvant prophylaxis [4, 5]. In one study the rate of scarring was 38% at presentation, while subsequent rates of scarring were low (2% and 9%) and unrelated to the presence or absence of VUR or breakthrough infection [4]. These two studies already demonstrated the difference between congenital renal mal-development and acquired scarring as a consequence of pyelonephritis. The introduction of routine antenatal ultrasound from the mid1980s onward, led to the recognition that much of the significant renal damage, often familial and seen in association with high grade VUR was the consequence of congenital abnormalities of the kidney and urinary tract (CAKUT) (Fig. 43.2b) [6]. This has led to questioning the role of acquired renal damage as a consequence of pyelonephritis as the major contributor to chronic kidney damage.

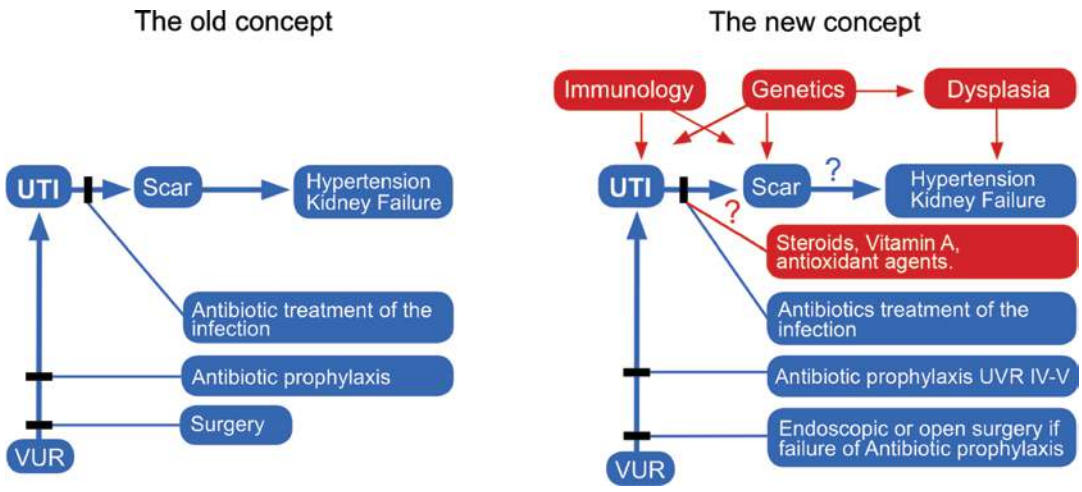

Fig. 43.2 The old concept (**a**). A casual relationship between febrile urinary tract infections and chronic renal failure was believed in the past to be the cause of major scarring. Vesicoureteric reflux was thought to be a major predisposing factor. Prompt antibiotic treatment of the acute infection was suggested to prevent renal scarring. Long term antibiotic prophylaxis and surgical correction of vesicoureteric reflux were employed to prevent recurrent urinary infections. The new concept (**b**). An enhanced understanding of genetic and immunologic mechanisms and prenatal ultrasound have revealed that major kidney damage appears to be congenital in origin. Trials have demonstrated surgery and antibiotic prophylaxis to be of limited if any benefit in preventing kidney damage. New treatments, such as the use of steroids and vitamin A, show promise in reducing the burden of post-infectious scarring

Clinical Presentation

Symptomatology of urinary tract infection varies considerably and is influenced by the child's age, level of infection, virulence of the organism and the inflammatory immune response. In newborns and infants, nonspecific symptoms such as prolonged neonatal jaundice, labile temperature, slow feeding and poor weight gain, irritability or listlessness with the infant unusually floppy are all hallmarks of infection. There is a propensity for infection in the very young to result in septicemia and sepsis, with possible adverse consequences such as seeding of infection at other sites, including meningitis.

Symptoms directly referable to the urinary tract such as frequency and pain on micturition are not usually recognised until around 2 years of age and beyond. Loin pain as a consequence of pyelonephritis, even if present, is not generally commented on by the child until 4–5 years of age. Cystitis can be associated with fever, however when this is 38.5 °C and above the likelihood of renal parenchymal infection is increased.

In older children, recurrent cystitis and, less frequently recurrent febrile UTIs, may be a feature of voiding disturbances, including hyperactivity and dysfunctional bladder emptying. Therefore, in these children, it is important to obtain a precise history of voiding patterns including incontinence, enuresis, frequency of micturition, urgency and bowel habits (see Chap. 47 for further discussion).

Diagnosis of Urinary Tract Infection

The diagnosis of urinary tract infection is contingent upon the culture of a single organism in significant numbers from an appropriately collected urine specimen. Laboratories use a standardized technique taking 0.001–0.002 mL (1–2 μL) of urine in a sterile loop, plating it out and on a culture medium and incubating in air at 35 °C with

the plates checked for the nature and number of colonies (Fig. 43.3). Dipslide cultures (Fig. 43.4) are an alternative to the loop technique, particularly when specimens are collected after hours, thus allowing earlier reporting of culture results. While urinalysis is a useful adjunct in the diagnosis, particularly as culture results may take 24–48 h, it can never be a substitute for urine culture. The cut-off level for significant growth of organisms was first set by Kass 60 years ago at $\geq 10^5$ organisms/mL studying pyelonephritis in pregnant women [7]. It was apparent to Kass at the time that such a cut-off would by necessity miss UTIs. This is no less so in the childhood population. A recent study of infants <1 year with a symptomatic UTI diagnosed by supra-pubic aspirate demonstrated 19% to have colony counts $<10^5$ of whom 87% had counts $<5 \times 10^4$ [8]. In an editorial comment on the paper it was noted that diagnosing a UTI on a strict cut-off level was inappropriate [9]. Most guidelines on investigation and management of UTI in children have avoided a detailed discussion of what constitutes an infection. Recently the Diagnosis of Urinary Tract infection in Young children (DUTY) study aimed at validating a clinical algorithm for the diagnosis of a UTI in children presenting with an acute illness to primary care. Collection was by pads in infants and clean-catch in older children. Agreement on UTI diagnosis was lower than expected between government and research laboratories and clean-catch encouraged as the preferred collection method [10]. A particular problem that creates difficulty with interpretation of urine cultures, resulting in false positive tests, is contamination during collection, which can be influenced by a number of factors. Contamination occurs when organisms from the genitalia, surrounding skin, urethra or periurethral area infect the specimen during collection. *Suprapubic aspiration* of urine and samples obtained by percutaneous nephrostomy are considered to be the "gold" standard for diagnosis of UTI, as they are the least likely to be contaminated, with the culture of any organism generally considered to be significant. Ideally suprapubic aspiration should be performed under ultrasound guidance, even then the invasive nature and lack of skill in the procedure often preclude its use. *Urethral catheterization* is the next most effective method of obtaining a reliable specimen for culture with a growth of 10^3–10^4 colonies per mL considered significant. Once again parents may consider this invasive and distressing for the child, with concerns that infection may be introduced by the procedure. A *voided clean catch midstream urine collection* has been long been considered the most practical method, particularly when children are toilet trained and can void on request. Colony counts of a single organism $\geq$50,000 colonies per mL accompanied by pyuria are considered diagnostically significant [11]. In our experience, mainly using midstream samples, we believe that both urinary leucocytes and a sig-

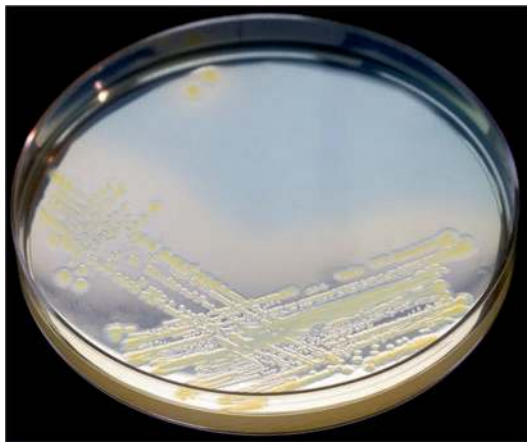

Fig. 43.3 This image shows bacterial colonies growing on an agar plate incubated at 35°C

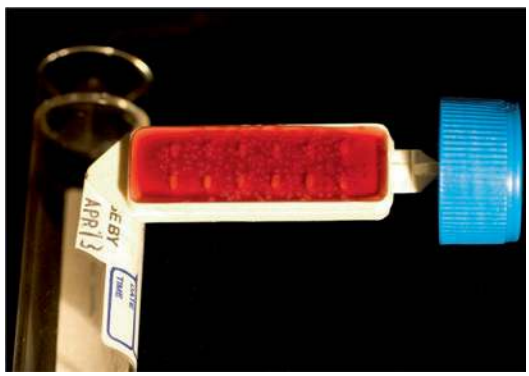

Fig. 43.4 Dipslide culture, which consists of a sterile culture medium on a plastic carrier that is dipped into the urine to be sampled and then incubated at 30°C for 48 h

nificant colony count are needed for the diagnosis of UTI [12]. The presence of pyuria is a useful means to assist in the differentiation between contamination, colonization and clinically significant infection. When sterile pyuria is detected with leukocyte counts >100 × 10^6/L, the urine should be tested for antibacterial activity, if negative, consideration should be given to the culture of fastidious organisms such as *Ureaplasma* and *Gardnerella* species that require specific growth media. When multiple organisms are cultured, there is an increased likelihood of contamination and the culture should be repeated.

Clean catch urine specimens can be obtained on occasion in infants and the very young, with the diaper removed and the parent waiting patiently for voiding to occur, however this is not always successful. One study described a success rate of 86.3% for obtaining a midstream urine specimen within 5 min in infants up to 30 days of age, using a bladder and lumbar stimulation technique [13]. *Bag collections* are practiced in a variety of settings and are of use as a screening procedure when the risk of infection is considered low or the child is afebrile. Controversy exists when bags are employed as the sole method of collection, with many claiming contamination rates are too high [14], such that a single positive specimen should be repeated, preferably with a catheter collection or supra-pubic aspirate. From a practical viewpoint, the use of bag collections is widespread, particularly in the primary care setting, where practitioners often do not have formal training in how to perform a suprapubic or catheter collection [15]. A bag specimen is particularly useful if negative for leukocytes on dipstick, in which case culture is unnecessary. If positive for leukocytes then a clean catch or catheter specimen for culture is appropriate. It has to be emphasized that if bag collection is the only method employed, strict protocols for the procedure are mandatory to reduce the contamination risk (how to wash the genitalia, how to apply the bag, the need to change the bag every 20–30 min). In our experience, when a count >10^5 of the same single organism in two consecutive carefully collected bag specimens is obtained, the likelihood of a genuine infection is extremely high.

Because of the delay in obtaining a urine culture result, the initial diagnosis and treatment is often guided by results of preliminary urinalysis and clinical symptoms. If a UTI is strongly suspected, particularly when accompanied by fever, antibiotic treatment should be initiated, based on the knowledge of local drug sensitivities, prior to availability of culture results. When urine microscopy is available this can be used to determine the number of white cells per mL in an un-spun urine specimen as well as document the presence of bacteria and non-glomerular hematuria that may occur with cystitis. Urine dipsticks provide a rapid, easy and cost-effective way of guiding initial management and treatment (Table 43.1). Leukocyte esterase activity in the urine is a sensitive indicator of pyuria while the nitrite test is based on the ability of most uropathogens to convert nitrite to nitrate. While a positive nitrite test is a good indicator of infection, it can be positive with asymptomatic bacteriuria and thus requires significant pyuria for confirmation [12], in addition, up to 4 h is required for the organisms to generate sufficient nitrite such that infants and those with urine frequency often have a false negative test. When more than one abnormality is found on urinalysis the likelihood of UTI is increased. Difficulties can arise with a single abnormality and subsequent borderline culture result, particularly when antibiotics have already been commenced.

Table 43.1 Interpretation and suggested practical approach following the result of nitrite and leukocyte esterase urine dipstick testing, performed immediately following collection (Adapted from Ammenti et al. [12] with permission)

Nitrite positive Leucocyte esterase positive	UTI very likely	Perform urine culture and start antibiotics empirically
Nitrite negative Leucocyte esterase positive	UTI likely	Perform urine culture and start antibiotics empirically
Nitrite negative Leucocyte esterase negative	UTI quite unlikely	Search for alternative diagnosis

Abbreviation: *UTI* urinary tract infection

There is a need for studies on appropriate urine sampling, interpretation of urine culture results (in particular the appropriate cut-offs for various methods of urine collection in children) and to determine if a negative urine leucocyte test can safely rule out a UTI or whether a culture is required.

Differentiating Upper from Lower Urinary Tract Infections

Differentiating between lower tract cystitis and upper tract pyelonephritis can be difficult in the acute setting. While fever and loin pain can result in a presumptive diagnosis of renal parenchymal infection it is not always the case. Currently there is no marker that can reliably predict the presence of acute pyelonephritis. While C-reactive protein and white cell count are often elevated they are insufficiently sensitive and specific for the diagnosis of renal parenchymal infection. Procalcitonin, a precursor of calcitonin, produced by the thyroid gland, and released in response to bacterial infections, appears to be the most robust blood test, but not a perfect predictor, of pyelonephritis and propensity for subsequent renal scarring [16]. Urinary markers that differ in pyelonephritis compared with cystitis include chemokine ligand CXCL1, CXCL9, CXCL12, INF gamma and IL15, however none has proved better than Procalcitonin [17]. Technetium-99m-labelled DimercaptoSuccinic Acid (DMSA) scans are considered the "gold standard" for confirmation of acute pyelonephritis (Fig. 43.5). DMSA scanning had been recommended as an initial investigation in the past, but did not receive wide uptake as it causes a significant radiation dose, needs to be performed within a week of the infection and is not available in many non-tertiary centers. Fever associated with the UTI has become indicative of likely pyelonephritis for clinical purposes [18, 19].

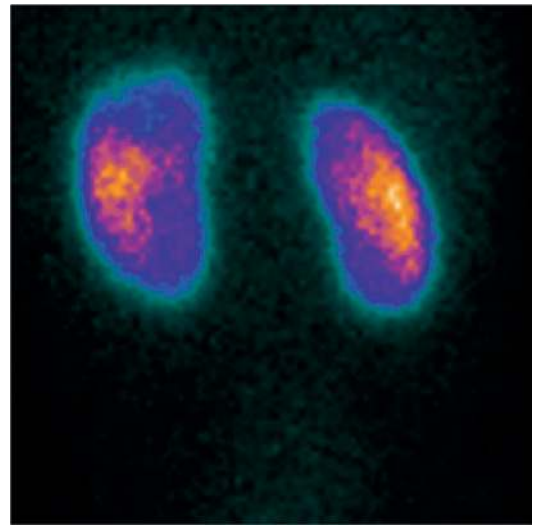

Fig. 43.5 Renal DMSA scintigraphy during the acute phase of a febrile urinary tract infection, showing acute pyelonephritis involving the left kidney

Pathogenesis

The kidneys and urinary tract are germ free environments under normal circumstances, while some infections may be blood born in origin, the majority are considered to be ascending in nature. When bacteria enter the bladder they are frequently washed out without causing infection [20]. Some develop asymptomatic bacteriuria while others develop cystitis with inflammation of the bladder mucosa. A few children will experience febrile UTIs with systemic activation of the immune inflammatory process.

A variety of host factors are thought to contribute to UTI in early childhood in the absence of significant renal tract anatomical abnormalities, these include dysfunctional bladder emptying prior to and after the development of urinary continence [21, 22], detrusor instability [23], constipation and fecal soiling prior to establishing bowel continence. Children with primary immunodeficiency do not appear particularly prone to

UTI, even those with frequent bacterial infections elsewhere, secondary to primary antibody deficiency states [24–26]. Information on UTIs in this group is scarce as the majority of infections are respiratory, gastrointestinal and skin. In the few instances where mention is made of UTIs in primary immunodeficiency diseases, associated renal tract abnormalities appear a feature. Similarly, with acquired immunodeficiency, such as organ transplantation, immunosuppression with steroids and chemotherapy, the issue of UTIs arises only in the context of catheterisation, instrumentation, stents involving the renal tract and not isolated immunosuppression per se. These observations support the contention that urine flow and integrity of the uroepithelium are the important factors in preventing infection. In addition, the intact uroepithelium appears to have an innate immune system with the intercalated cells secreting antimicrobial peptides that demonstrate potent bactericidal activity toward uropathogens [27]. Recent interest has focussed on uromodulin (UMOD), a glycoprotein secreted by the kidney into the urine. Eighty percent of humans have a UMOD promoter variant that results in a doubling of the urinary UMOD concentration, resulting in a reduced predisposition to UTIs [28, 29]. UMOD protein filaments aggregate around uropathogens causing bacterial clumping; it is likely that this inhibits adhesion, allowing clearance of the organisms by micturition [30].

Certain bacterial characteristics predispose to infection. In excess of 80% of infections are caused by *Escherichia coli*. These are the predominant bowel organisms found in close proximity to the urethral orifice. In addition, they have P fimbriae that enable uroepithelial adherence even in the presence of adequate urine flow. When children have neurogenic bladders or renal tract malformations that lead to urine stasis or residual urine after voiding, non-attaching bacteria may cause infection.

When uropathogens infect kidney parenchyma localized inflammation occurs, triggering the innate immune system through a variety of pathways. Recognition of bacteria initiates toll like receptor signalling that in turn causes an immune response involving nuclear factor κβ, cytokines and chemokines. Interleukin 8 (IL-8) is an important chemokine that activates neutrophils via receptors CXCR1 and CXCR2. Children with polymorphisms of IL-8, CXCR1 and CXCR2 have reduced trans-epithelial migration of neutrophils, defective clearance of bacteria and demonstrate a predisposition to recurrent pyelonephritis [31–33].

Full recovery can occur, however if the inflammation is prolonged, scarring can result, though the exact predisposing factors and mechanisms remain ill understood, a number of possible genetic determinants have been identified [34]. Interleukin 6 is one factor that plays a role in the immune response and has been shown to be a marker of children who develop later scarring [35]. Functional variants in genes that encode for vascular endothelial growth factor and transforming growth factor β1 and factors that regulate fibrosis in response to tissue inflammation have also been implicated in renal parenchymal scarring following pyelonephritis in children [36]. Ethnicity has been proposed as an additional risk factor: a meta-analysis demonstrated that post pyelonephritic scarring in children varied by region from 27% in Australia to 49% in Asia [37].

Several pharmacological approaches to limit renal scarring have been explored. Three randomized controlled trials (RCTs) on the use of **steroids** yielded conflicting results. One study of oral methylprednisolone compared with placebo in 84 children with a first episode of pyelonephritis on DMSA scan demonstrated a significant reduction in scarring at 6 months (33% vs. 60% $p < 0.05$) in those given steroid [38]. The second study comparing dexamethasone with placebo in 52 children with pyelonephritis showed no benefit in scarring [39]. The most recent double blind RCT evaluated the efficacy of an oral 3-day course of dexamethasone in reducing kidney scarring in children 2 months to 6 years of age [40]. Unfortunately, only 254 of the 546 children randomized had the primary outcome evaluated with a late DMSA scan. The absolute risk reduction was 5.9% (95% confidence interval: −2.2; 14.1), which did not reach statistical significance.

Vitamin A has been promoted as an agent that might alleviate scarring following acute pyelonephritis. It has also been demonstrated to impact urinary tract morphogenesis, with a deficiency or excess resulting in CAKUT [41]. A meta-analysis including 4 RCTs of 248 children concluded that Vitamin A may be of benefit, however the studies were of low methodologic quality [42]. A recent high-quality double-blind placebo RCT assigned 90 girls (mean age 5, range 2–12 years) with DMSA scan-confirmed acute PN to a 10-day course of oral vitamin A (1500 U/kg/day) or placebo. Symptoms were briefer in the treatment group. A late DMSA scan demonstrated worsening in 8 (21%) on vitamin A vs. 17 (48%) on placebo ($P = 0.003$), while 23 (64%) on vitamin A and 8 (21%) on placebo demonstrated improvement in photopenia ($P < 0.0001$) [43].

In experimental models a variety of agents including montelukast, a leukotriene CysLT1 receptor antagonist that reverses oxidative effects [44], COX-2 inhibitors [45], melatonin [46], an inhibitor of neutrophil infiltration, and losartan, that down regulates TGFβ production [47], have all shown potential as therapeutic agents, though all lack clinical corroboration. More needs to be understood regarding the renal parenchymal inflammatory response and mechanisms of scar formation if effective strategies are to be developed to prevent or reduce scarring.

Bacterial Virulence

Uropathogenic *Escherichia coli* (UPEC) are a subgroup of the *Escherichia coli* that form the normal bowel flora. They express a range of virulence mechanisms that make them well suited to invade the renal tract and kidneys, resist the innate immune responses and initiate a damaging inflammatory cascade. Specific adhesins that form part of fimbriae or pili on the external surface of the organism facilitate attachment to uroepithelial cells [48]. Type 1 pili found on virtually all UPEC predispose to bladder infection, while P pili are associated with acute pyelonephritis [49]. Once they invade the uroepithelial cells, additional virulence factors, termed autoinducers, can coordinate the formation of an intracellular biofilm like cluster of rapidly multiplying organisms, that are protected from the host immune system. The bacteria can go on to infect other cells or remain dormant for a period with the ability to cause recurrent infections [50]. Additional virulence factors transmitted by plasmids promote tolerance to antibiotics [51]. Understanding these host pathogen interactions is of increasing importance at a time where drug resistance is a serious issue. Strategies specifically targeting virulence factors are being investigated as an alternative to antibiotics [52–54] as is the colonization of at-risk patients with non-pathogenic *Escherichia coli* in the hope that they might keep uropathogenic organisms at bay [55].

Organisms Causing Infection

Escherichia coli is the causative organism in 80–90% of UTIs that occur in the outpatient setting [56, 57]. The remaining 10–20% of infections are caused by a variety of organisms including *Klebsiella, Enterococcus, Enterobacter, Proteus* and *Pseudomonas species.* Infection with atypical organisms such as *Pseudomonas* increase the risk that underlying renal pathology exists, such as a neurogenic bladder or significant anatomic abnormalities [58]. In the inpatient setting *E. coli* account for approximately 60% of infections with the higher incidence of atypical organisms a reflection of underlying pathology as well as urinary tract procedures undertaken in this context [59].

Candida UTIs are predominantly a concern for neonatal intensive care units. The infections are associated with prematurity, respiratory distress, congenital heart disease, major renal tract abnormalities and sepsis, while systemic corticosteroids use is a probable additional risk factor. Mortality has been reported as high as 30% and is more likely when systemic spread has occurred, with the co-morbid conditions also playing a role [60].

Granulomatous interstitial tuberculous nephritis is a recognised entity that can occur on rare occasions in children, almost always in devel-

oping countries, and often associated with co-morbid conditions such as human immunodeficiency virus (HIV) [61] or underlying renal disease that predisposes to the infection [62]. It should be considered when sterile pyuria occurs in association with tuberculosis elsewhere in the body [63]. *Schistosomiasis* is a common tropical blood fluke infection, particularly in sub-Saharan Africa. A fresh water snail is the intermediate host, with infection following exposure to the infected water; children have the highest burden of disease [64]. The organism enters through the skin and can infect the ureters and bladder causing hematuria. It is being seen with increasing frequency in high income countries with the arrival of travellers and refugees from endemic areas [65].

Treatment of the Acute Episode

With their advent, antibiotics have become the cornerstone of treatment for UTIs resulting in dramatically improved outcomes. In the early twentieth century infants and young children hospitalized with acute pyelonephritis frequently died as a consequence [66]. Despite the recognized effectiveness of antibiotics in treating UTIs and impeding their parenchymal localization, until the mid-1990s there was a lack of research regarding the appropriate agent, manner of administration and duration of treatment required. Uncomplicated afebrile UTIs were and remain for the most part treated with oral antibiotics that have a high renal excretion and a restricted spectrum of activity. A recent systematic review of antibiotic treatment for uncomplicated lower UTI in children concluded that short course oral therapy (3–7 days) was as effective as long course (7–10 days) treatment with single dose therapy not recommended until further studies are undertaken to determine its effectiveness [67]. In contrast, for febrile UTIs, particularly when occurring in infants and younger children, hospital admission and broad-spectrum intravenous antibiotics became the norm. Between 1995 and 2004 five trials demonstrated that short courses of intravenous antibiotics (up to 4 days) followed by oral therapy, were as effective as longer courses of intravenous treatment (7–14 days) [68–72]. Four studies involving a total of 1344 infants and children as young as 1 month of age with a first febrile UTI went further, demonstrating that oral antibiotics alone were as effective as intravenous antibiotics followed by oral antibiotics, the studies showed no difference in time to resolution of fever or subsequent renal damage [56, 57, 73, 74]. Current guidelines for treatment of a febrile UTI recommend oral antibiotic administration on an outpatient basis, with hospital admission and parenteral administration restricted to those children who appear to be severely ill, vomiting or where poor compliance is a risk (Table 43.2) [75, 76]. The choice of antibiotic should be guided by local resistance patterns and the lead organism, *E. coli*. Where good β lactam and cephalosporin sensitivity has been demonstrated, these are the preferred antibiotics. Of concern, there is an increased prevalence of *E. coli* producing extended spectrum beta-lactamase (ESBL) [77]. Commonly used antibiotics are listed in Table 43.3. Provided an organism is sensitive to the drug chosen, no antibiotic has been shown as superior to another. Hence, we encourage cephalosporins (cefixime or ceftibuten for the oral route and cefotaxime or ceftriaxone for iv administration) in children with severe infections. Cephalosporins are recognized to have a more rapid onset of action, making the possibility of resistance a less important issue. When a febrile infection occurs in children receiving antibiotic prophylaxis, a different antibiotic is required, because of an almost certain resistance to the prophylactic agent.

Table 43.2 Treatment of complicated and uncomplicated febrile urinary tract infections

Uncomplicated febrile UTI ↓ Oral antibiotic on an outpatient basis	Complicated febrile UTI ↓ Parenteral antibiotic—hospital admission
Good general condition	Septic child
Able to drink	Vomiting
Absent to mild dehydration	Moderate to severe dehydration
Good compliance	Possible poor compliance

Table 43.3 Antibiotics commonly used for the treatment of febrile urinary tract infections in children. (Adapted from Montini et al. [6] and Ammenti et al. [12]). Antibiotic choice should be based on local antimicrobial sensitivity patterns

Intravenous treatment	*Dose*	*Comments*
Penicillins		
Ampicillin-Sulbactam	100 mg/kg/day of ampicillin in 3–4 doses	
Amoxicillin-clavulanic acid	100 mg/kg/day of amoxicillin in 3–4 doses	
Cephalosporin		
Cefotaxime	20–40 mg/kg four times per day	Increasing resistance
Ceftazidime	30–50 mg/kg three times per day	Good coverage for Pseudomonas
Ceftriaxone	50 mg/kg once per day	Advantage of once daily dosing Contraindicated in neonates, especially prematures
Aminoglycoside		Useful if beta-lactam allergy. Nephrotoxic. Must monitor with serum levels and adjust dosage accordingly. A recent meta-analysis supports single daily dosage[a]
Gentamycin	6–7.5 mg/kg once per day or 2–2.5 mg/kg three times per day	
Amikacin	15 mg/kg once per day or 7.5 mg/kg twice per day	
Oral treatment	*Dose*	*Comments*
Trimethoprim-sulfamethoxazole	4 mg/kg twice per day (dose expressed as trimethoprim)	High resistance rates Risk of allergic reaction
Amoxicillin-clavulanic acid	25–50 mg/kg twice per day (dose expressed as amoxicillin)	Increasing resistance
Cephalosporin		Increasing resistance
Ceftibuten	9 mg/kg twice per day the first day, once daily thereafter	
Cefixime	8 mg/kg twice per day the first day, once daily thereafter	
Quinolone		Treatment of complicated UTIs as a second choice. Increasing resistance Increased risk of musculoskeletal adverse events
Ciprofloxacin	10–20 mg/kg twice per day (maximum 750 mg per dose)	

[a]Contopoulos-Ioannidis DG, Giotis ND, Baliatsa DV, Ioannidis JP. Extended interval aminoglycoside administration for children: a meta-analysis. Pediatrics 2004;114(1):e111–e118

Infants in the first month of life who present unwell with unstable temperature, lethargy, poor feeding or prolonged jaundice, and are thought to be septic, are a particular group that warrants intravenous antibiotics after appropriate cultures, including blood and urine, are taken. Sepsis in the first 72 h is most commonly group B streptococcus, although *E. coli* infections can occur in this period and become more common thereafter. There is a lack of controlled trials in this age group, as neonatal sepsis is recognized as a serious illness, particularly when it occurs in low birth weight preterm infants. Contributing factors to the potential severity of bacterial infection in infants include an immature immune system, and in the case of preterm infants, reduced placental transfer of protective maternal antibodies. Thus, intravenous penicillins combined with aminoglycosides are commonly used on an empiric basis. Care should be taken to monitor aminoglycoside levels because of their potential nephrotoxicity, particularly in infants with impaired renal function secondary to renal dysplasia.

Imaging After a Febrile Urinary Tract Infection

There is a lack of consensus as to the recommendations for imaging after a fUTI, although recent improved understanding of UTIs and their sequelae has resulted in a lessening of the nature and number of procedures indicated (Table 43.4). Published protocols give guidance on the investi-

Table 43.4 Imaging tests routinely recommended by the most recent published guidelines in children following a first febrile urinary tract infection

Guideline	Ultrasound	VCUG	DMSA	Prophylaxis
NICE (2007, updated 2017)	Atypical, <6/12 age	No unless <6 months of age with positive US or atypical UTI	Yes >6/12 post UTI	No
AAP (2011)	Yes	No unless abnormal US	No	No
ISPN (2020)	Yes	No unless abnormal US or bacteria other than UPEC	Consider if major abnormalities at US	Considered for VUR IV–V
CARI (2014)	Yes, if: no second or third trimester US, <3/12 age or atypical UTI	No unless abnormal US	No	No
Canadian (2014)	Yes	No unless abnormal US	No	No

gation of febrile UTIs in infants and younger children; however, there is little advice regarding afebrile UTIs or infections occurring in older children.

Ultrasonography

Ultrasonography is a non-invasive procedure that can detect a variety of anatomical abnormalities, but is dependent on the expertise of the radiologist. In recent years antenatal ultrasound has proved of value in detecting most of the significant CAKUT prior to birth, including renal hypodysplasia, pelviureteric junction obstruction, vesicoureteric obstruction often as a result of ureteroceles and posterior urethral valves [78]. In three prospective trials involving 864 children investigated after a first febrile UTI, ultrasonography did not reliably detect changes associated with reflux or predict subsequent scarring. Minor abnormalities were found in 12–14% of cases that had little influence on subsequent outcomes [79–81].

The American Academy of Pediatrics (AAP) recommends ultrasonography in all children under 2 years of age after an initial febrile UTI [11], as does the Italian Society of Pediatric Nephrology (ISPN) [75]. The recently published Italian guideline identifies specific ultrasound abnormalities which represent an indication to perform VCUG: mono- or bilateral renal hypoplasia, abnormal parenchymal echogenicity, ureteral dilatation, kidney pelvis epithelial thickening, pelvi-calyceal dilatation, and bladder abnormalities [12].

The National Institute for Health and Care Excellence (NICE) guidelines restrict ultrasonography further to those <6 months of age, unless the infection is atypical or additional risk factors are present [76]. The Australian guidelines even restrict ultrasound to <3 months of life or to children with an atypical UTI or absent antenatal ultrasound [82]. Given the low detection rate of clinically significant findings following an uncomplicated UTI in childhood, we enquire as to whether a reliable second or third trimester antenatal ultrasound is available to be reviewed and limit ultrasound to those where this is not the case (Fig. 43.6). If the infection appears atypical, there is evidence of renal function impairment, inadequate urine stream or repeated infection, we believe ultrasonography to be indicated [83, 84]. For older children ultrasonography is indicated for repeated febrile and nonfebrile UTIs after a precise history of voiding patterns has been taken, particularly in girls.

The ultrasound examination should assess bladder filling and emptying, post-micturition residual urine and bladder wall thickness when full and empty. Normal values are usually less than 3 mm when the bladder is full and less than 5 mm when empty. In case of significant abnormal findings on bladder ultrasound, urodynamic studies should be considered.

Voiding Cystourethrography

Voiding cystourethrography generally requires the bladder instillation of a radiopaque, radioactive or echogenic contrast medium via urethral

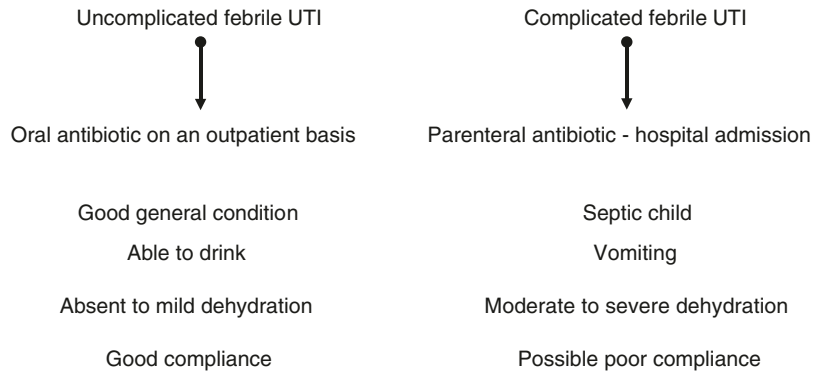

Fig. 43.6 Treatment of complicated and uncomplicated febrile urinary tract infections

catheterization, followed by serial imaging. It can demonstrate VUR during the filling and voiding phases, as well as demonstrate urethral obstruction due to posterior urethral valves in boys. Much controversy has centred on the need or otherwise for this study, given the distress this invasive procedure can cause children. Those advocating cystourethrography cite a strong association between the severity of reflux and presence of renal damage [83], which is being increasingly recognized as congenital rather than acquired in origin [85, 86]. As there is a lack of evidence that intervention is beneficial for all but those few children with high grade dilating reflux, in terms of medical or surgical procedures [87], published protocols support a selective approach to this investigation. In children following a febrile UTI in the first 2–3 years of life, they recommend this study if the ultrasound is positive or the UTI atypical (Table 43.4). We believe that this investigation is rarely indicated following a first febrile UTI and should be restricted to those few children with significant bladder abnormalities, ureteric dilatation on ultrasound or inadequate urine stream (Fig. 43.6). For older children with febrile UTIs and those with recurrent cystitis, we believe voiding cystourethrography is rarely indicated, as a precise history of voiding patterns and an ultrasound are usually sufficient to establish the diagnosis and treatment (see Chap. … for further discussion).

Renal Scintigraphy

Renal imaging with DMSA requires the intravenous administration of a radio-active isotope which localizes to the renal parenchyma, the procedure is highly sensitive, with decreased uptake that may represent inflammation as seen in acute pyelonephritis (Fig. 43.5) or may represent scarring (Fig. 43.8a). The main concern is the radiation dose of approximately 1 mSv. The procedure has been used to confirm pyelonephritis when performed in close proximity to a febrile UTI, or from 6 to12 months after to determine if scarring has resulted. The delay is important for the determination of scarring, as uptake defects can persist for some months following the acute infection, without necessarily indicating scarring. The technique may also detect congenital renal hypodysplasia (Fig. 43.8b), which can sometimes be difficult to differentiate from acquired scarring, however the former usually appears as a small kidney with uniform uptake of isotope while the latter is more likely to present as a loss of smooth contour with a focal uptake defect (Fig. 43.8a, b).

The AAP guidelines do not recommend an acute or late DMSA scan following a febrile UTI [11], while a late DMSA scan is recommended by NICE when the infection is atypical [76] and by the ISPN where ultrasound shows abnormalities or there is evidence of VUR [75]. In both cases the purpose is to detect any scarring. An

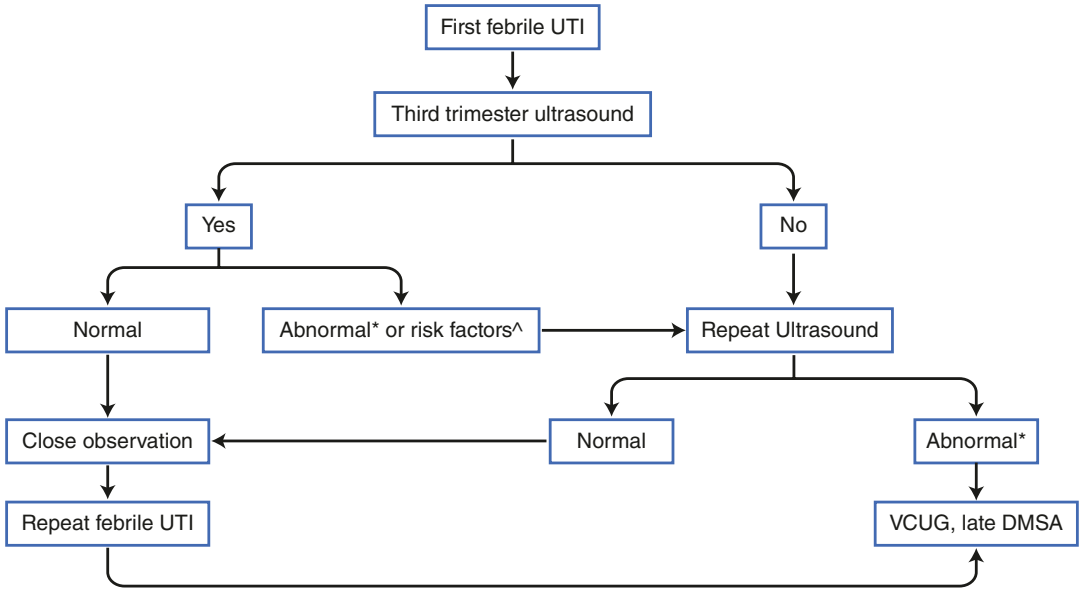

Fig. 43.7 Diagnostic flow chart of the suggested diagnostic protocol in a child 2 months to 3 years of age, following a first febrile urinary tract infection

alternative that was never widely accepted was the "top down" approach whereby a DMSA scan is performed in the acute phase of the illness, followed by cystourethrography if the scan is positive. Two studies claimed a strong correlation between high grade dilating VUR and abnormal scans. In contrast, a later study demonstrated 14/46 children with grade III–IV reflux to have a normal DMSA scan during the acute infection [88]. Some investigators have recommended a DMSA scan 6–12 months following an infection as the most appropriate investigation, as this would detect those children with permanent renal scarring that requires follow-up [81, 89]. When UTIs are afebrile, DMSA scans are not indicated.

Technetium-99m-labelled diethylenetriaminepentaacetic acid (DTPA) and technetium-99m-mercaptoacetyltriglycine (MAG 3) are radionuclides that as well as being taken up by the kidney have an early excretory phase. As such that they are useful for assessing the presence and severity of any obstruction, and are indicated when other imaging raises this possibility. They are not used primarily for the detection of pyelonephritis or scarring.

Other Imaging Modalities

Plain abdominal X-ray may detect radio-opaque renal calculi, however most calculi can now be detected on ultrasound**. Intravenous pyelography** has for the most part been superseded by ultrasound and radionuclide scintigraphy. **Magnetic resonance urography** is a newer modality that gives excellent anatomical detail as well functional imaging without an attendant radiation dose. It appears to have some ability to indirectly assess obstruction, by measuring cortical transit times, when used in conjunction with contrast material. While at the present time it cannot be recommended as a first line procedure, in future as the technology becomes more available and cost effective, it may supplant a range of current imaging procedures (Fig. 43.8) [90]. A major limitation in younger children is the need for sedation or general anesthesia.

Impact of Reduced Imaging

Some concern has been expressed that the trend toward reduced imaging may be accompanied by adverse risks, with voiding cystourethrography being the most contentious procedure. Several studies have addressed this issue.

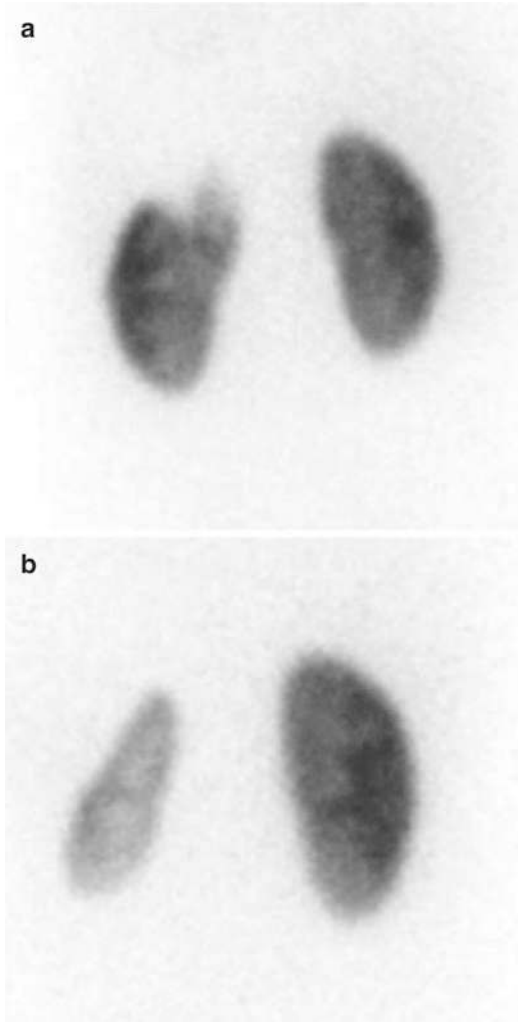

Fig. 43.8 Renal DMSA scintigraphy demonstrating a left upper pole scar (panel a) and a left hypodysplastic kidney (panel b)

Following publication of the NICE guidelines, two studies assessed their impact [91, 92]. Schroeder et al. compared the more selective imaging algorithm (NICE) with the formerly employed comprehensive algorithm (AAP 1999) [76, 91, 93]. The change led to a reduction in cystourethrograms performed following a febrile UTI from 99% to 13% and ultrasounds from 99% to 67%. The recurrence rate of UTI and detection rate of grade 4 and 5 VUR did not change, and the use of prophylactic antibiotics diminished. Deader et al. performed a retrospective analysis of 346 children who had an ultrasound performed at their institution following a UTI, to determine if significant renal pathology would have been overlooked had the procedure been restricted to those <6 months of age in accordance with NICE. Three scars confirmed on DMSA and one ureterocele would have been missed, such that the more restrictive guidelines regarding ultrasound appeared safe and provided significant cost savings [92]. Similarly, Jerardi et al. instituted a rapid uptake of the revised AAP guidelines [11] that significantly reduced the number of investigations performed, without any significant difference in the rate of abnormalities detected pre and post-intervention [94]. La Scola et al. evaluated the yield, cost and radiation dose of five different investigative guidelines if they had been applied to 304 children 2–36 months of age with a first febrile UTI that had been comprehensively evaluated in a RCT [95]. Scarring would have been detected by those that recommend a late DMSA scan in all cases [18], while guidelines that recommend a late DMSA in selected circumstances missed approximately half of the scars [75, 76] and all scars were missed in those guidelines that do not recommend a DMSA scan [11, 96]. Four of the five protocols failed to reliably detect high grade VUR, and proved even less effective when all grades of reflux were considered [11, 75, 76, 96]. Conversely the protocol that would detect all scarring had the highest cost and radiation burden [18], while the more selective protocols including the AAP guidelines, are the least costly with the lowest radiation dose.

Antibiotic Prophylaxis

Recent prospective studies have demonstrated antibiotic prophylaxis to be of little or no benefit in preventing recurrent infections, or reducing the risk of scarring in the majority of children following an uncomplicated first febrile UTI. Antibiotic prophylaxis has been widely recommended in the past following pyelonephritis, particularly where VUR was present, on the assumption that it would reduce the recurrence rate of infections and prevent renal scarring [93]. Eight randomised prospective studies involving 2390 children and

adolescents evaluating the effectiveness of prophylaxis have been published between 2006 and 2015 [97–104]. While a direct comparison between the studies is not possible, due to their disparate populations, duration of follow-up and investigations undertaken, it is reasonable to draw a number of conclusions. All studies had recurrent UTI as a primary end-point. Four studies in patients with absent or predominantly low grade reflux showed rates of recurrence to be similar in the prophylactic compared with no treatment groups [97–100], two of these studies demonstrating a trend toward increased infections in the children with grade III reflux who were not on prophylaxis [99, 100]. One smaller study demonstrated an increase in UTIs on prophylaxis compared with controls [104]. A further study, the Swedish Reflux Trial, restricted to 203 infants with higher grade (III–IV) dilating reflux, demonstrated a clinically significant reduction in febrile UTIs on prophylaxis compared with controls (19% vs. 57%, $p < 0.001$) over a 2 year period in girls, but not boys [102]. The two largest and only studies to be placebo controlled, the PRIVENT and RIVUR trials involving 1183 children showed prophylaxis to result in a statistically significant though clinically insignificant reduction in UTIs, with 14–16 patient years of prophylaxis needed to prevent one symptomatic infection and 22 patient years of treatment to prevent one febrile infection [101, 103]. In both studies the maximum benefit of prophylaxis occurred in the initial 6 months. A systematic review of antibiotic prophylaxis for prevention of recurrent UTI in children demonstrated a small benefit of low dose antibiotic prophylaxis in preventing recurrent UTI, at the expense of increasing bacterial resistance to the treatment drug with subsequent infections. The analysis did not address the severity of VUR as a risk factor neither did it evaluate scarring as an adverse outcome [105].

Seven studies looked at late renal scarring on DMSA scan as a secondary endpoint. Only one paper, restricted to infants with high grade reflux, demonstrated a benefit of prophylaxis in preventing scarring, once again only in girls [87]. This benefit was not confirmed in the larger RIVUR trial, comparing antibiotic prophylaxis to placebo, which recruited 607 children (92% female) following a UTI, 46% of whom had grade III–IV dilating reflux [103]. No study was sufficiently powered to detect a possible benefit of prophylaxis in prevention of renal scarring. To address this, a systematic review and meta-analysis of 1427 subjects from 7 RCTs was undertaken and demonstrated no prevention of renal scarring by antibiotic prophylaxis, as did a sub-analysis restricted to the 1004 subjects with VUR [106].

These findings suggest that antibiotic prophylaxis is of minimal if any benefit in infants and children with absent or grades I–III reflux with a low recurrence rate for infection [101]. In a single study prophylaxis appeared to be of benefit in reducing recurrent pyelonephritis and scarring in the female infants and young children with high grade (III–IV) dilating reflux [102], although the finding in relation to scarring was not confirmed in the RIVUR trial with larger numbers of randomised girls who had the same degree of reflux [103]. In the few children with recurrent febrile UTIs who might benefit from prophylaxis, there is no consensus at what age or for what duration it should be prescribed.

Based on current evidence, we believe that antibiotic prophylaxis should be prescribed for 2 years in young girls and for 1 year in young boys with high grade reflux. Nitrofurantoin appears the most effective agent; however, the side effect profile and risk of poor compliance appear to outweigh any benefit of the medication, such that cotrimoxazole, amoxiclavulanate and the cephalosporins are generally preferred.

Antibiotic Prophylaxis in Children with Major Urologic Malformation, Spina Bifida, Neurogenic Bladder and Intermittent Catheterisation

Urinary tract infections are common in children with major urologic malformations that pertain particularly to the bladder. Children with ongoing bladder dysfunction for a variety of reasons including surgical resolution of posterior urethral valves, those with neurogenic bladders as a consequence of spina bifida or spinal cord injury, and children with bladder exstrophy, many of whom have undergone multiple surgical procedures and

are on clean intermittent catheterisation, are at risk of long-term bladder colonisation and recurrent UTIs. The role of antibiotics and prophylaxis remains unclear in these patients. In the only RCT of antibiotic prophylaxis in children on clean intermittent catheterization, those that continued prophylaxis had a significantly higher incidence of UTIs when compared with those that ceased treatment [107]. Furthermore, a meta-analysis of adolescents and adults with spinal cord dysfunction did not support the use of prophylactic antibiotics [108]. Despite these findings, there is a lack of uniformity in management of children with neurogenic bladder dysfunction, with a remarkable variation in the prescription of prophylactic and therapeutic antibiotics in this population.

Given the difficulty of eradicating organisms from the bladder of patients on long-term intermittent catheterization, an alternative approach with some promise is to colonize the bladder with an avirulent strain of *E. coli* using infected catheters [55]. A further study of children with neurogenic bladder showed they most commonly carried avirulent commensal clones [109]. Uropathogenic clones, when present, were associated with prolonged carriage, however this was not associated with symptomatic disease or deterioration of the upper urinary tract. Thus, urine culture and antibiotic treatment of any infection detected in these children should on current evidence be restricted to those with definite symptoms.

Infants and young children with chronic kidney disease due to dysplasia are at particular risk of early deterioration to the point of requiring kidney replacement therapy, with some evidence that the rate of deterioration is accelerated in those with recurrent febrile UTIs [110]. A large multicentre RCT, the PREDICT trial, is currently underway evaluating the usefulness of antibiotic prophylaxis in this at risk population.

Surgical Correction of VUR

Surgical correction of VUR can be achieved by either re-implanting a tunnelled ureter through the bladder wall or endoscopic injection of a bulking agent adjacent to the vesicoureteral orifice. The reported success rate for re-implantation is 98% (95% CI 95–99) and 83% for a single attempt at endoscopic treatment, with uncertainty regarding the permanency of the endoscopic approach [111]. Surgical correction is no longer routinely recommended, but may be considered when breakthrough febrile UTIs occur on prophylaxis, particularly in association with high grade dilating reflux in females.

A recent study, the Swedish infant high-grade reflux trial, randomized 77 infants <8 months of age with VUR grade IV–V to antibiotic prophylaxis or endoscopic correction of VUR with prophylaxis until VUR resolution was confirmed. The study showed no benefit of surgical intervention over prophylaxis in the risk of UTI recurrence or renal function deterioration. The study did not include a no-treatment control group to assess whether the interventions were of benefit. Of interest, over the 1-year follow-up 21% of the infants on antibiotic prophylaxis had a reduction in VUR severity to grades 0–II [112].

Additional Therapies

Circumcision

There are no RCTs on routine neonatal circumcision for the prevention of UTIs in males [113]. Two systematic reviews including predominantly cohort studies, reached opposite conclusions. One review concluded that routine circumcision to prevent UTI was not indicated in normal boys with the number needed to treat to prevent one UTI calculated at 111, however it could be considered in those with recurrent UTIs or high grade reflux, where the benefits appear to outweigh risks of the procedure [114]. A second review concluded that circumcision was indicated in all boys, as it reduced the lifetime risk of UTI, with a little over four low risk operative procedures to prevent one infection [115].

Cranberry Juice

Some studies suggest that cranberry juice may be of benefit in reducing recurrent UTIs, presumably by inhibiting bacterial adhesion to uroepithelial cells. A Cochrane review of 24 randomized

controlled or quasi randomized controlled trials failed to demonstrate any significant benefit of cranberry juice, concentrate or capsules/tablets in the management of recurrent UTI in a variety of circumstances, including children and those with neuropathic bladder or spinal injury [116].

Probiotics

Studies have been undertaken to assess the possible benefit of probiotics in reducing or preventing recurrent UTIs. Most studies have been undertaken in women with variable results [117]. The studies are difficult to assess, as they use different strains of organisms, predominantly lactobacilli that have a variable effect on intestinal flora. One retrospective study demonstrated a possible benefit of probiotic prophylaxis similar to antibiotic prophylaxis in prevention of UTIs over a 6 month period, however the paper had significant methodologic shortcomings such that further studies are needed before any recommendations can be made on probiotic use [118]. Given the low quality of studies, a systematic review failed to show a role of probiotics in reducing recurrent UTIs, however a benefit cannot be ruled out prior to larger scale prospective RCTs [119].

The treatment of **constipation** and soiling in at risk children, as well as strategies to manage **dysfunctional voiding**, are considered by many to be worthwhile in the prevention of recurrent UTI. Unfortunately, there are no RCTs that assess the efficacy of any particular intervention.

Long-Term Clinical Consequences

Uncomplicated UTIs are common in children. When associated with fever there is an increased probability of renal parenchymal involvement, which can result in permanent renal scarring. In the absence of scarring there is no documentation of any adverse long-term outcomes. The frequency and severity of scarring as a consequence of acute pyelonephritis, the age at which it occurs and the long-term sequelae have been points of conjecture, as has the risk of consequent chronic kidney disease (CKD), hypertension and pre-eclampsia. The introduction of routine antenatal ultrasound over the past 20–30 years, has demonstrated that much of the renal parenchymal damage previously attributed to pyelonephritis, is the result of congenital mal-development of the kidneys, often associated with significant urinary tract abnormalities such as high grade VUR or obstruction (Fig. 43.2b).

This recognition has led to a critical re-evaluation of the potential for pyelonephritis to cause scarring and result in adverse outcomes. A systematic review of renal scarring reported a prevalence of 15% following a UTI [120]. The risk and severity of scarring as a consequence of a single episode of pyelonephritis appears unrelated to age, when infants and young children are compared with older children [121]. In a meta-analysis involving 1280 children and adolescents following a UTI from 9 studies, a scar developed in 191, those with an abnormal ultrasound, fever >39 °C or an organism other than *E. coli* were at higher risk of kidney damage [122]. A review looking at long term consequences of UTI in children noted that despite numerous publications there are no clear data as to the outcomes [123]. They noted that earlier publications, often retrospective and from selected populations, had a tendency to record frequent adverse outcomes. More recent studies, particularly those that are population based, and with the benefit of prenatal ultrasound to exclude congenital kidney disease, reported a much lower incidence of harmful sequelae. In regard to kidney function they reported that only 0.4% of the 1029 children in prospective studies with normal function at the outset reported a decrease on follow-up. Conversely, virtually all the children with decreased kidney function at the conclusion of studies had scarring or kidney dysplasia at the beginning. There was a low risk of hypertension. In five studies involving 713 children where blood pressure was measured at the beginning and completion of follow-up (which ranged from 5 to 20 years), the incidence of hypertension increased from 2.4% to 4.6% [124–128]. In the studies reporting a high incidence of hypertension, up to 35%, scarring and kidney damage was almost always present on enrolment. In contrast, one study that followed two groups of children after a first UTI for 16–26 years found no significant differences in ambulatory blood pres-

sure monitoring between the 53 individuals with scars when compared with 51 controls without scars [129]. A further paper addressing the obstetric outcome of 72 parous women followed from their first UTI in childhood to a median age of 41 years noted that pregnancy complications were few. Irrespective of the presence or absence of kidney damage, none were diagnosed with hypertension prior to their first pregnancy. Hypertension was diagnosed in 10 of 151 pregnancies, all in women with renal damage of whom 4 experienced preeclampsia [130]. A well conducted population based controlled study evaluated pregnancy-related complications in 260 women who experienced childhood UTIs compared to a control group of 500 mothers without a childhood UTI [131]. There was no difference in essential or gestational hypertension, pre-eclampsia or pyelonephritis during their first pregnancy between the UTI group (40%) and the controls (41%). Thus, the data appears to exclude a major influence of childhood UTIs on subsequent growth and pregnancy related complications.

Summary

Children with normal kidneys at birth and no obstruction to drainage would appear to be the ones at minimal risk of developing chronic kidney insufficiency or other adverse outcomes following an uncomplicated febrile UTI. The infection can be treated with oral antibiotics and the child monitored to ensure recurrent episodes do not occur. Children with significant congenital abnormalities of the kidneys and urinary tract, appear at risk of progressive kidney impairment. Whether dysplastic kidneys are more prone to severe damage as a consequence of pyelonephritis remains to be determined. Further research is underway that will clarify a number of unresolved issues: the need for antibiotic prophylaxis in children with high grade reflux with and without kidney hypodysplasia, the use of new biomarkers to diagnose UTI and to differentiate upper from lower tract infections, the optimal duration of antibiotic treatment during the acute phase, and the appropriate cut-offs for culture results in children. Long term follow-up of children who have been prospectively studied following a first febrile UTI with normal kidneys, as well as those with prenatally diagnosed hypodysplasia, will be important to determine the late risks such as hypertension, pre-eclampsia, and chronic kidney insufficiency.

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Vesicoureteral Reflux

44

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Introduction

Vesicoureteral reflux (VUR) is the retrograde flow of urine from the bladder to the kidneys. VUR may be classified as primary, which is a congenital defect in the vesicoureteral junction anatomy, or secondary, which is the result of persistent high intravesical pressures that overwhelm the vesicoureteral junction. VUR has been implicated in renal injury prior to birth as well as postnatal development of urinary tract infections (UTIs) and further renal damage. Primary VUR is most commonly identified in infants and children, and historically has been most prevalent in infants 0–24 months of age. Although much is known about the diagnosis, medical, and surgical management of VUR, many questions remain regarding the potential of VUR to cause infections and renal injury.

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Embryology

Anatomic Factors

Ureteral development has been studied to understand the anatomic factors that may lead to primary VUR. The development of periureteral sheaths, intravesical ureteral muscles and trigonal muscles have been studied for potential contribution in VUR [1]. Based on observations in 11–27 week fetuses, it has been determined that the superficial trigone is derived from the intravesical ureteral muscles and the deep trigone is derived from the deep periureteral sheath of the ureter. Fixation of the ureters in the appropriate location is important for the development of a normal trigone and non-refluxing ureters. The intravesical submucosal length of the ureter and the oblique path of entry of the ureter into the bladder have been identified as critical factors in the prevention of VUR (Fig. 44.1).

Development of renal scarring in children appears to be independent of the presence or absence of VUR. Anatomic factors in the ureterovesical junction, however, may play a role in the degree of renal injury produced by VUR in that higher grade VUR that occurs with lower bladder pressure is associated with increased risk of nephropathy [2].

Other anatomic anomalies outside the ureterovesical junction anti-reflux mechanism, such as periureteral diverticula or duplications of the

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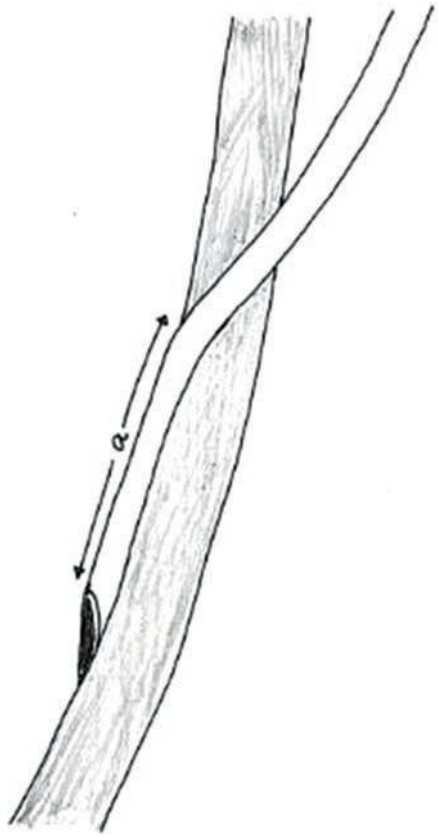

Fig. 44.1 Ureterovesical junction anatomy contributes to the anti-reflux mechanism of the compression valve, which includes the length of the tunneled ureter, angle of the ureteral insertion, and fixation of inlet and outlet points

collecting system, can contribute to persistence of VUR, increasing the risk of renal damage and the requirement for surgical correction.

Extra-anatomic Factors

Embryological development of the ureteral bud from the mesonephric duct is dependent on multiple factors. Signaling by glial cell line derived neurotrophic factor (GDNF)has been shown in the mouse model to induce the formation of ureteral buds [3]. Misexpression of GDNF has been shown to be associated with the development of multiple ureteral buds. Additionally, GDNF is focally expressed in the appropriate location of ureteral bud development. If GDNF is expressed in an ectopic location, the ureteral bud will develop in this ectopic location, leading to lateral or medial localization of the ureter in the bladder predisposing to VUR or obstruction. Additionally, trigonal development is dependent on apoptosis induced by a vitamin A signaling pathway [4]. Normal development of the trigone is also necessary to provide appropriate support to the distal ureter. Studies have shown that symmetric muscle contractions and unidirectional peristalsis also play a significant role in the competence of the ureterovesical junction [5].

Associated Conditions

Many anatomic and genetic conditions are associated with the presence of VUR in children. The most commonly noted anatomic conditions are multicystic dysplastic kidney (MCDK), renal agenesis and renal or ureteral ectopia and duplication. VUR is also a common occurrence with many syndromic conditions.

Multicystic Dysplastic Kidney (MCDK)

Contralateral VUR is the most common abnormality present in children with MCDK [6]. VUR has been noted in 12–28% of contralateral kidneys in children with MCDK [7]. The impact of this contralateral VUR continues to be debated. One study has indicated that contralateral renal growth is compromised in the presence of VUR [8]; however, other studies have revealed that VUR into the contralateral renal unit is usually low grade and does not lead to renal compromise [9]. Study of the natural history of VUR in the presence of MCDK indicates that in most boys and 40% of girls there will be eventual spontaneous resolution.

Renal Agenesis

As with MCDK, VUR is the most common abnormality noted in the contralateral kidney in children with unilateral renal agenesis.

Management of VUR in the context of a solitary kidney is not different from that in patients with two kidneys [10].

Ectopia and Duplication

Dilating VUR occurs in up to 26% of children with renal ectopia and hydronephrosis [11]. VUR is the most commonly associated anomaly noted in children with renal ectopia. The presence of renal ectopia does not seem to reduce the potential for VUR to resolve spontaneously [12].

Ureteral duplication is also associated with the presence of VUR, typically into the lower pole of the duplex system. The ureteral orifice is displaced proximally and laterally in children with duplex systems and plays a role in the development of VUR into the lower pole moiety. Following endoscopic management of ureteroceles associated with duplex systems, VUR into the lower poles unilaterally or bilaterally may be unmasked, and may even occur into the upper pole as an iatrogenic entity. Many patients with duplex systems will eventually require surgical management for their VUR [13]. Typically, surgery in these patients is indicated for the associated conditions that are present (i.e. ureteroceles, ectopic ureters, etc.).

Ureteral ectopia may also be noted into the bladder neck and urethra, leading to VUR during voiding. Bilateral or single system ureteral ectopia is associated with reduction in bladder growth and capacity and requires surgical management with ureteral reimplantation and possible later bladder neck reconstruction to provide continence [14]. Prognosis in this condition is based on development of adequate bladder capacity.

Syndromes

Syndromes that have been associated with the presence of VUR include the VATER-VACTERL syndrome, Townes-Brock syndrome (*SALLI* mutation), cat-eye Syndrome (tetrasomy, chromosome 22), Casamassima-Morton-Nance syndrome, renal coloboma syndrome (*PAX*2 mutation), branchio-oto-renal syndrome (*EYE1* mutation) and Frasier syndrome (*WT1* mutation).

Incidence

Although it is difficult to quantify a disease process that has the ability to be transient, several studies have sought to estimate the prevalence of VUR among children. In a meta-analysis, the incidence of VUR was classified based on initial presentation. The prevalence of VUR in children undergoing cystography for UTI was 30%, as compared to 17% in children who underwent radiographic imaging for other reasons such as hydronephrosis [15].

Guidelines from the American Academy of Pediatrics (AAP) and the National Institute for Health and Care Excellence (NICE) appear to have had an impact on the evaluation of children with UTI. A reduction in the utilization of voiding cystourethrogram (VCU) has led to decreases in the rates of VUR identified. This was reflected in a study that demonstrated a decline in the incidence of VUR in children 0–2 years of age from 38/100,000, to 25/100,000, following publication of the 2011 AAP guidelines. Additionally, reduction in the performance of VCUs was also noted in children 3–10 years of age [16], suggesting a reluctance to screen older children as well.

Age

VUR is more commonly identified in younger children. In a retrospective review of 15,504 children, the incidence of VUR was 35.3% in children less than 2 years of age, 22.7% in those 2–6 years of age, 15.3% in those 7–11 years of age, and 7.9% in those 12–21 years of age [17].

Gender and Race

There is a gender disparity between the patients diagnosed following identification of hydronephrosis on antenatal ultrasonography and those

diagnosed following initial UTI. The majority of infants with VUR following identification of antenatal hydronephrosis are male [18]. It has also been noted that the incidence of dysplasia is greater in male infants with VUR. Girls form a majority of patients presenting with VUR identified following UTIs, except for the first 6 months of life, when UTI is more common in boys [19].

African-American girls have a lower potential for development of VUR as compared to Caucasian girls. This difference in incidence was also noted in infants diagnosed with VUR following identification of antenatal hydronephrosis [20]. Additionally, few African American girls presenting with VUR following a UTI have high grade VUR [17]. The incidence of scarring, however, is higher in African-American girls than Caucasian girls, although progression of scarring is less in African American girls. Additionally, time to spontaneous resolution of VUR is shorter in African American girls [21]. The incidence of VUR in Hispanic girls is comparable to Caucasian girls [22].

Presentation

VUR is identified in four groups of children—those identified during evaluation of antenatally identified hydronephrosis, those with other congenital anomalies, those following a febrile or symptomatic UTI and those evaluated due to VUR in a child or parent.

Antenatal Diagnosis

The widespread utilization of antenatal ultrasonography has made early detection of hydronephrosis and subsequent diagnosis of VUR possible prior to the occurrence of UTIs. Fetal pelvic diameter of 7 mm during the third trimester or 10 mm at postnatal examination is considered an indication for further investigation, including possible evaluation for VUR. About 10% of such patients evaluated for hydronephrosis on antenatal ultrasonography are diagnosed with VUR [23]. Cohorts of infants with VUR diagnosed following antenatal identification of hydronephrosis have greater numbers of boys [24]. Additionally, compared to children with VUR following a UTI, there are more patients that have low grade VUR, with greater propensity for spontaneous resolution. Boys with even high grades of VUR (IV–V) have a 29–37% rate of spontaneous resolution in the first year of life [25]. This potential for VUR resolution is attributed to the resolution of a mixed pattern of voiding, with coordinated voiding interspersed with high pressure voiding due to increased sphincteric activity.

Urinary Tract Infections

Most children are diagnosed with VUR following an initial febrile UTI. Since UTIs are most prevalent during the first 2 years of life, the majority of VUR diagnosis is made in infants and young children. In a cohort of 1953 patients with UTI <2 years of age undergoing VCU, VUR was noted in 30% [26]. This incidence was similar to that noted by Hoberman et al. [27]. In more recent studies, the overall incidence of VUR has been 15–20% [28].

Siblings with VUR

There remains debate on the benefit of routine screening of siblings of index patients with VUR. In one study, 88% of siblings had VUR. However, index patients had high grade VUR, and siblings screened were less than 3 years old, and were generally symptomatic with UTI [29]. Therefore, restricting evaluation for VUR to siblings that present with an infection may have a better yield in identifying children with VUR that may benefit from intervention.

Genetics of Reflux

There is increasing evidence for a genetic basis for primary VUR. The reported incidence of VUR in siblings of an affected patient varies

from 27% to 45% [30]. A higher incidence of VUR has also been reported in children of parents with a history of VUR [31]. VUR is most certainly genetically heterogenous. Autosomal dominant inheritance with variable expression or multifactorial inheritance has been implicated for VUR and reflux nephropathy (RN). In a study of 88 families with at least one individual with primary VUR, the authors concluded that a single major locus was the most important causal factor [32]. Kaefer and colleagues found 100% concordance in monozygotic twins and 50% concordance among dizygotic twins [33]. One gene associated with apparent autosomal dominant VUR has been mapped to chromosome 1 [34], though two of the families studied showed negative linkage to this locus, further confirming the genetic heterogeneity of VUR.

Association of Urinary Tract Infections

VUR is believed to be the primary risk factor for pyelonephritis, although some studies dispute this association [35]. The International Reflux Study in Children (IRSC) reported recurrent UTI in 28% of children with medically managed severe VUR [36]. The risk of UTI recurrence is related to the degree of VUR: 6–8% for VUR grade I and II; 27% for grade III; and 43% for grade IV [37]. The usual organisms that cause UTI originate from fecal flora that colonize the perineum, and the organisms that cause recurrent UTI can be found on perineal cultures prior to the onset of UTI [38]. Escherichia coli (E. coli) is the most frequent organism, being responsible for approximately 80% of UTIs, the rest being due to Klebsiella, Enterobacter, Citrobacter, Proteus, Providencia, Morganella, Serratia and Salmonella species [39]. In prospective study of infants with a first UTI, the incidence of non-E. coli bacteria were associated with an increased VUR severity (12% with VUR I–II, 30% with VUR III–V) [28]. A variety of bacterial virulence factors increase the ability of E. coli to cause a UTI. The presence of P fimbriae allows E. coli to adhere to the epithelial cells of the urinary tract, while other virulence factors increase tissue damage and protect E. coli from serum bactericidal activity [35].

Diagnosis

There remains significant debate regarding which children should be evaluated for VUR, both those identified following antenatally identified hydronephrosis, and those that had a UTI. This is partially due to concerns about the efficacy of antibiotics in the prevention of UTI. Multiple radiographic modalities have been successfully utilized for the diagnosis of VUR.

Renal Bladder Ultrasound

The AAP guidelines recommend ultrasonography as the first imaging modality for evaluation of children ages 2–24 months presenting with UTI. Ultrasound is the initial modality for evaluation of *any* child that is being evaluated for possible VUR. This permits evaluation of the upper tracts to determine the presence of anomalies (e.g., duplication, hydronephrosis, MCDK, agenesis). Ultrasound should always include evaluation of the ureters and bladder before and after voiding to determine if there are lower tract changes that might suggest the possibility of VUR (i.e. diverticula, ureteral dilation) or other lower tract anomalies (ureteroceles, megaureter) that may predispose to UTI. Although the presence of abnormalities on ultrasound may increase the likelihood of VUR, there are no specific findings that have a definite correlation [40].

Voiding Cystourethrogram

The gold standard for the diagnosis of VUR is the radiographic voiding cystourethrogram (VCU). VCU requires urethral catheterization and fluoroscopy. This modality allows grading of VUR as standardized by the IRSC. The AAP and NICE UTI Guidelines have suggested limiting further evaluation of children presenting with a first UTI. This has led to a significant decrease in the

utilization of VCU since the publication of the guidelines. Although VCU is the best procedure for the identification of VUR, discrepancy can be noted in the grading of VUR even among experienced readers and when multiple filling cycles are utilized [41]. The timing for the performance of VCU has been debated. It was felt that early VCU may lead to increase in the diagnosis of VUR due to the "instability" of the VUR in the child with a recent UTI. Recent studies have demonstrated that performing the VCU within a week of presenting with a UTI leads to improved compliance with performing the study and does not change the potential for the identification of VUR [42].

The discomfort of catheterization is a contributor to the reluctance to perform VCUs. The use of sedation improves the tolerance for the procedure without changing the potential for the diagnosis of VUR [43]. Intermittent fluoroscopic evaluation reduces the exposure of radiation during the procedure, further improving the safety of the study [44]. The use of one or a few doses of antibiotics may help to prevent UTI due to catheterisation.

VCU is also used for the follow-up of VUR to determine its persistence or improvement. There is a trend to reduce the frequency of performance of VCU during follow-up of children with VUR. Additionally, VCU has been typically omitted following surgical correction of VUR, due to the high success rates achieved with most techniques.

In an effort to reduce the radiation associated with the use of standard (conventional) VCU, radionuclide cystogram (RNC) has been performed. This study uses technetium-99m (Tc-99m) sodium pertechnetate. It requires catheterization, but utilizes less radiation than a standard VCU. RNC has excellent correlation to conventional VCU [45]. The major limitation of this procedure is the inability to grade VUR as recommended by the IRSC. Using RNC, grading is limited to mild, moderate and severe. It is an excellent modality for the follow-up of VUR and for the determination of resolution of VUR. It is also used as the modality of choice for the identification of surgical success in the correction of VUR.

Dimercaptosuccinic Acid Scan

The major concern with the presence of VUR is the development of infections and subsequent renal scarring [46]. A nuclear scan with Tc-99m dimercaptosuccinic acid (DMSA) is the best modality for identifying renal scarring in the kidneys [47]. The difficulty is to determine if the scars that are identified are the result of prenatal hypodysplasia or secondary to recurrent UTI [48]. Some have suggested using a DMSA scan as the primary test for the evaluation of children presenting with UTI. The absence of renal involvement on DMSA scan performed during the acute phase of a febrile UTI makes higher grades of VUR unlikely [28].

Experimental Diagnosis Modalities

Voiding Ultrasonography

Another alternative to VCU is contrast-enhanced voiding urosonography with intravesical contrast, a method increasingly used in Europe. This method has the advantages of not using ionizing radiation and obtaining simultaneous images of the renal parenchyma. However, the use of this method for the evaluation of the male urethra is controversial [49, 50]. Urosonography has been recommended as a primary diagnostic modality for the diagnosis of VUR to reduce radiation exposure [51]. This modality is not widely available; the reduction in radiation exposure with current fluoroscopic techniques and the requirement for catheterization have limited its utilization.

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) may be used in the diagnosis of renal scarring, and less frequently is used in the setting of voiding imaging. MR voiding cystography (MR VCU) has the benefits of not requiring catheterization, simultaneously imaging the upper tract, and no radiation exposure. However, in younger children sedation may be required. In a small study, MR VCU was

found to be 90% sensitive and 96% specific for detecting VUR [52]. When compared to the gold standard DMSA scan for detecting renal scars, magnetic resonance urography has a much higher interobserver agreement and may provide superior detection of renal scars due to its ability to differentiate swelling from renal scarring [53].

Grading of VUR

Grading of VUR was standardized in 1982 [54] using the radiographic VCU, by the International Reflux Study Committee (IRSC). This system of grading divides VUR into five grades (Fig. 44.2). Grading of VUR correlates with the degree of renal scarring as well as the potential for spontaneous resolution. Lower grades of VUR have greater potential for spontaneous resolution independent of the age at diagnosis [55]. In addition, the grade of VUR is a consideration in the appropriate choice of management (endoscopic vs. open surgical reconstruction) [56]. Unfortunately, there is significant interobserver variability when grading VUR by VCU. In an analysis comparing a local radiologist with the interpretations of two blinded reference radiologists, VUR grade was agreed upon unanimously in only 59% of ureters [57]. The ureter may also be dilated without renal calyx dilation, which could lead to inconsistent grading.

Bladder and Bowel Dysfunction

Bladder and bowel dysfunction (BBD) is the combination of lower urinary tract symptoms (urgency, frequency, hesitation, straining, and withholding maneuvers) with bowel symptoms such as painful defecation, encopresis and constipation [58]. At baseline this is a common disorder. In a prospective study of children diagnosed

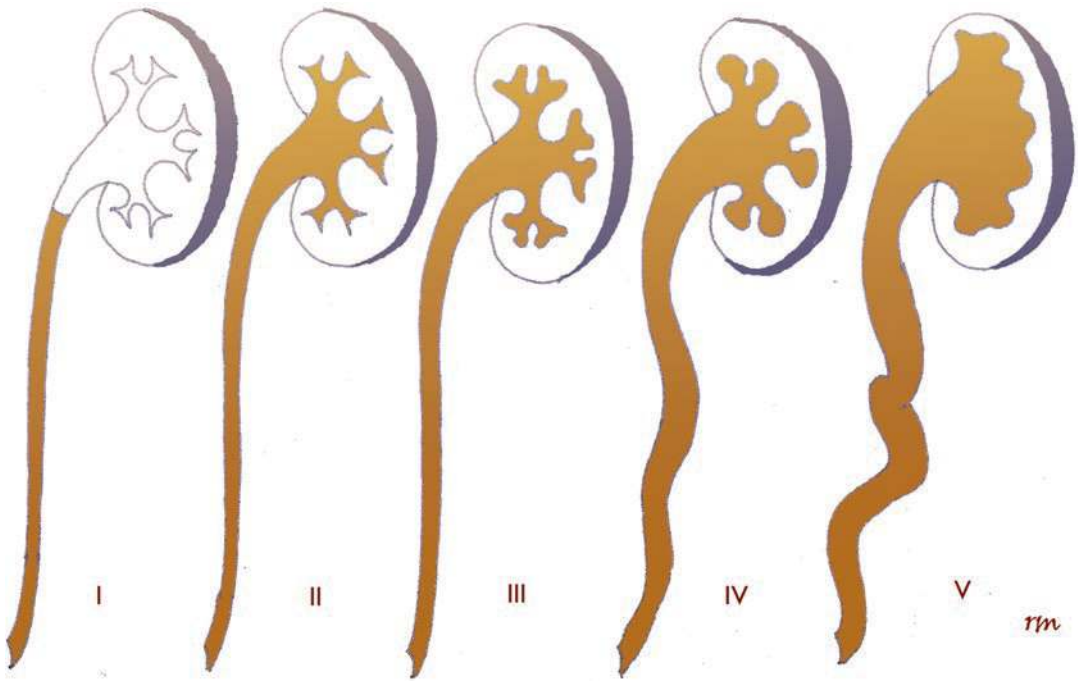

Fig. 44.2 International grading system for vesicoureteral reflux. Grade *I*—contrast in the non-dilated ureter; Grade *II*—contrast in the non-dilated ureter and renal pelvis; Grade *III*—mild dilation of the ureter and renal pelvis with minimal blunting; Grade *IV*—moderate tortuosity of the ureter and dilation of the renal pelvis and calyces; Grade *V*—gross dilation of the renal pelvis and calyces with significant ureteral tortuosity. (Grading based on International Reflux Study. *Pediatrics* 1981; 67:392–400; Figure copyrighted by Dr. Ranjiv Mathews)

with VUR, the presence of BBD significantly increased the rate of recurrent UTIs from 20% to 51% [59].

Potential for Reflux Resolution

The potential for spontaneous resolution of VUR is the basis for its conservative non-operative management. All grades of VUR have the potential for resolution, although the likelihood of resolution is based on the grade and presentation of VUR [60]. Overall 39% of refluxing ureters will have spontaneous resolution [61].

Multiple studies have evaluated the rate of resolution of the various grades of VUR. In one study, resolution of Grade I VUR was 82%, Grade II VUR was 80% and Grade III was 46% [62]. Similar rates of resolution have been noted in other studies evaluating the medical management of VUR [63]. Resolution rates over 5 years of Grade IV and V VUR were 30% and 11%, respectively [60]. In a study from Sweden, a negative correlation was found between bladder dysfunction and spontaneous improvement of VUR grades III and IV [64]. Boys are more likely than girls to have spontaneous resolution of high-grade VUR [61].

Potential for Renal Injury

A variety of factors influence the probability of scarring in children with VUR and UTI. The role of VUR, initially proven in piglets [65], has been shown in multiple clinical studies [66, 67]. Moreover, children with higher grades of VUR have an increased likelihood of developing renal scarring [68, 69]. Renal damage is more common in infants with UTI and VUR because of their unique kidney papillary morphology [70].

Factors affecting the probability of renal scarring in children with VUR and UTI include delay in the treatment of UTI, recurrent UTI and bacterial virulence [71]. Finally, there is evidence that genetic factors predispose patients with VUR to scarring as demonstrated by studies of angiotensin converting enzyme gene polymorphisms [72], and by studies of the IL-8 receptor CXCR1, which have identified a genetic innate immune deficiency with a strong link to acute pyelonephritis and renal scarring [73, 74].

Reflux Nephropathy

Several studies have shown that scarring develops at the same site as previous infection [75]. The pathogenesis of renal scarring following acute pyelonephritis is not well understood. The process is an inflammatory response, with chemotaxis and phagocytosis, release of lysosomal enzymes and superoxides, production of peroxide and hydroxyl radicals, tubular ischemia and reperfusion injury [76, 77].

Reflux nephropathy (RN) is the primary diagnosis in 5.2% of children undergoing renal transplantation according to a North American registry [78]. High grade VUR confers the greatest risk for kidney damage [79], and males with RN appear to have a poorer outcome than females [79, 80]. It is likely that the most severe renal damage associated with VUR is congenital hypoplasia/dysplasia and not postinfectious scarring. In a study from Sweden, boys were more inclined to have congenital lesions while girls were more likley to have acquired RN associated with prior infections [80]. While previous literature reported that younger children had the highest risk for new kidney scacrring, the Randomized Intervention for Vesicoureteral Reflux (RIVUR) trial and other studies reported that the risk for aquired scarring is higher in older children [79, 81, 82]. A lower threshold for identification of VUR in younger children may potentially have a protective role in preventing renal scarring. Despite succesful surgical management, patients with higher grades of VUR at diagnosis are more likely to progress to chronic kidney disease as compared to those with mild or moderate VUR, reflecting the possible role of prenatal dysplasia [83]. Long-term consequences from acquired renal damage, such as renal insufficency, hypertension and pregnancy complications, are considerably less than previously thought [84–86].

Management of VUR

A close relationship between pyelonephritis and VUR was demonstrated by Hodson and Edwards in 1960 [87]. VUR was believed to be detrimental to the kidneys and surgical procedures to correct VUR were developed during the 1960s [88]. However, the high rate of spontaneous resolution of VUR led to more limited surgical intervention [89]. Long-term antibiotic prophylaxis was implemented to protect children with VUR from renal damage induced by infection [90].

The potential for VUR to resolve spontaneously in many patients has changed the paradigm of management from one of immediate surgical correction to initial medical management with antibiotic prophylaxis [91]. Another alternative is watchful waiting. In current clinical practice, treatment decisions are made individually based on VUR grade, previous febrile UTIs, renal damage, bladder and bowel function, adherence to prophylaxis if given, and the parents' preferences. Surgical treatment is typically reserved for patients without spontaneous resolution and with recurrent infections.

Antibiotic Prophylaxis

The main objective of treatment in children with VUR is the prevention of recurrent UTI and renal parenchymal damage. Antibiotic prophylaxis was introduced in 1975 [91]. Controlled trials demonstrated the effectiveness of daily, low dose trimethoprim-sulfamethoxazole (TMP-SMZ) or nitrofurantoin in preventing UTIs [92]. Breakthrough infections are common in children with VUR, with rates ranging from 25% to 38% [93]. Side effects are not uncommon; these include gastrointestinal disturbances, skin rashes, hepatotoxicity and hematological complications [94].

There is no significant outcome difference between medical and surgical management in the incidence of renal scarring. The IRSC European cohort included 300 children with VUR randomly allocated to medical or surgical management. Follow-up with intravenous urography and DMSA scintigraphy over 5 years revealed no difference in the development of new renal scars or the progression of existing scars [95]. Similar results were reported by the Birmingham Study [96].

Recently, several randomized, controlled studies comparing antibiotic prophylaxis and no treatment were performed [97–101]. The results of these studies were summarized in a meta-analysis published in the latest AAP clinical practice guidelines [102]. These studies were unable to show a beneficial effect of antibiotic prophylaxis. However, most children in these studies had no VUR or low grades of VUR, underlining the fact that for most patients, prophylaxis is unnecessary. For children with higher grades of VUR, especially grades IV–V, there may be a benefit. The RIVUR trial, which randomized children with VUR to placebo or prophylaxis, demonstrated a 50% reduction in the risk of recurrent infections with the use of prophylaxis (TMP-SMZ). Additionally, the RIVUR trial showed that in children presenting with a second UTI, older children and those with grade IV VUR, there was greater potential for development of new renal scarring. Given these risk factors, it would suggest that there is a role for prevention of UTIs as a means to prevent new renal scarring [79]. A reanalysis of the RIVUR study divided patients into low risk and high-risk categories (high risk defined as grade IV VUR, female grade I–III, and uncircumcised males with grade I–III, with concurrent BBD) and showed that antibiotic prophylaxis significantly decreased UTI recurrence in the high risk patients. Thus, the treatment of high-risk patients confers greater benefit [103]. The necessity to analyze boys and girls separately was illustrated in the Swedish Reflux Trial, where a beneficial effect of prophylaxis was seen in girls with grades III–IV VUR, but not in boys [101].

Treatment of Bladder and Bowel Dysfunction

Since children with BBD have a higher risk of recurrent UTIs, it is imperative to treat BBD. Adequate hydration, timed voiding, pelvic

floor muscle awareness and a bowel regimen alleviate the urinary and bowel symptoms associated with BBD [58]. BBD increases the risk of UTI in VUR patients regardless of antimicrobial prophylaxis, decreases the likelihood of spontaneous VUR resolution, and may decrease the efficacy of surgical treatment of VUR [59, 64].

Surgical Management

The major indications for surgical intervention in children with VUR include recurrent UTI despite appropriate antibiotic prophylaxis, worsening of renal scarring during follow-up, and grade V VUR, which is unlikely to resolve spontaneously. Surgical correction of VUR may be more important in patients with a single renal system. Surgical management decisions, however, should be individualized based on potential risk to the renal units.

Minimally Invasive Treatment Options

Minimally invasive options for the treatment of VUR are endoscopic and laparoscopic techniques, with robotic techniques applied to improve the results with laparoscopy.

Endoscopic Treatment

Endoscopic techniques use bulking agents to increase resistance at the ureteral orifice to prevent VUR. Polytetrafluoroethylene (Polytef) has been used successfully for correction of VUR since the early 1980s [104]. Polytef had excellent surgical success that was maintained over time; however, the concern of particle migration prevented approval in the United States (US) and led to the gradual decline in use worldwide. Polydimethylsiloxane has been utilized in Canada and has been associated with no migration and high success rates [105], but this agent has also not been approved for use in the US. Bovine cross-linked collagen has been used in the US and, although initial results were acceptable, long-term recurrence of VUR was frequent due to absorption of collagen over time [106, 107]. Other agents that have been tried include expanded chondrocytes and placement of balloons; however, these techniques require multiple procedures [108, 109].

Dextranomer hyaluronidase (Dx/HA, Deflux™) has been utilized for bulking of the ureters for treatment of VUR (Fig. 44.3a, b). Initially reported by Stenberg and Lackgren in 1995 [110], worldwide experience has grown rapidly. The overall cure rates with Dx/HA are 94% for grade 1, 85% for grade II, 78% for grade

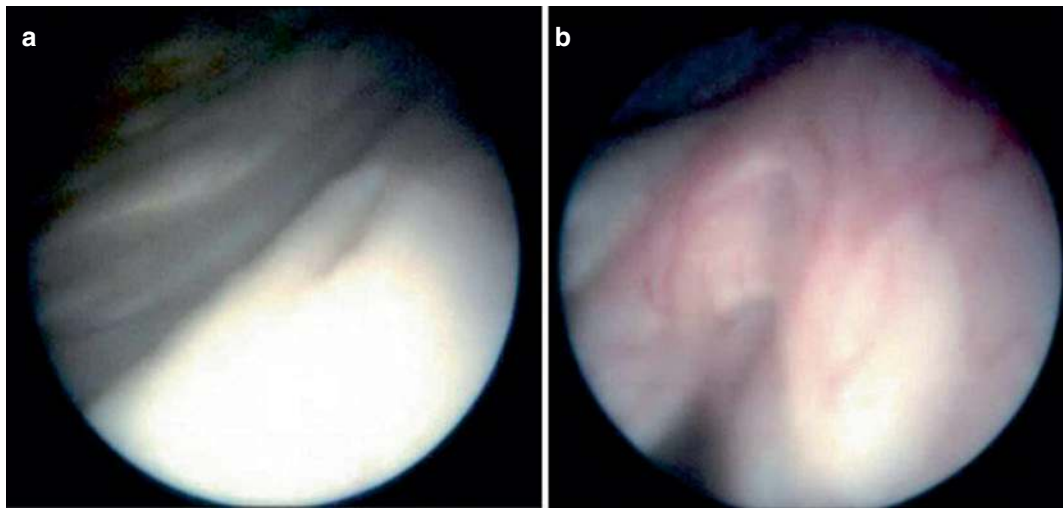

Fig. 44.3 (**a**, **b**) Injections are placed in one of several locations either within or near the ureter to increase coaptation of the ureterovesical junction. **a** depicts a ureteral orifice before injection, and **b** depicts after injection

III and 71% for grade IV VUR [111]. Dx/HA is effective in patients that fail prior treatment and in those that have associated urologic anomalies like ureteroceles and duplex systems. Patients that fail endoscopic management are still candidates for open surgical reconstruction. Late recurrence of VUR was noted in 20% of patients that had follow-up [112].

The efficacy and relative simplicity of the use of Dx/HA for the correction of VUR has led some to question the current paradigm of VUR management [113]. It has been suggested that Dx/HA should be used as a first line treatment for VUR. This procedure requires the use of general anesthesia, which is a significant consideration in infants.

Laparoscopic and Robotic Treatment

Laparoscopy has been successfully utilized for the surgical correction of VUR [114]. Laparoscopic techniques allow small incisions to be used and have the potential to reduce discomfort and length of hospital stays. The technique initially involved an extravesical approach for the correction of VUR [115]. Intravesical and transvesical techniques have since been reported [116]. The presumed benefit of reduction in hospital stay and smaller incisions have been eclipsed by the advent of improved endoscopic management with Dx/HA. The recent advent of the use of robotic techniques has shortened the length of the procedure and has made laparoscopic surgery for VUR more universally accepted. In a single surgeon direct comparison of open versus robotic approach to ureteral reimplant, surgical complications did not differ between the groups. There was a decrease in pain medication usage in the robotic group, albeit a 12% longer operative time in the robotic group [117]. However, in larger comparisons, there is more variation in reports of surgical complications in the robotic approach [118]. Wide variation in usage, success and complication rates of robotic surgery can be attributed to a learning curve and reported outcomes [119].

Open Surgical Techniques

Open surgery remains the gold standard for the surgical correction of VUR. The technique devised by Politano-Leadbetter combined an intra and extravesical technique and has been used widely with great success [87]. This technique, however, has been supplanted by the two techniques described below. In general, open surgical techniques are associated with 90–95% success rates and most can now be performed with a 1–3 day hospital stay. The success rates are so consistent across multiple studies that the use of routine post-procedure VCU has been abandoned. Despite high success rates for the correction of VUR, along with a decrease in the incidence of pyelonephritis [120], there has been no reduction in the incidence of renal scarring during follow-up.

Intravesical (Cohen) Cross-trigonal Reimplantation

Since the initial description of this technique, it has been rapidly adopted by most pediatric urologists due to the consistency of surgical outcomes and low rates of complications [121]. This technique involves the dissection of the ureters within the bladder and then the ureters are placed in submucosal tunnels created across the trigone of the bladder. Over time, significant improvement in pain management has permitted reduction in hospital stays, reduction in the need for stenting and suprapubic tube placement, and high patient satisfaction rates [121]. Because of the high rate of success, this technique is the most frequently performed and taught procedure for the correction of VUR. It is routinely used for the correction of bilateral VUR. It also allows for other bladder anomalies (e.g., ureteroceles, bladder diverticula) to be corrected concurrently. Potential complications associated with this technique are the development of contralateral VUR following correction of unilateral VUR, ureteral obstruction and residual VUR [122].

Extravesical (Lich-Gregoir) Reimplantation

This technique allows reimplantation without entry into the bladder. The ureters are dissected prior to the entry into the bladder and reimplantation is performed by placing the ureters into troughs created in the bladder wall [123]. It has been utilized most frequently for the

management of unilateral VUR as there is a concern that some patients that have had bilateral reimplantation using this technique have had secondary transient neuropathic bladder dysfunction requiring temporary intermittent catheterization [124]. This technique also has a high success rate for the correction of VUR. Many patients can be managed with a 24 h hospitalization as bladder spasms are less frequently noted.

Controversies and Conclusions

The routine use of antenatal ultrasound has increased identification of hydronephrosis. This has permitted early identification of high grade VUR, potentially permitting reduction in renal injury from postnatal infection. In children with VUR identified during the evaluation of antenatal hydronephrosis, there is a pattern of boys with dilating VUR and renal dysplasia and girls with mild VUR and normal renal units [125]. A similar pattern of renal injury has also been noted in a population-based cohort of children followed after a first episode of UTI [80]. However, it remains unclear if the renal damage identified is congenital or acquired. The ability to distinguish renal scarring from dysplasia on DMSA renal scan is at the center of this debate. Newer imaging modalities will potentially help to differentiate these two entities.

The AAP and NICE guidelines have recommended limiting evaluation for diagnosis of VUR to those children presenting with recurrent infections, or those with abnormalities identified on ultrasound. These recommendations have led to a significant reduction in the numbers of children being identified with VUR. Additionally, studies have demonstrated that these recommendations have led to some children with high grade VUR being missed [126]. The long-term impact of this remains a concern.

The role of antibiotic prophylaxis in the prevention of recurrent infections also remains debated. We would certainly recommend prophylactic regimens for those children in higher risk groups as identified by the RIVUR study and other studies.

Surgical management of VUR has been shown to have high success rates for the resolution of VUR, but patients with BBD may continue to develop lower tract infections despite abatement of VUR.

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Obstructive Uropathies

45

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Introduction

Obstructive uropathy is the partial or complete blockage to the flow of urine, which can occur as the consequence of an anomaly at any level of the urinary system: the ureteropelvic junction, distal ureter, ureterocele, urethra, or extrinsic compression by other structures (e.g., blood vessels, tumors).

As most of these conditions are congenital, they are currently routinely diagnosed on prenatal ultrasound (US) in developed countries. Early in fetal development, urinary tract obstruction starts a complex sequence of events affecting renal growth and development, and eventually leading to renal impairment. Congenital urinary tract obstruction is associated with a significant reduction in the number of nephrons and is the primary cause of end-stage renal disease (ESRD) in children [1, 2].

Detection of these conditions during pregnancy varies according to gestational timing at which screening is performed. Thanks to its safety, excellent anatomical resolution, wide accessibility and low-cost, US is the first examination to perform before and after birth [3].

From the beginning of the second trimester of gestation, the renal pelvis becomes detectable and appears as a sonolucent area in the middle of the kidney. Pyelectasis and hydronephrosis are the most common signs of obstruction detected on US. Other signs of obstructive uropathies are cysts, abnormal corticomedullary differentiation, increased echogenicity of the renal parenchyma, ureteral dilatation, pelvic or ureteral wall thickening, increased bladder size or wall thickness or presence of ureterocele [4–6].

Pyelectasis, defined as the dilation of the renal pelvis, and hydronephrosis, defined as the dilation of both the pelvis and calices, are evaluated in the sections of fetal abdominal transverse planes by measuring the anteroposterior diameter (APD) of the renal pelvis. APD may vary depending on the gestational week.

A third-trimester renal pelvis APD diameter of 7 mm is the most widely used criterion to select patients requiring postnatal investigation [4, 7]. Fetal distension of the urinary collecting system is often a dynamic and physiologic process which resolves spontaneously after birth [3, 8–10]. Among the prenatal mild pyelectasis cases, only a small proportion are associated with a serious problem in the postnatal period. However, in some cases pyelectasis can signal

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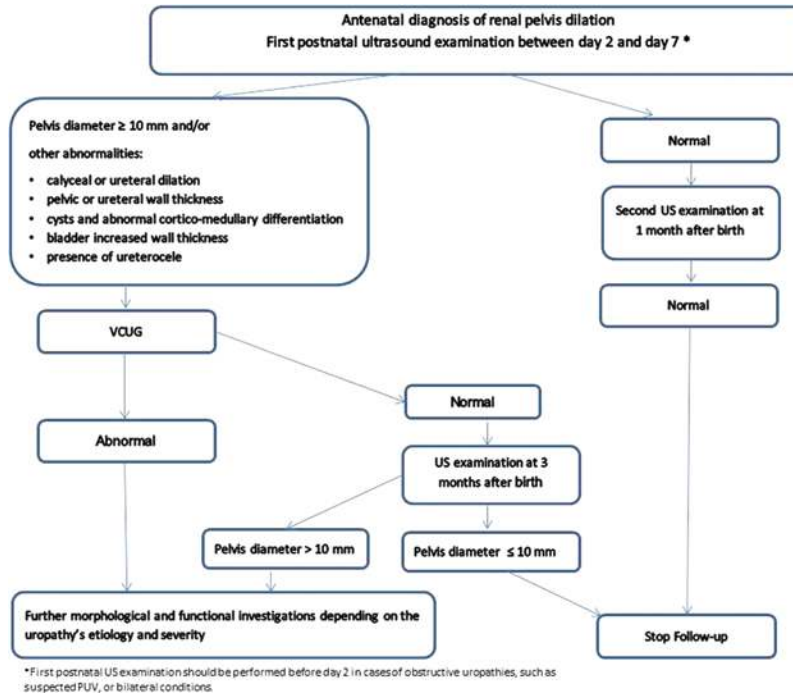

Fig. 45.1 Algorithm for antenatally detected urinary tract dilation and postnatal imaging strategy

the presence of severe urinary tract pathology [11], especially in patients with significant hydronephrosis [7]. Renal pathology is confirmed postnatally in 12–14% of mild, 45% of moderate and 90% of severe pyelectasis cases detected in the second and third trimesters of pregnancy [12].

After birth, a renal pelvis APD of 10 mm is the most commonly accepted upper limit threshold value of normal [5] while a renal pelvis APD >15 mm is often associated with significant uronephropathies [4, 6, 13]. Based on the American [4] and European recommendations [14] and on our experience [3, 6, 7], we have suggested an algorithm for a pragmatic postnatal imaging strategy [15] (Fig. 45.1).

Common Obstructive Uropathies

The presentation and management may be divided into upper and lower urinary tract obstruction. Upper urinary tract obstruction is often unilateral and therefore less critical for overall renal function. Lower urinary tract obstruction, especially of the bladder outlet, is usually associated with a more serious prognosis.

The most frequent congenital upper urinary tract conditions are ureteropelvic junction obstruction (UPJO), ureterovesical junction obstruction (also referred to as primary megaureter) and renal duplication anomalies. Posterior urethral valves (PUV) in boys are the most common cause of lower urinary tract obstruction.

The clinical management of these pathologies remains a challenge for nephrologists and urologists, due to the wide spectrum of severity and clinical progression and the difficulty in predicting long-term prognosis [1].

The different imaging and clinical features as well as current treatment options of the most common obstructive uropathies are reported below.

Ureteropelvic Junction Obstruction

UPJO is the most common cause of persistent prenatal hydronephrosis, occurring in 5–20% of children with antenatally diagnosed renal pelvis

dilation [3]. It is usually unilateral, more common on the left side and more often observed in males [16]. Bilateral UPJO has a higher risk of additional kidney anomalies and potential renal function impairment, and often has a poor prognosis when associated with oligohydramnios and hyperechoic renal parenchyma in utero. UPJO is primarily caused by an aperistaltic segment, or crossing vessels at the level of junction between the pelvis and the ureter [17]. Rarely, obstruction is caused by an epithelial polyp, a benign growth of urothelium blocking the lumen at, or distal to, the UPJ. UPJO is highly suspected when there is significant pyelectasis, often >15 mm, in the absence of any dilation of the ureter (Fig. 45.2), and once vesicoureteral reflux (VUR) has been excluded by voiding cystourethrography (VCUG). In severe obstruction, caliceal dilation, hyperechoic parenchyma and sometimes a perirenal urinoma may be seen. On US, perirenal urinoma appears as a perinephric pseudocyst confined to the Gerota's fascia [12, 18]. Although the development of a urinoma is rare, it is most common when there is an ipsilateral dysplastic kidney [12].

The postnatal management of children with antenatally detected UPJO remains controversial [19, 20]. The primary question is how to identify asymptomatic children with hydronephrosis at risk for loss of renal function if managed by observation alone. To date, there is no consensus about the criteria for surgery, even within the same center [21, 22].

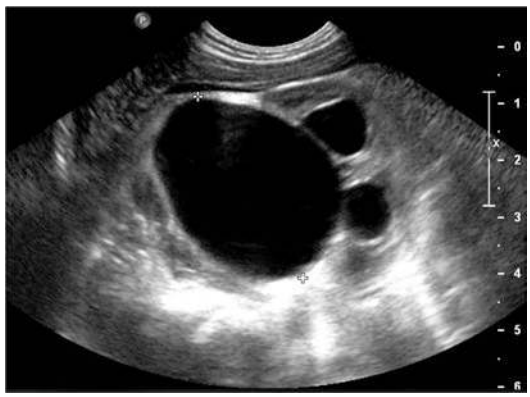

Fig. 45.2 Ultrasound of severe dilatation in a ureteropelvic junction obstruction

In 2016, the Cochrane review by Weitz et al. [23] focusing on unilateral UPJO evaluated the effects of surgical versus conservative management in newborns and children under 2 years of age. Unfortunately, the study was limited by the small sample size and short follow-up time and therefore was unable to clarify the optimal therapy for young children with unilateral UPJO. One year later, Weitz published a systematic review [21] on more than 1083 patients from 20 studies, with the aim of determining the effect of non-surgical management of unilateral UPJO. Although this review was also biased by the great heterogeneity of the included studies, it showed that more than 80% of the cases had improved drainage pattern over time, that about 20% of the patients were at risk for split renal function deterioration, and that nearly 30% underwent surgical intervention [21]. In order to definitively resolve the ongoing controversy and define the optimal management of unilateral UPJO, a randomized controlled trial with sufficient statistical power and adequate follow-up would be required.

A major challenge is that children with UPJO are often clinically asymptomatic and the criteria for surgery are primarily related to sonographic and isotopic parameters. In addition to the severity of the hydronephrosis on US, important predictors of the need for intervention are impaired differential renal function (DRF) and renal drainage on renogram [19, 21, 24]. MAG3 renogram is the gold standard for the evaluation of differential renal function and severity of obstruction. The Society of Fetal Urology and the Pediatric Nuclear Medicine Council of the Society of Nuclear Medicine have provided a consistent methodology for renography [25]. The Paediatric Committee of the European Association of Nuclear Medicine guidelines [26] has underlined the potential pitfalls in the acquisition, processing and interpretation of isotopic examinations.

Pediatric urologists decide on the need for pyeloplasty primarily based on poor drainage [25, 27–29] as indicated by delayed cortical transit time, which is the passage of the tracer from

the outer cortex to the collecting system [30]. The cortical transit is generally fast, and fairly homogeneous kidney filling can be observed in approximately 2 min. In cases of delayed cortical transit, the tracer is retained in the outer cortical rim and the remaining kidney remains hypoactive for several minutes. Severely delayed cortical transit is an important indication for surgery to avoid renal function deterioration [24, 31].

A sensible clinical approach can be summarized as follows:

- Absolute indications for surgical treatment include symptoms such as febrile urinary tract infections (UTIs), hematuria, and kidney stones. Surgery is also indicated in a solitary kidney with evidence of reduced overall renal function.
- For unilateral UPJO, observation with close monitoring is the prudent approach for the large majority of cases, even in those with severe hydronephrosis initially on US.
- A MAG3 renogram should be performed to exclude worsening drainage in the event of a significant increase of the pelvic diameter on US.
- Early surgical intervention is recommended in cases of severe hydronephrosis with impaired split renal function and/or delayed cortical transit.

Megaureter

Megaureter is a ureteral diameter greater than 8 mm [32] and represents nearly one fourth of all causes of urinary tract obstruction in children [32], with cases presenting initially on prenatal US. In utero, megaureter appears as a serpentine fluid-filled structure with or without dilatation of the renal pelvis and calices [6]. Primary megaureter (pMU) is a ureteral dilation caused by an obstruction at the junction between ureter and bladder. pMU can be caused by both an aperistaltic (adynamic) segment in the terminal ureter causing a functional obstruction or, less commonly, by anatomic causes such as congenital distal ureteral strictures or valves [32]. Secondary megaureter represents an obstructive process due to elevated intravesical pressure associated with an underlying bladder or bladder outlet condition. Common causes include high-grade VUR, neurogenic bladder and PUV. It is important to distinguish between primary and secondary megaureter, as in the latter treatment is directed at the underlying pathology and not at the ureter itself [32]. Differentiating primary and secondary megaureter relies on a VCUG.

With the exception of cases associated with the highest grades of hydroureteronephrosis, the prognosis of pMU is generally good and spontaneous resolution usually occurs within the first 3 years of life. The likelihood of requiring surgical correction increases with ureteral diameter exceeding 10 mm [33]. Regarding the management of pMU, two controversies remain. The first relates to the need for continuous antibacterial prophylaxis (CAP) to prevent UTIs while awaiting resolution. The second relates to indications for surgery.

Some authors recommend CAP in the first 6 months of life [34], although for others CAP is not considered mandatory, in the absence of recurrent UTIs and/or VUR [7]. Unfortunately, the risk of pyelonephritis in newborns with pMU is not clear [35]. A systematic review [36], including 16 studies and 749 patients, found a prevalence of UTIs in patients with pMU greater than 14% and a calculated number needed to treat for patients on CAP to prevent one UTI over the course of 1–2 years of 4.3. The authors recommended CAP in children with pMU selected for non-surgical management, at least in patients with poor emptying or greater ureteral dilatation, and for children in the first months of life [36].

When considering indications for surgery, Rubenwolf et al. reviewed a two-decade experience of pMU, and showed a constant decline of surgical interventions due to the favorable outcomes of conservative management in the majority of children [34]. However, in cases of severe hydronephrosis or a retrovesical ureteral diameter greater than 10 mm, resolution may take longer, and more commonly requires surgery [6]. The British Association of Pediatric Urologists (BAPU) recommends surgical intervention only

in case of recurrent febrile UTIs, impaired renal function associated with severe or progressive hydronephrosis, or a drop in differential function on serial renograms [37]. In these cases, the BAPU recommends megaureter repair in patients over 1 year of age even though the procedure may be challenging in small children [37].

A sensible clinical approach can be summarized as follow:

- For a patient with a megaureter, VCUG should be performed in order to differentiate between primary and secondary megaureter.
- In children with asymptomatic pMU, close surveillance is recommended.
- Continuous antibiotic prophylaxis can be recommended in newborns with the highest grades of hydronephrosis and children with a history of a febrile UTI.
- Surgical intervention is mainly required in case of recurrent febrile UTIs, impaired renal function associated with progressive hydronephrosis and/or deteriorating split renal function on serial renograms.

Duplex Kidney

Duplication of the renal collecting system is a congenital defect that involves a kidney drained by two ureters that may be completely or partially separated [38]. Most patients with duplex kidneys have no significant clinical symptoms. When there is no hydronephrosis or renal impairment, a duplex kidney should be considered a normal variant [39]. However, it can also be pathological, associated with the presence of VUR and/or obstruction. Fetal urinary tract dilatation is associated with renal duplication anomalies in under 5% of cases [39]. In those, VUR classically involves the lower pole ureter and tends to be of higher grade as compared to single system reflux [38, 40]. Obstruction of the upper pole may also present secondary to an ectopic insertion, or more often due to a ureterocele, which on US appears as a thin-walled anechoic cystic dilation of the intravesical submucosal ureter (Fig. 45.3). However, ureteroceles can sometimes escape

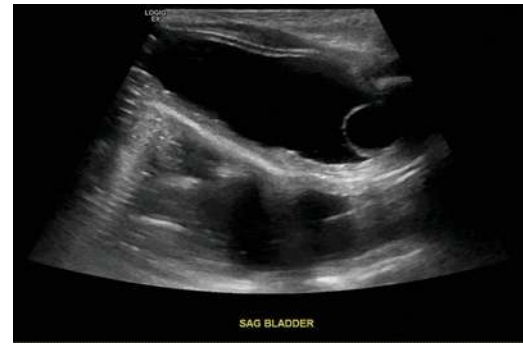

Fig. 45.3 Ultrasound of a ureterocele appearing as a thin-walled anechoic cystic dilation of the intravesical submucosal ureter

sonographic detection, as an overdistended bladder can compress the ureterocele or occasionally the ureterocele itself may be mistaken for the bladder.

In utero, duplex kidneys may be seen as two noncommunicating renal pelves, dilated structures within one pole, and a cystic dilation in the bladder, representing a ureterocele [41].

Postnatal investigations are US and VCUG [42]. Most authors agree that the surgical approach to pathological duplex systems is largely dictated by the anatomic etiology, clinical evolution, and the degree of function in the affected renal moiety [39, 42]. Children with a pathological duplex collecting system and/or ureterocele are also at higher risk of UTIs despite the use of prophylactic antibiotics [43].

A practical clinical approach can be summarized as follows:

- In children with dilated duplex system, US and VCUG are recommended after birth in order to diagnose VUR, assess renal parenchymal anatomy, and detect the presence of a ureterocele.
- In complex cases, prophylactic antibiotics should be started. Isotopic studies are recommended in order to evaluate renal function in the affected renal moiety.
- Decompressive surgery should be planned in cases of an obstructive ureterocele associated with good function, albeit depending on the clinical circumstance. Surgical options include

ureterocele puncture, ureteral reimplantation, and connecting an obstructed ureter to a non-obstructed, non-refluxing ureter. Poor renal function associated with a ureterocele may be an indication for removal of the affected moiety in cases with recurrent UTIs or lower urinary tract obstruction.

Posterior Urethral Valves

PUV are membranous folds fanning distally from the prostatic urethra to the external urinary sphincter and represent the most common cause of lower urinary tract obstruction in boys, affecting 1 in 4000–8000 infants [44]. This congenital malformation, depending on the severity of obstruction and the time of diagnosis, causes a wide spectrum of clinical manifestations. When suspected very early in pregnancy, as in the first or early second trimester, PUV carry a very poor prognosis [45] and are associated with high fetal and neonatal mortality. Impaired urine output leads to oligohydramnios and, ultimately, pulmonary hypoplasia and renal failure [46, 47]. With milder obstruction, the outcome is less predictable. In general, the obstruction leads to hypertrophy of the detrusor muscle, which often affects compliance and raises intravesical pressure. This elevated bladder pressure may be transmitted to the ureters, with or without VUR. This predisposes patients to an increased risk of UTIs, incontinence, and progressive renal impairment [44].

In utero, PUV should be suspected with the following findings: bilateral hydroureteronephrosis, abnormal renal cortex, failure of the bladder to empty, oligohydramnios, and an enlarged, thick-walled bladder with a dilated posterior urethra producing a keyhole configuration (Fig. 45.4). In rare cases, extravasation of urine can be recognized as a urinoma or urinary ascites due to bladder rupture [48] (Fig. 45.5).

Fetal urinary electrolytes and β-2 microglobulin are the most used biological markers to predict postnatal renal function. Fetal urine should be hypotonic, with osmolality less than 210 mEq/L. The combination of raised osmolality and a β-2 microglobulin greater than 4 mg/L suggests irreversible renal dysfunction, and is a contraindication of shunt placement to restore amniotic volume [49].

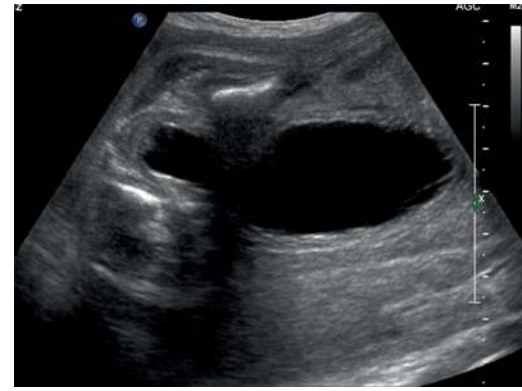

Fig. 45.4 Ultrasound of posterior urethral valves in a 32 weeks gestation fetus. It shows an enlarged thick-walled bladder with a dilated posterior urethra producing a keyhole configuration

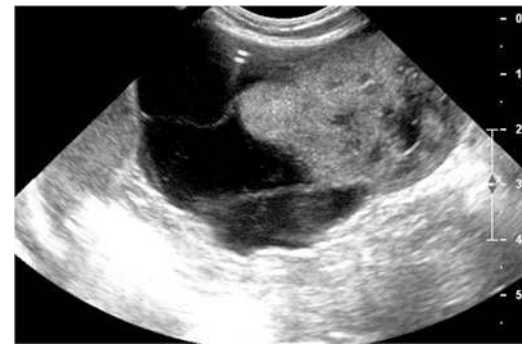

Fig. 45.5 Ultrasound of a perinephric urinoma in the context of severe posterior urethral valves

Once PUV are suspected prenatally, management warrants the prompt involvement of a multidisciplinary team in a fetal and pediatric urology referral center. Various options can be discussed according to the severity of presentation, including termination of pregnancy, in utero therapy or follow-up with planned (active or palliative) postnatal management.

For decades, a variety of in utero approaches to therapeutically relieve obstructing posterior urethral valves have been utilized: open surgical technique of fetal vesicostomy [50], direct fetoscopic valve resection [51, 52], and vesicoamniotic shunting [53].

Vesicoamniotic shunting is the method most commonly used for bladder drainage. It involves the placement of a double pig-tailed catheter under US guidance with the distal end in the fetal bladder and the proximal end in the amniotic cavity to allow drainage of fetal urine [54, 55]. Its principal benefit is prevention of early neonatal pulmonary insufficiency and death. However, the risks of premature labor, perforation of fetal bowel or bladder, and fetal or maternal hemorrhage or infection are significant [56]. In 2013, the PLUTO (Percutaneous vesicoamniotic shunting in Lower Urinary Tract Obstruction) study was completed [57]. In this trial, fetuses diagnosed with lower urinary tract obstruction were randomly assigned to either vesicoamniotic shunting or conservative management. Despite its small sample size, PLUTO's results suggest that survival in the neonatal period was higher with vesicoamniotic shunting than with conservative management. However, there was substantial morbidity in both groups, with only two out of seven shunted survivors having normal renal function at 1 year of age. These results indicate a low likelihood of patients with severe PUV surviving with normal renal function, irrespective of management [58].

In summary, the experience with the intrauterine shunting technique as currently practiced suggests that postnatal survival may be increased, but that a significant improvement of postnatal renal function cannot be expected. Hence, the goals of care in patients with PUV are prompt urological care following a full gestation delivery with the aim of maximizing bladder and renal function.

The long-term outcome of PUV is far from satisfactory, with 15–30% of patients reaching ESRD during childhood [59, 60]. Indeed, PUV are the most frequent cause of chronic renal disease in boys and account for about 17% of children with ESRD [61]. In a retrospective study of more than 100 patients with PUV, the factors associated with a higher risk of CKD and ESRD were antenatal diagnosis, prematurity, abnormal renal cortex with loss of corticomedullary differentiation on initial US and elevated plasma creatinine at 1 year of age [62]. The decreased number of total nephrons present at birth leads to hyperfiltration injury, exacerbation of the underlying inflammatory process, renal fibrosis and, ultimately, renal failure.

In addition to renal impairment, the severity of bladder dysfunction in PUV patients varies widely, as the clinical spectrum ranges from severely pathological, high pressure bladders to late presentation with mild lower urinary tract symptoms, incontinence and recurrent UTIs. Indeed, children not diagnosed prenatally may present in infancy with urosepsis, poor urinary stream and/or failure to thrive. Older children may also present with urinary symptoms of incontinence, poor flow or retention. In a systematic review [63] of the outcomes of PUV in nearly 1500 patients, urodynamic bladder dysfunction was seen in more than half of the patients after endoscopic treatment of PUV, and nearly one in five cases had urinary incontinence.

A reasonable clinical approach can be summarized as follows:

- All cases antenatally suspected with PUV should be discussed by a multidisciplinary team, including obstetrician or maternal fetal medicine specialist, radiologist, pediatric nephrologist and urologist. All cases should then be referred to a pediatric center with a neonatal intensive care unit with nephro-urological expertise.
- Once the risks and benefits have been assessed, if an antenatal therapeutic intervention is proposed, the procedure should be performed selectively in qualified centers.
- In all children suspected of PUV, catheter drainage of the bladder should be performed at birth. Fluid status, serum electrolytes, and renal function should be monitored closely. Prophylactic antibiotics should be started.
- VCUG should be performed as soon as possible after birth. A voiding view of the urethra with the catheter removed is crucial for definitive evaluation of the urethra. Antibiotics should be administered 1 day prior and 1 day after the VCUG to prevent UTIs.
- The standard of care for PUV is endoscopic valve ablation when the child is medically

stable. Circumcision is also recommended to reduce the risk of infection.

- If catheter drainage improves hydronephrosis and plasma creatinine, then valve ablation is all that is necessary.
- If catheter drainage improves hydronephrosis, but renal function does not improve, this suggests renal dysplasia.
- If with catheter drainage the hydronephrosis and renal function do not improve, an upper tract diversion should be considered.
- If the newborn is too small (under 2 kg), the urethra might not safely allow standard resectoscope introduction. In these cases, alternative techniques such as vesicostomy, Fogarty balloon-based intervention, or laser ablation can be performed in order to alleviate or bypass the obstruction until the child is large enough for definitive treatment.
- Due to the high risk of chronic kidney disease in this patient population, patients should have close, long-term follow-up of renal function.
- Urodynamic studies are particularly valuable in order to evaluate bladder storage, pressure, compliance, emptying, and post-void residual volume, and help guide medical and surgical management.
- In children with ESRD, full assessment of the bladder is crucial before transplantation in order to prevent a hostile bladder from damaging the renal allograft. Moreover, with immunosuppression, the risks of transplant pyelonephritis and subsequent renal allograft damage need to be considered. If urological intervention is required, then reconstruction, such as bladder augmentation, should be performed pretransplant.

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Renal Calculi

46

Larisa Kovacevic and Paul Goodyer

Introduction

Pediatric nephrolithiasis has shown a dramatic increase in incidence [1], hospitalization and complication rates over the past two decades [2], and is no longer considered a rare or benign disease. Shifts in epidemiology, etiology, and stone composition have been noted and are related, at least in part, to the modern life-style [3, 4]. New trends include: an increasing annual incidence among two specific age groups (young and adolescent children), and among girls and African-Americans [5]; a change in the etiology from infectious to metabolic [6], and in the main metabolic focus from hypercalciuria to hypocitraturia [7], along with alterations in stone composition (ammonium and urate to calcium) [8]. Additionally, there is an increasing body of evidence supporting the association of nephrolithiasis with other conditions such as hypertension, coronary heart disease, atherosclerosis, diabetes and low bone mineral density, supporting the hypothesis that nephrolithiasis has a multisystemic involvement rather than being a single disease [9–11]. Moreover, the serious consequences of kidney stones are increasingly recognized based on new data on its association with chronic kidney disease (CKD) [12, 13]. Altogether, this emphasizes the need for comprehensive re-evaluation of pediatric stone management, which will help identify the modifiable risk factors at an early stage, guide treatment, and allow better prevention strategy.

There are several unique characteristic features of pediatric stone disease compared to adult stone formers: heterogeneous clinical presentation, high frequency of an underlying etiology (anatomic and genetic in infants and younger children, metabolic in older children), great variability of risk factors in relation to age, gender and race, and high rate of recurrence.

In this chapter, we will address the differences between children and adults and recently observed trends in pediatric nephrolithiasis, as well as advances in pathophysiology and treatment strategies, and future directions.

Epidemiology

The true incidence of pediatric nephrolithiasis is not known because many cases are either misinterpreted or undiagnosed due to the lack of symptoms. The estimates obtained from population-based observational studies indicate a range from 36 to 57 stone cases per 100,000 children

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[14, 15]. Overall, the incidence of pediatric nephrolithiasis is lower than in adults, and this may be due to higher concentration of urinary inhibitors of stone formation such as citrate and magnesium [16]. The presence of certain urinary macromolecules (UMM) in children, containing higher proportions of fibronectin and glycosaminoglycans, also exhibit crystal-cell adhesion inhibitory activity compared with adult UMMs [17].

A change in epidemiology and a 4–16% annual increase in incidence of pediatric nephrolithiasis were noted over the past two decades, with the greatest increased rates among children between 12 and 17 years old, females, and African-Americans. Although the cause of the increasing incidence of pediatric nephrolithiasis is not entirely clear, potential explanations focus on changes in dietary habits (increased intake of sodium and decreased water intake), an increase in antibiotic usage and other lithogenic drugs, and the increased use and sensitivity of imaging.

The prevalence, incidence and risk of nephrolithiasis varies with gender, race, climate/geographic location, dietary habits and socioeconomic factors.

Gender

The strong male gender predominance found in adults with nephrolithiasis is not seen in children [18]. An age-dependent gender distribution was reported in the United States, with boys being more affected in the first decade of life and girls in the second decade [19]. This could be due to the main risk factor of stones identified in these groups, namely obstructive urinary malformations in boys and urinary tract infections in post-pubertal, sexually active girls. Recent data show a higher prevalence in girls compared with boys (52% versus 48%) [20], and a male-to-female ratio of 1:1.4, a difference that becomes more pronounced in adolescence [2, 20]. Additionally, female gender imposes a relative risk (RR) of 1.5 for hospitalization for nephrolithiasis [21, 22]. This marked increase seen in females has special significance due to the association of nephrolithiasis with cardiovascular disease and fractures. The incidence of stone disease shifts towards a male predominance around 26 years of age, which continues throughout adulthood.

Age

During a 12-year period, one study reported the lowest incidence in children aged 0–3 years (0.6 per 100,000) and the highest in children aged 14–18 years (34.9 per 100,000) [6]. The same study showed that children aged 14–18 years had a 10.2-fold greater risk for nephrolithiasis and hospitalization compared to children 0–13 years of age [7]. Ureteral stones are more common in older children, whereas younger children are more likely to develop renal stones [19, 20]. Moreover, it seems that age is not predictive of spontaneous passage of stones, but stones less than 5 mm in size are more likely to pass compared to stones more than 5 mm [23, 24].

Race/Ethnicity

Nephrolithiasis is more common in non-Hispanic white children [5, 15, 17], followed by Hispanic and African American children [5, 7, 8, 14, 15]. However, it is unclear whether these differences are due to genetic differences or dietary habits.

Regional Differences

The incidence of pediatric nephrolithiasis varies by geographic area (rural vs. urban), and among countries, and is due to differences in climate, diet, socio-economic factors, and genetics. A higher incidence of kidney stones was found in children living in western countries (5–10% of that in adults) and in rural communities. In developed countries, stones are found mainly in the kidney and ureter, and are predominantly calcium-oxalate or calcium phosphate. In underdeveloped and developing countries, bladder stones are commonly seen, and usually consist of uric acid or ammonium [25].

Turkey and Thailand are endemic areas with the highest incidence of renal stones. "Stone belts" have been described in different parts of the world, and include Southeast US (Virginia, North Carolina, Georgia, Tennessee, and Kentucky), Sudan, Egypt, Saudi Arabia, the United Arab Emirates, Iran, Pakistan, India, Turkey, Myanmar, Thailand, Indonesia and Philippines. This is probably due to the hot, dry climate causing dehydration, and high rates of consanguinity.

Pathogenesis

The initiation and growth of calculi requires high urinary solute concentration (supersaturation) and low urinary volume. Supersaturation represents the ratio of a salt's concentration in urine to its solubility; a ratio of more than 1 favors crystal formation, while a ratio less than 1 allows the crystals to dissolve. Further growth and aggregation is favored by ionic strength, urinary pH, and the concentration of promoters and inhibitors of crystallization (Table 46.1). These aggregates may occlude the tubular lumen and serve as an initial nidus (the free particle theory) [26]. This mechanism is particularly important for stone formation in cystinuria, where high urinary levels of cystine initiate intratubular nucleation. Adherence to the epithelial renal tubule cells of the urinary tract is required to allow for crystal growth (the fixed particle theory) [27], a process especially important in patients with brushite and apatite stones. Injured renal tubular cells by either toxins, infection or medication (e.g., calcineurin inhibitors and gentamycin) favor crystal attachment. The expected urine washout of crystal aggregates is impaired by stasis caused by congenital anomalies of the urinary tract.

Table 46.1 Promoters and inhibitors of stone formation

Promoting factors	Inhibiting factors
Calcium	Citrate
Oxalate	Magnesium
Sodium	Pyrophosphate
Urate	Tamm-Horsfall protein
Cystine	Osteopontin
	Prothrombin fragment-1
	Bikunin
	Inter-alpha-inhibitor
	Alpha-1-microglobulin
	Calgranulin
	Fibronectin
	Matrix Gla protein
	Renal lithostathine
	Glycosaminoglycans (i.e. heparan sulfate)

In the Randall's hypothesis, apatite plaques or other sources of uroepithelial damage (infection, foreign body) represent the nidus for calcium oxalate stone formation [28]. Randall's plaque originates from the basal membrane of the thin loops of Henle, expands through the interstitium and protrudes into the papillary vasculature causing injury and repair in an atherosclerotic like fashion (vascular theory of Randall's plaque formation) [29]. This theory accounts for stones which appear to be embedded in the papillary wall and is supported by the physiology of renal papilla: turbulent flow, high osmolality and hypoxia [30, 31]. The reported association between vascular disease (hypertension, atherosclerosis, myocardial infarction) and nephrolithiasis [32] and the tendency of unilateral stones to develop on the sleeping side due to increased renal flow [33] are additional evidence supportive of the vascular theory.

Initial Presentation, Evaluation and Management

The presenting symptoms of nephrolithiasis in children differs from that in adults and depends on the child's age. Typical renal colic and gross hematuria are more often seen in older children and adolescents, who have higher rates of ureteral stones and spontaneous passage [6]. Flank pain may identify the position of stones: trapped at ureteropelvic junction (costovertebral angle tenderness), passing down the ureter (lateral flank tenderness) or trapped at the ureterovesical junction (lower abdomen or groin pain). In contrast, younger children may present with irritability, vomiting, failure to thrive, nonspecific

Table 46.2 Initial evaluation of a child with nephrolithiasis

Medical history
Diuretic use in premature newborns, urinary tract abnormalities, urinary tract infections, intestinal malabsorption (Crohn's disease, bowel resection, cystic fibrosis), immobilization, diabetes mellitus, hypertension
Medications (antibiotics, anticonvulsants, diuretics, corticosteroids, chemotherapy, antacids, protease inhibitors)
Dietary history
Daily intake of fluid, sodium, potassium, calcium, oxalate and protein; special diets (vegetarian, meat). Use of vitamins C or D, herbal products, special therapeutic diets (e.g. ketogenic diet)
Family history of stone, hematuria, or renal failure (pedigree to establish mode of genetic transmission)
Physical examination: obesity, growth failure (distal renal tubular acidosis), dysmorphic features (William's syndrome), rickets (Dent disease), lower urinary tract stasis (spina bifida)
Urine
Urinalysis (pH[a], specific gravity or osmolality, glucose[b], protein[c]) and culture
Urine solute to creatinine ratios in random urine: spot urine
Qualitative cystine screening
Urine solute concentrations and excretion rates in timed 24-h urine collection (volume, calcium, phosphorus, oxalate, citrate, uric acid, sodium, potassium)
Serum chemistry: calcium, phosphorus, magnesium, alkaline phosphatase, sodium, potassium, chloride, bicarbonate, uric acid, creatinine, urea nitrogen

[a] Urine pH higher than 6 favors calcium phosphate precipitation; higher than 7 suggests urease producing organisms and struvite stones; lower than 6 favors cystine or uric acid stones
[b] Glycosuria and proteinuria indicates tubular dysfunction
[c] Low-molecular weight proteinuria is consistent with Dent disease

abdominal pain, microscopic hematuria and urinary tract infection [34].

Some form of hematuria is present in about half of children with stones [35]. Dysuria or frequency is seen in about 10%, while urinary retention/bladder pain may be seen in others. Other features include failure to thrive, hypertension and kidney failure; stones are occasionally reported in association with enuresis, penile edema, and anorexia. Rarely, children present with acute anuria caused by bilateral obstructive stones.

The initial assessment of children with nephrolithiasis is presented in Table 46.2; a proposed algorithm for the initial management of suspected nephrolithiasis is shown in Fig. 46.1. At presentation in the emergency room or during primary outpatient consultation, patients should have a careful medical history, family history and physical examination as outlined in Table 46.2. Blood should be drawn for measurement of serum creatinine, urea nitrogen, electrolytes, uric acid, calcium, alkaline phosphatase, parathyroid hormone and 1,25-dihydroxy vitamin D. Urine should be obtained for standard urinalysis and microscopy to screen for specific crystals in the urinary sediment (Table 46.3). Urine should also be screened for the ratio of calcium, oxalate, uric acid and cystine to creatinine (measures of excess excretion of these solutes) and for the ratio of citrate and magnesium to creatinine (measures of suboptimal levels of these stone inhibitors) (Table 46.4). Fractional excretion of sodium can be calculated to identify renal salt-losing states.

Diagnostic Imaging

The goals of initial imaging are to confirm the presence of a stone, detect whether it is obstructing urinary flow, estimate stone size (and likelihood of passing), ascertain whether the stone contains calcium and identify any anatomic abnormality (congenital or acquired) which might cause local urinary stasis. Ultrasonography and a plain abdominal radiograph showing kidney/ureter/bladder (KUB) are the mainstay of radiological imaging in children (Table 46.5) [36, 37]. Color Doppler ultrasonography provides information about the "twinkling effect" that is characteristic of small stones and should be used in patients who show no stones by B mode ultrasonography. A simultaneous KUB should be performed to identify ureteral stones and assess the calcium content of the stone.

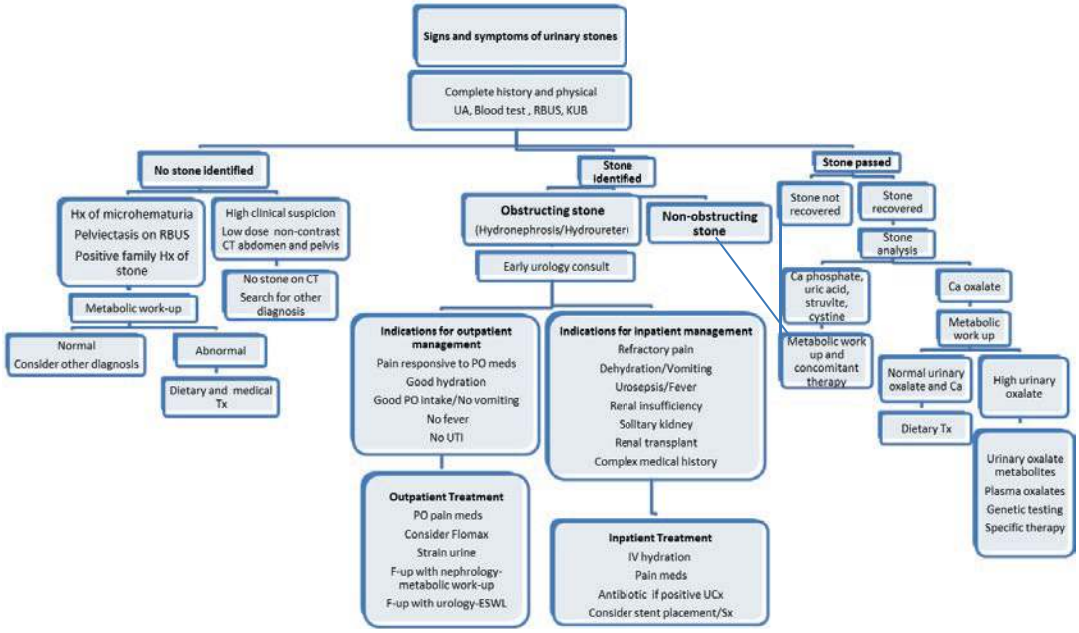


Fig. 46.1 Algorithm for the initial management of suspected nephrolithiasis

Table 46.3 Stone composition in children

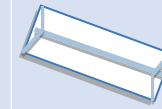
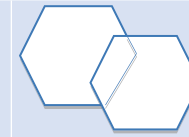
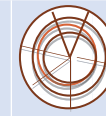
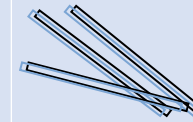
Crystal type (stone incidence)	Possible causes	Appearance	Urine pH	Appearance
Calcium oxalate Monohydrate (Whewellite-80%)	Primary hyperoxaluria	Dumbbell or oval	Acidic	
Calcium oxalate dehydrate (Whedellite-20%)	Hypercalciuria Hyperoxaluria Hypocitraturia	Envelope	Acidic	
Uric acid (0.5–1%)	Hyperuricosuria (primary or secondary)	Varying sizes and shapes—rhomboids, parallelograms and rosettes	Acidic	
Struvite (triple phosphate) (Struvite 5–7%, Calcium phosphate 0.5–6%)	Infection	"Coffin lid"	Alkaline	
Cystine (1–5%)	Cystinuria	Hexagonal	Acidic	
2,8 dihydroxyadenine	Adenine phosphoribosyltransferase deficiency	Round, reddish brown with dark outlines and central spicules	Acidic	
Drug related -sulfa -acyclovir		Needle like crystals		

Table 46.4 Normal value for urinary excretion of metabolites

	Age	Random (mg/mg)	Random (mmol/mmol)	Timed (all ages)
Calcium	0–6 months	<0.8	<0.8	<4 mg/kg per 24 h (<0.1 mmol/kg per 24 h)
	7–12 months	<0.6	<0.6	
	>2 years	<0.2	<0.2	
Oxalate	0–6 months	<0.26	<0.26	<40 mg/1.73 m^2 per 24 h (<0.5 mmol/1.73 m^2 per 24 h)
	7–24 months	<0.11	<0.11	
	2–5 years	<0.08	<0.08	
	5–14 years	<0.06	<0.06	
	>16 years	<0.32	<0.32	
Cystine	>6 months	<0.075	<0.075	<60 mg/1.73 m^2 per 24 h (<250 μmol/1.73 m^2 per 24 h)
Uric acid	<1 year	<2.2	<1.5	<815 mg/1.73 m^2 per 24 h (<486 mmol/1.73 m^2 per 24 h)
	1–3 years	<1.9	<1.3	
	3–5 years	<1.5	<1.0	
	5–10 years	<0.9	<0.6	
	>10 years	<0.6	<0.4	
Citrate[a]	0–5 years	>0.2–0.42	>0.12–0.25	>310 mg/1.73 m^2 per 24 h (>1.6 mmol/1.73 m^2 per 24 h) in girls and
	>5 years	>0.14–0.25	>0.08–0.15	>365 mg/1.73 m^2 per 24 h (>1.9 mmol/1.73 m^2 per 24 h) in boys
Magnesium	>2 years	>0.13	>0.13	>0.8 mg/kg (>0.04 mmol/kg)

[a] A range for normal random citrate values is presented in the table to account for regional variations

Table 46.5 Radiologic imaging of the urinary tract

Radiologic imaging	Advantages	Disadvantages
Ultrasonography[a] (Sensitivity 77–90% Specificity 88–94%)	Wide availability, easy to perform, no pain, no radiation, no anesthesia, can be repeated, low cost Shows stones of all compositions Shows the anatomy of the kidney and bladder Identifies associated hydronephrosis	Operator dependent Body habitus and bowel gas may affect stone visualization Can miss ureteral calculi Can miss papillary or calyceal stones, and small calculi (<5 mm) Overestimates stone size
Plain abdominal radiography (Sensitivity 45–58%)	Detects large ureteral stones not seen on ultrasonography: Detects calcium (radiodense), struvite and cystine stones (intermediate radiodensity)	Misses radiolucent stones: uric acid, xanthine and indinavir Risk of malignancy with radiation
Noncontrast computed tomography[b,c] (Sensitivity and specificity 90–100%)	Detects radiolucent and small stones Provides anatomic details	Can miss indinavir and small distal stones Risk of malignancy with radiation Potential need for sedation
Intravenous pyelogram	Indicated in medullary sponge kidney	

[a] First choice in the initial assessment
[b] Gold standard
[c] New pediatric protocols have decreased the radiation exposure by 60–90%

Although computed tomography (CT) scan without contrast is the gold standard for assessing nephrolithiasis in adults, it is used sparingly during the initial evaluation in children to minimize radiation and to avoid the need for sedation. However, low radiation dose, non-contrast CT scan protocols have been developed for pediatrics and are mainly indicated when the patient is symptomatic and stones are suspected but not seen by ultrasound. A single center study found

that a history of previous stones, vomiting, and the presence of blood on urinalysis were the strongest predictors for finding kidney stones on unenhanced CT scans [38]. Contrast agents can mask the presence of a stone and should be avoided on the initial CT scan; however, contrast may be useful in defining additional anatomical abnormalities.

Stone Analysis

If acute nephrolithiasis is suspected, patients should be asked to pass urine through a sieve to capture stones for analysis. This should continue until acute symptoms have resolved and/or passage of the stones are confirmed by repeat imaging. The composition of all recovered stone fragments should be analyzed by either infrared spectroscopy or X-ray diffraction, and components exceeding 5% should be reported. This should be done with each passage of stone since the composition may differ from the initial presentation. About two thirds of stones are composed of more than one substance. The composition of stones varies in children, although calcium oxalate is the most common [39, 40] (Table 46.3).

Differential Diagnosis

Abdominal or flank pain may be found in children with infections, such as gastroenteritis, urinary tract infections (UTI), appendicitis, ovarian torsion, intussusception, constipation, and pneumonia. UTI can concomitantly exist with nephrolithiasis. Worsening UTI or failure to improve within 24–48 h following antibiotic therapy may indicate the presence of renal stone, renal abscess, or underlying anatomic abnormalities/obstruction, and requires imaging of the urinary tract.

Gross hematuria may be caused by UTI, irritation of the meatus or perineum, trauma, and glomerular disease. Cola-colored urine, urinary sediment, and the possible presence of hypertension and/or edema indicate glomerulonephritis.

Acute Management

When a child presents with an acute stone episode, the immediate treatment goals are pain relief, nausea/vomiting control, rehydration, and treatment of associated infection if present. Nonsteroidal anti-inflammatory medications may be used with caution, provided renal function and hydration are adequate. If this is insufficient, oral or intravenous narcotics (e.g. morphine 0.3 mg/kg oral q3–4 h or 0.05 mg/kg intravenous q2–4 h) can be used for pain control, depending on oral tolerance. The preferred antiemetic agent is ondansetron due to its minimal side effects; metoclopramide hydrochloride or prochlorperazine are acceptable alternatives.

Once the diagnosis of nephrolithiasis is established, it must be determined if the patient can pass the stone spontaneously or whether surgical intervention is required. The size of the stone and its orientation are the critical determinants, with stones up to 5 mm having a high likelihood of spontaneous passage in children of all ages. With adequate pain control, uncomplicated unilateral stones causing only minimal or partial obstruction can be managed conservatively for several weeks before surgical intervention is considered. During this period, medical expulsive therapy (MET) for smaller ureteral stones has been used, especially in older children, with some success. However, the evidence to support this form of therapy in children is conflicting. There are a few randomized trials showing no efficacy in children [41] or adults [42]. In contrast, others concluded that MET is effective in pediatric patients [8, 43]. The most commonly used agent is tamsulosin, which causes relaxation of ureteral smooth muscle with inhibition of ureteral spasm and dilatation of the ureter. A prospective cohort study by Mokhless et al. demonstrated that ibuprofen and tamsulosin administered at bedtime (0.2 mg for children <4 years of age, and 0.4 mg for children >4 years of age) to children with distal ureteral stones led to significantly increased percentage of stone passage (88% vs. 64%), shorter passage time (8 vs. 14 days), and less need for analgesia (0.7 vs. 1.4 days) [44]. Tamsulosin is safe and has been approved by the US Food and Drug

Administration in pediatric patients. Alternatively, alpha blockers or calcium channel blockers can be used to facilitate the passage of ureteral stones under 10 mm in size [45]. Calcium channel blockers act by decreasing the intracellular calcium concentration, which induces relaxation of the ureteral smooth muscle. Alpha adrenergic receptors are abundant in the smooth muscle of the distal third of the ureter and ureterovesical junction and their blockage promotes relaxation of the ureteral wall.

In most cases, passage of the stone takes days or weeks and is usually managed as an outpatient, with careful medical oversight of pain control, expulsive therapy when stones are in the ureter and treatment of UTI if indicated. However, some children may need hospitalization. In general, this is restricted to those with an urgent need for upper tract decompression (nephrostomy tube or a lower tract stent), severe pain requiring intravenous analgesia, or need for intravenous antibiotic therapy for a UTI, as in urosepsis.

Evaluation for Risk of Recurrent Nephrolithiasis

Following the initial presentation and management, children with suspected or proven stones should be referred for evaluation of primary factors that might predispose to recurrence of nephrolithiasis, and provided with appropriate preventative measures. Ideally, this involves a clinic setting that can provide access to urologic expertise, genetic testing, nutritionist support and appropriate metabolic laboratory investigation. The risk of recurrent renal stones in children is high, with approximately 50% presenting with recurrent symptomatic stone within 3 years from the first episode of nephrolithiasis [46]. Those who have an identifiable metabolic abnormality are at fivefold higher risk compared to those without [23]. The rate of recurrence is also higher in those with a positive family history of stones in first-degree relatives.

Compared to adults, a predisposing cause for stone formation is found in about 2/3 of children and includes metabolic (33–95%), anatomic (8–32%) and infectious factors (2–24%), alone or in combination [47–49]. About 50% of children under 10 years of age with stone disease have an underlying metabolic condition [23] caused by a renal, enteric or endocrinologic abnormality. The chance of finding a metabolic risk factor is higher in infants, patients with a positive family history or consanguinity, and those with recurrent kidney stones [25]. The risk of CKD and end-stage kidney disease (ESKD) is doubled in recurrent stone-formers compared to non-stone formers [8].

Thus, the primary goal of the "stone work-up" in children is to identify hyperexcretion of specific solutes that are likely to drive stone formation and to characterize the urinary content of inhibitors that normally protect against precipitation of lithogenic salts. When possible, a timed 24-h urine collection should be obtained to confirm the results of initial screening with spot urine samples.

Due to day-to-day variation in diet and fluid intake, at least two initial 24-h urine collections should be done. Urine creatinine should be checked for the completeness of urine collection (>15 mg/kg/day). The timed collection should be performed without altering the child's usual fluid intake, diet or activity, in the absence of a urinary tract infection, and at least a month after the spontaneous passage of stone or surgical intervention. Results can be interpreted with respect to weight, body surface area and urine creatinine (Table 46.4). When 24-h timed urine collections are not possible, shorter collection periods or repeat spot urine specimens measuring solute to creatinine ratios are acceptable. Normal values vary by age and prandial state (Table 46.4).

Blood analyses for calcium, uric acid, parathyroid hormone (PTH) and for assessment of renal tubular function (Table 46.2) should be performed at the time of the urine evaluation. Calcium homeostasis should be assessed in detail among children with hypercalcemia or hypercalciuria. Primary hyperparathyroidism is rare in children, but suppression of PTH offers a clue to states of vitamin D excess.

A fundamental assumption underlying the management of recurrent nephrolithiasis is that

stones form when the urine is supersaturated with certain lithogenic salts (e.g. calcium oxalate, calcium phosphate). A 24-h urine identifies conditions that cause the urine to be supersaturated: (a) excessive excretion of calcium, oxalate, uric acid and cystine; (b) suboptimal excretion of citrate and magnesium; (c) unfavorable urinary factors such as pH, electrolyte concentrations. Supersaturation for calcium oxalate, calcium phosphate and uric acid are predicted by the well-established Equil2 program. Cystine saturation of the urine is established empirically by measuring the capacity of the urine to dissolve cystine crystals after 48 h. Serial determinations of supersaturation might be predictive of recurrence risk and, therefore, useful in monitoring successful interventions. Reduction of initial calcium oxalate supersaturation by 50% in stone-formers is a reasonable target. There are several publications supporting the utility of this approach in adults [50], but clinical trials in children are lacking, with the exception of cystinuria [51].

Other programs for calculating supersaturation of urine have been developed and adapted for use on personal computer platforms and smartphones [52]. A new web-based platform offers (for a fee) individual access to software that estimates supersaturation for calcium oxalate, struvite (NH_4MgPO_4-$6H_2O$), brushite ($CaHPO_4$-$2H_2O$), uric acid and cystine, using urinary parameters widely available in clinical labs.

Surveillance

Repeat renal ultrasound is needed to diagnose stone recurrence or increasing size of previous stones. The frequency of these tests depends on the presence and severity of the metabolic abnormality, the number of stones and recurrence rate. A child with a single stone and no evidence of an underlying metabolic abnormality will require less frequent monitoring than a child with multiple stones and a significant metabolic problem known to be at greater risk for recurrent nephrolithiasis (primary hyperoxaluria or cystinuria). Compliance with high fluid intake should be monitored by measuring the urine specific gravity.

In an asymptomatic child, a repeat kidney ultrasound is usually done 6 months after the initial episode. If the ultrasound shows no stone recurrence or change in residual stone size, the study can be performed yearly. Metabolic work-up is repeated 4–6 weeks after therapy has been initiated. If the metabolic abnormality was corrected, repeat studies should be done at 6 months, and then yearly. Re-evaluation is needed if metabolic abnormalities persist. A multidisciplinary approach through a combined "Stone Clinic" including nephrology, urology, and dietary services serves these children well for long-term management.

Types of Kidney Stones

Calcium-Based Stones

Isolated hypercalciuria (excretion of >4 mg calcium/kg/day) is the most common cause of stones in children (Table 46.4). Early work in adults by Coe and Pak led to the suggestion that patients with calcium nephrolithiasis could be subdivided into those with "renal hypercalciuria" (decreased calcium reabsorption in the renal tubules), those with "absorptive hypercalciuria" (excessive vitamin D-dependent hyperabsorption), and those with "resorptive hypercalciuria" (parathyroid hormone-driven resorption of calcium from bone). While these classifications refer to important mechanisms in calcium homeostasis, it has become increasingly clear that the majority of patients don't fit easily into one category, and the stone work-up has shifted to other approaches.

Urinary stones develop about three times more often in adults with a family history of nephrolithiasis [53]. Twin studies indicate that the heritability of stone disease ranges from 46% to 63% [53, 54]. In a family study of children with hypercalciuria, Goldfarb et al. noted evidence of heritability in more than 50% [13]. However, it has been difficult to identify a primary genetic cause in most patients with calcium stones. Large genome-wide association studies in adults

suggest that the heritable calcium stone trait is complex, influenced by multiple common variants of genes implicated in rare monogenic forms of nephrolithiasis. Among 3773 adult calcium stone-formers from Denmark and Iceland, homozygosity for a single nucleotide polymorphism (SNP) close to the claudin-14 gene conferred a 1.64 RR of developing stones among Icelanders [55], but could not be confirmed in the Danish group [56]. In large Japanese [57], Chinese [58] and British/Japanese cohorts, multiple SNPs were identified that conferred modest increased risk of nephrolithiasis (RR usually about 1.2), but there was little overlap between studies. Thus, the genetic factors predisposing to calcium nephrolithiasis in humans appear to be complex and remain elusive.

Increasingly, there is a focus on urinary factors that influence the saturation of lithogenic calcium salts. One major risk factor is urinary citrate excretion [7]. Hypocitraturia is defined in children as a urinary citrate excretion rate <310 mg/1.73 m^2/day in girls and 365 mg/1.73 m^2/day in boys, or less than 400 mg/g of creatinine in a 24-h urine collection (Table 46.4). Others have suggested even lower limit values (250 mg/1.73 m^2/day in girls and 180 mg/1.73 m^2/day in boys) due to decreased urinary citrate excretion per kg body weight with increasing age. Citrate is best interpreted in a 24-h urine collection because urinary citrate excretion appears to be influenced by diet and prandial state. It should not be measured during an active UTI because it may be artifactually low. Children have more citrate excretion than adults. Citrate combines with calcium in the tubular lumen to form a soluble complex resulting in less free calcium available to combine with oxalate. Moreover, citrate exerts a direct or indirect inhibitory effect on crystal aggregation, growth, and adherence to renal tubular cells. The indirect effect is due to enhancement of the inhibitory effect of Tamm-Horsfall protein on stone formation. Hypocitraturia is seen in patients with metabolic acidosis and hypokalemia, as well as in those with complete form of distal renal tubular acidosis (RTA).

Oral citrate supplementation is an effective strategy for treating adults with calcium-based kidney stones [59]. Two prospective studies in children showed that oral potassium citrate reduces the risk of stone recurrence by about 50% [60, 61]. Doses typically range from 0.5 to 1.0 mEq/kg/day in hypercalciuric patients; doses up to 2–3 mEq/kg/day may be required in children with dRTA. It is available in tablet and oral solution; a palatable, flavored form is commercially available.

The rationale for this approach is based on the well-established affinity of calcium for citrate (vs. oxalate or phosphate) and the relative solubility of calcium citrate. Individuals excreting suboptimal urinary citrate have increased risk of nephrolithiasis [62]. Srivastava et al. showed that hypercalciuric children with stones have higher calcium/citrate ratios (0.65 mg/mg) in urine than hypercalciuric non-stone formers (0.23 mg/mg) or controls (0.17 mg/mg) [63]. The authors suggested that calcium/citrate ratios of <0.33 mg/mg might decrease stone risk.

However, the decision to introduce citrate therapy for hypercalciuric children is sometimes complicated. There is theoretic concern about the potentially deleterious effect of citrate in raising urinary pH. Shen et al. found that oral supplements with potassium citrate (2 mEq/kg/day) for 5 days had marginal effect on urinary citrate levels, but did increase urinary pH [64]. An interesting resolution to this conundrum was proposed by Rodgers et al. who reported that the therapeutic effect of oral citrate may be due to formation of a pH-dependent soluble calcium/citrate/phosphate complex, and that clinical benefit is derived from the increase in urine pH, rather than from a change in citrate concentration [65].

Another well-established treatment for calcium stones is the use of a thiazide diuretic, sometimes with a potassium-sparing diuretic. The rationale for this therapy is to generate a mild volume deficit, which stimulates paracellular calcium reabsorption in the thick ascending limb of the loop of Henle. A thiazide diuretic consistently reduces urine calcium level in children [61, 66–68]. The RR of recurrent stones (3.3), episodes of hematuria (2.5) and osteopenia (3.0) are increased in those with hypercalciuria [68]. Interestingly, thiazide diuretics reduce stone

recurrence even when the pre-treatment urinary calcium is within the normal range [67, 69]. However, while hydrochlorothiazide (0.5 mg/kg bid) reliably decreases urinary calcium, long-term adherence is reported in only one third of patients and hypercalciuria recurs in 44% [68]. Furthermore, a thiazide diuretic may induce hypokalemia, requiring oral potassium supplements or the addition of a potassium-sparing diuretic.

Uric Acid Stones

The oldest known urinary tract stone was a bladder stone found in a 7000 year-old mummy in upper Egypt; the stone had a mixed composition, including uric acid [70]. About 11.7% and 7% of stones in adult males and females, respectively contain uric acid and it may be the dominant component, but it is often mixed with calcium salts [71]. In middle eastern regions, uric acid accounts for up to one third of stones [72], but only 1% in India [73].

On a typical Western diet, humans excrete about 10 mg/kg/day of uric acid, 50% derived from turnover of nucleic acids and 50% from the diet [70]. However, the dietary component can be doubled on diets containing high amounts of meat, fish and certain vegetables (e.g. asparagus, mushrooms) [70]. Since the pKa of uric acid is about 5.3, it tends to precipitate in acidic urine to form identifiable geometric crystals (Table 46.3). However, uric acid crystals easily dissolve at the higher urine pHs during the normal "alkaline tide" each day. Thus, it has been proposed that uric stones may form in those with sustained acidic urine, perhaps among individuals with blunted renal ammoniagenesis.

In children, pure uric acid stones are rare, and are seen mostly in settings where there is overproduction of uric acid such as tumor lysis syndrome, lymphoproliferative and myeloproliferative disorders, and genetic disorders (Lesch-Nyhan syndrome and glycogen storage disorders). However, mixed composition stones containing some uric acid are seen in 2–8% of children with nephrolithiasis and hyperuricosuria (>815 mg/1.73 m^2/day) (Table 46.4). Uric acid excretion is highest in infancy; pinkish-brown uric acid crystals may precipitate in the diaper and are often mistaken for blood, but are not associated with stone formation.

Children with uric acid-containing stones can be managed with oral citrate (0.5–1.0 mEq/day in divided doses) to maintain urine pH above 6.5, while also offsetting lithogenic calcium salt supersaturation. Sometimes an extra evening dose is required to manage overnight aciduria. In children with malignancy, use of a xanthine oxidase inhibitor (e.g. allopurinol or febuxostat) and recombinant rasburicase are remarkably effective, and have largely eliminated elevated uric acid as a cause of complications in tumour lysis syndrome.

Monogenic Forms of Nephrolithiasis

In a study of children (age at first stone <18 years) consecutively referred to a kidney stone clinic, patients were screened for 30 genetic disorders predisposing to kidney stones. In 20.8% of these cases, a causative mutant gene was identified. Thus, the work-up of pediatric patients presenting with urinary tract stones should include a focused effort to diagnose monogenic forms of nephrolithiasis.

Primary hyperoxalurias (incidence 1:120,000) are recessive, hereditary errors of glyoxylate metabolism, leading to overproduction and excessive urinary excretion of oxalate [74]. Hyperoxaluria is defined as a urinary oxalate excretion rate that is greater than 40 mg/1.73 m^2 per 24 h (Table 46.4), and is found in about 10–20% of children. The oxalate to creatinine ratio on a spot urine sample can also be used, but the normal values are age- and assay-dependent. Primary hyperoxaluria, type 1 (PH1), caused by biallelic mutations of *AGXT*, is the most common (80%), and about one third of these are responsive to oral pyridoxine (5–10 mg/kg/day). Excess oxalate in urine drives calcium oxylate crystallization within tubules, while elevated plasma oxalate causes tissue deposition in many tissues, including the renal parenchyma (nephrocalcino-

sis), leading to progressive renal insufficiency [75]. About one quarter of PH1 patients develop ESKD by 20 years of age. Most develop kidney stones in early childhood, and sometimes present with failure to thrive and stones in the first year of life. Those with milder mutations may be relatively asymptomatic until middle age. PH1 stones have a pale, yellowish surface, unlike the darker surface of those with idiopathic calcium oxalate stones.

PH2 (biallelic mutations of *GHPRH*) constitutes about 10% of the primary hyperoxalurias and is milder than PH1. PH2 patients usually excrete excess oxalate and glycerate in the urine [76]. PH3 (about 12% of hyperoxalurias), due to biallelic mutations of *HOGA*1, results from mutations of a mitochondrial enzyme that normally generates glyoxylate from 4-hydroxy-2-oxoglutarate (HOG). Although the pathomechanism is not entirely understood, PH3 urine has excessive oxalate and HOG [77].

Screening for the hyperoxalurias should be performed in all children with early onset nephrolithiasis. Normal urine oxalate/creatinine ratio ranges are age dependent (Table 46.4). Genetic diagnosis of primary hyperoxalurias involves mutation screening for the panel of *AGXT*, *GRHPR* and *HOAG1*.

Cystinuria is caused by mutations of the recessively inherited *SLC3A1* or the incompletely dominant *SLC7A9*, found in about 5% of children with renal calculi. These genes encode the two protein partners of the luminal reabsorptive transport mechanism for cystine and the dibasic amino acids in the renal proximal convoluted tubule. Cystinuria genotype can often be inferred from the level of urinary cystine in parents of affected children, since *SLC3A1* is recessive (urine cystine is normal in the heterozygote), whereas loss of one *SLC7A9* allele partially compromises cystine reabsorption (heterozygotes have moderate cystinuria).

Children with biallelic mutations of *SLC3A1* or *SLC7A9* excrete cystine in the range of 3000–5000 μmol half-cystine/g creatinine vs. normal levels of <150 μmol/g creatinine. Over 24 h, normal cystine excretion is <60 mg half-cystine/1.73 m^2/day. Microscopic hexagonal cystine crystals are pathognomonic. Crystal volume is thought to correlate with stone risk and commonly give rise to asymptomatic bladder debris visible on ultrasound. In one cohort, 1.1% required dialysis at a mean age of 35 years and 15.6% had an estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2 [78].

During the first 1–2 years, children who have heterozygous mutations in *SLC7A9* excrete cystine in the homozygous range, but with tubular maturation and elongation over the first 2 years, it decreases to an intermediate level, varying between 300 and 1500 μmol half-cystine/g creatinine. Although their urine cystine levels are usually below the threshold of solubility, *SLC7A9* heterozygotes may occasionally form stones in childhood during periods of dehydration. Halbritter et al. found that *SLC7A9* heterozygotes were the most common cause of nephrolithiasis among 101 pediatric stone-formers recruited from a stone clinic [79]; adult heterozygotes may comprise nearly half of the cystinuria patients in a stone clinic.

Pre-symptomatic children who excrete cystine in the stone-forming range (>1200 μmol/L) should be monitored carefully by annual ultrasonography and serial urine cystine/L measurements [80], but interventions are usually restricted to increased fluid intake (2–2.5 L/m^2/day) and avoidance of excessive salt intake. Among stone formers, alkalinization of the urine is usually initiated (particularly overnight), and some clinicians recommend low salt intake and limited animal protein diet. With frequent or obstructive stones, tiopronin (10–15 mg/kg/day in divided doses), which reduces free cystine, should be introduced. Malieckal et al. have shown in adults that a dose of 1 g/day reduces excretion of free cystine and increases cystine capacity of urine from −39 to +130 mg cystine/L [81].

Distal RTA is caused by dysfunction of the transport mechanism in alpha-intercalated cells of the cortical collecting tubule that pumps hydrogen ions (H^+) into the lumen and normally allows excretion of the daily metabolic acid load (about 2 mEq/kg/day). In adults, dRTA is seen with various autoimmune diseases (e.g. Sjögren

syndrome and systemic lupus erythematosus) and acquired tubular injury from drugs (e.g. lithium). In children, however, dRTA is usually the result of mutations in genes encoding subunits of the "vacuolar type" ATPase (*ATP6V1B1*, *ATP6V0A4*, *ATP6V1C2*) that secretes H^+ out of the cell across the apical membrane, the basolateral anion exchanger (*SLC4A1*) that pumps bicarbonate across the basolateral cell surface or the transcription factors that regulate the process (*FOX1*, *WDR72*) [31]. Mutations of *ATP6V1B1* and *FOX1* are associated with early deafness, while *ATP6V0A4* mutations cause later onset deafness. Heterozygous mutations of the *ATP6V1B1* [82] and *ATP6V0A4* have been linked to "incomplete" (mild) dRTA and kidney stones in adults, but this has not been described in children with nephrolithiasis.

dRTA presents in infancy, with normal anion gap, hyperchloremic metabolic acidosis and failure to thrive. Urine pH is inappropriately high (6–8) and urinary anion gap is inappropriately positive in the setting of a metabolic acidosis (reflecting absence of urinary $NH4^+$). Sustained acidosis causes dissolution of bone mineral and hypercalciuria. The acidosis also causes consumption of citrate by the mitochondrial citric acid cycle, so that urinary citrate levels are low. Thus, as the tubular fluid is progressively concentrated in the collecting ducts, when pH is high and citrate levels are low, urine becomes supersaturated with lithogenic calcium salts, particularly calcium phosphate (brushite). Brushite crystals are unusually large and may completely obstruct individual renal papillae, accounting for the characteristic medullary nephrocalcinosis on ultrasound. Crystals arriving in the renal calyces may coalesce to produce stones.

Given the pathogenesis of calcium phosphate stones in dRTA, management involves correction of acidosis, hypokalemia and hypocitraturia with potassium citrate 1–2 mEq/kg/day in 3–4 divided doses. In early infancy, slightly higher doses (2–3 mEq/kg/day) may be needed. In children with mild dRTA and erythrocyte abnormalities caused by heterozygous mutations of *SLC4A1*, lower doses of potassium citrate (0.5–1.5 mEq/kg/day) are needed.

Dent Disease is an X-linked recessive form of nephrocalcinosis and nephrolithiasis caused by mutations of *CLCN5* (Dent1, about 60% of cases) or *OCRL* (Dent2, about 15–20% of cases), causing endolysosome dysfunction in the kidney [83, 84]. Progressive proximal tubule damage leads to a partial Fanconi syndrome, with low molecular weight proteinuria in affected males, with some developing rickets. Hypercalciuria and nephrocalcinosis are common (99%) in young males, but only about 25% develop stones (calcium oxalate and calcium phosphate) in childhood [85]. Urinary oxalate and citrate are usually within the normal range [85]. About half develop ESKD between the third and fifth decades of life. Females may be mildly affected, but kidney stone and progressive renal insufficiency are uncommon.

The optimal treatment of Dent disease is unclear but, since hypercalciuria, nephrocalcinosis and nephrolithiasis are prominent early features, Raja et al. tested a combination of chlorthalidone and amiloride to treat a cohort of Dent disease patients. They found that the diuretics reduced hypercalciuria and decreased calcium phosphate and calcium oxalate supersaturation by 35% and 25%, respectively [86]. However, it is unclear whether this strategy can slow the progressive loss of GFR [86].

Bartter Syndrome, a recessively inherited disorder, is caused by mutations in genes (*SLC12A1*, *KCNJ1*, *ROMK*, *CLCNKB*, *BSND*) that cause dysfunction of the apical transport mechanism for sodium, potassium and chloride in the thick ascending limb of the loop of Henle (TALH). Massive salt and water loss may cause polyhydramnios, hyponatremia and failure to thrive in infancy and hyperreninemic hypokalemic alkalosis due to compensatory mechanisms in the distal nephron. Since TALH dysfunction causes hypercalciuria by blocking paracellular reabsorption of calcium, Bartter syndrome patients may develop stones in childhood, but this is rare. Halbritter found no mutant Bartter genes in their screen of 106 serial children with nephrolithiasis recruited from specialized stone clinics [79]. However, nephrocalcinosis is common.

Adenine Phosphoribosyl Transferase Deficiency is due to biallelic mutations of *APRT*, which causes recurrent stones from high urinary levels of 2,8 dihydroxy adenine (DHA) and progressive renal insufficiency [87]. The stones are radiolucent and urinary crystals have a Maltese cross pattern [88]. Excessive DHA production is treated with xanthine oxidase inhibitors (allopurinol and febuxostat) and these therapies can reduce new stone formation [89]. Patients with mistargeting mutations are often responsive to pyridoxal phosphate (10 mg/kg/day).

Idiopathic Infantile Hypercalcemia is a syndrome of 1,25-dihydroxy vitamin D excess caused by biallelic mutations of *CYP24A1* (encoding a key enzyme of the renal vitamin D degradative pathway) or *SLC34A1* (encoding the sodium-phosphate co-transporter, NaPi2). Infants with *CYP24A1 mutations* present with hypercalcemia due to 1,25-dihydroxy vitamin D excess, failure to thrive, episodes of vomiting and dehydration and sterile pyuria. Initial treatment usually involves strategies to limit intestinal calcium absorption such as prednisone (0.5–2.0 mg/kg/day) to suppress vitamin D receptor expression in the small intestine, oral phosphate supplements (25–50 mg phosphate/kg/day) to bind dietary calcium. Oral supplements of vitamin D should be discontinued. As a longer-term strategy, bisphosphonates can be used to limit the flux of calcium from bone. Infants usually show marked clinical improvement and normal weight gain once serum calcium is normalized. Calcium stone formation presumably correlates with normalization of urinary calcium excretion, but this has not been formally studied. Interestingly, the need for medical therapy tends to diminish in the first years of life, although the mechanism is unknown.

Hereditary hypophosphatemic rickets with hypercalciuria (HHRH) is caused by biallelic mutations of *SLC34A3*, which encodes the sodium-phosphate co-transporter NPT2c in the renal proximal tubule. Infants present with metabolic bone disease resembling X-linked hypophosphatemic rickets (hypophosphatemia, bowing of legs) but with higher levels of 1,25-dihydroxy vitamin D. The latter drives hyperabsorption of dietary calcium, suppression of PTH and hypercalciuria. About one third of these patients develop calcium phosphate (brushite) stones [90]. Treatment usually consists of oral phosphate supplements to repair bone (without oral vitamin D as would be given in X-linked hypophosphatemic rickets) but the long-term effect on nephrolithiasis has not been studied.

Stones and Structural Abnormalities of the Urinary Tract

Conditions associated with urinary stasis cause crystal and stone formation, and include primary megaureter, polymegacalicosis, medullary sponge disease, autosomal dominant polycystic kidney disease, ureteropelvic junction obstruction, ureterocele, horseshoe kidney, bladder exstrophy, neuropathic bladder and surgically reconstructed or augmented bladders. Polymegacalicosis is a congenital abnormality with a higher number (more than 12) and larger in size calyces, and can be isolated or part of a syndrome.

Stones and Urinary Tract Infection

UTIs occur in about 1/4 of children with nephrolithiasis and may be either the cause or the effect of a kidney stone. The distinction between the two is important for further management, but can be difficult to distinguish, but the stone analysis may provide helpful clues. Usually, the stone associated with infection has a mixed composition, with the surface made of struvite and the core containing calcium-oxalate. In contrast, the stone induced by infection (so-called infectious or triple-phosphate stone) is entirely made of struvite (magnesium ammonium phosphate). The infectious stone is commonly seen in boys younger than 5 years of age with obstructive uropathy (e.g. ureteropelvic junction obstruction, urethral valves, primary megaureter). Urease

produced by bacteria such as Proteus, Klebsiella, Pseudomonas, and enterococci metabolizes urea into ammonium and bicarbonate. This creates a favorable milieu for struvite stones, which can further grow into the renal calyces, and produce "staghorn" calculi with high morbidity. It is important to note that patients with struvite stones and negative urine culture should be evaluated for Ureaplasma urealyticum infection. Other patients prone to struvite stones are those with a neurogenic bladder.

An important consideration in children with urinary tract stones and urinary tract infections is that organisms within the stone may produce a biofilm that excludes certain antibiotics. Therapeutic options include a protracted course of an antibiotic, an antibiotic with good biofilm penetration and aggressive efforts to remove the stone.

Drug-Induced Nephrolithiasis

Drug-induced nephrolithiasis is uncommon in children, and causes 1–2% of stones, but its recognition is important for optimal management. Besides the specific drug, other contributory risk factors include higher drug dose, prolonged duration of treatment, poor hydration, the urine pH (which will affect the drug solubility), and the age of the patient (younger patients are at higher risk).

The responsible drugs are classified based on the mechanism involved in stone formation. The first category involves poorly soluble drugs that directly or through their metabolites induce urine supersaturation and increase the risk of crystallization. More common examples are triamterene (used in the treatment of Liddle syndrome), indinavir (used in HIV treatment) and antibacterial drugs, including third-generation cephalosporins (such as ceftriaxone and cefotaxime), sulfa drugs, nitrofurantoin, broad-spectrum penicillins, and fluoroquinolones. Usually these stones are radiolucent and therefore cannot be seen on plain X-rays unless they also contain calcium. The second category are drugs that cause metabolic changes in the urine, such as furosemide, acetazolamide, topiramate, zonisamide and allopurinol. Furosemide causes inhibition of calcium reabsorption and hence hypercalciuria, while acetazolamide inhibits the proximal tubular reabsorption of bicarbonate leading to metabolic acidosis, alkaline urine and hypocitraturia. Anticonvulsants such as topiramate and zonisamide have carbonic anhydrase inhibitory activity causing acidosis that leads to bone resorption and hypercalciuria, as well as hypocitraturia. Allopurinol increases urinary excretion of xanthine, which has low urinary solubility (Fig. 46.2).

Intoxication with ethylene glycol following accidental or intentional ingestion of antifreeze leads to excessive production of oxalic acid causing calcium oxalate deposition in the kidneys and resulting in acute renal failure. Other substances reported to cause similar problems include xylitol (a constituent of parenteral nutrition), methoxyflurane (anesthetic agent), ascorbic acid, piridoxilate, and food high in oxalate (carambola or start fruit, sorrel and rhubarb).

Specific Pediatric Populations at Risk for Nephrolithiasis

Premature and low birth weight infants have an increased risk for nephrolithiasis and nephrocalcinosis for several reasons: metabolic disturbances related to parenteral nutrition (hypercalciuria and hyperoxaluria), use of loop diuretic (e.g. furosemide) and nephrotoxic drugs [25], and renal tubular immaturity causing changes in urine composition. About half of these patients show spontaneous resolution of nephrocalcinosis within several years, although a few patients may have persistent hypercalciuria.

Inflammatory bowel disease and other diseases of the gastrointestinal tract associated with malabsorption can cause metabolic disturbances leading to stone formation. These patients can develop hyperoxaluria (due to increased enteric

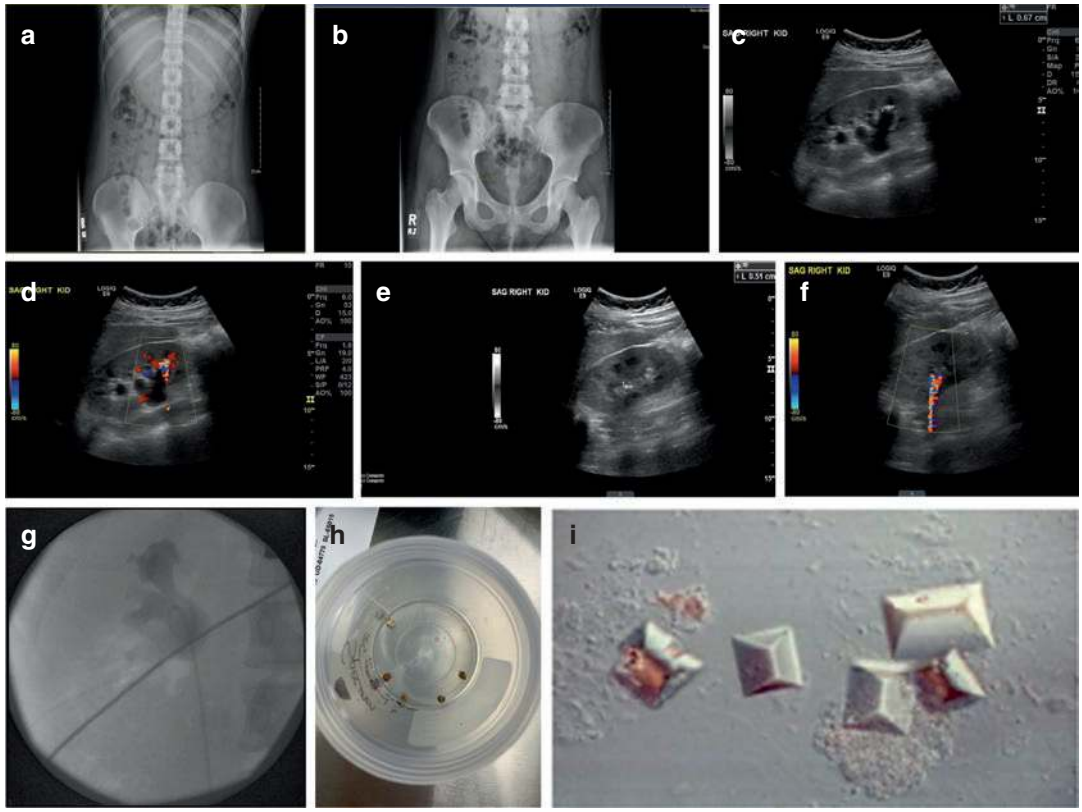


Fig. 46.2 Plain X-ray in an 18-year-old girl showing a 5 mm calcific density overlying the mid to lower pole of the right kidney consistent with calculus (**a**), and a 4 mm calcific density in the pelvis on the right side presumably in the distal ureter or bladder (**b**). Renal ultrasound showed moderate hydronephrosis of the right collecting system (**c**) and a 0.67 cm in the lower pole (**c**). Color ultrasound demonstrated acoustic shadowing behind the stone, creating the "twinkle artifact" (**d**). An additional stone was noted in the mid-polar region of the right kidney (**e**, **f**). Despite right hydronephrosis, no ureteral stones were detected by ultrasound. However, cystoscopy confirmed that there was a right obstructing ureterovesical junction stone and this required ureteral stent placement (**g**). Retrieved stones were composed of 80% calcium phosphate and 20% calcium oxalate (**h**). Urinalysis showed triple phosphate crystals (**i**). (**a**) A 5 mm calcific density overlying the mid to lower pole of the right kidney consistent with calculus (arrow). (**b**) A 4 mm calcific density in the pelvis on the right side presumably in the distal ureter or bladder (arrow). (**c**) Stone measuring 0.67 cm in the lower pole of the right kidney and right hydronephrosis. (**d**) Acoustic shadowing behind the stone creating the "twinkle artifact". (**e**) Stone measuring 0.51 cm in the midpole of the right kidney. (**f**) Acoustic shadowing behind the stone creating the "twinkle artifact." (**g**) Cystoscopy demonstrating placement of a right ureteral stent with its proximal pigtail in the region of the right renal pelvis, and the injection of contrast into a dilated right renal collecting system. (**h**) Macroscopic appearance of the surgically removed stones that showed mixed calcium phosphate and calcium oxalate composition. (**i**) Triple phosphate crystals on contrast microscopy (160× magnification)

absorption of oxalate), decreased urine citrate and hypomagnesuria (due to stool loses of bicarbonate and magnesium), hyperuricosuria (due to increased cell turnover), and low urinary volume induced by diarrhea. Patients with cystic fibrosis can develop calcium oxalate nephrolithiasis and nephrocalcinosis. Uric acid stones may also form due to acidic urine.

Patients with neurological disease represent another category at risk for renal stones. Risk factors in this population include reduced fluid intake, medications that predispose to stones (anticonvulsants), ketogenic diet used for the treatment of seizures, and decreased or absent ambulation.

Obesity has been associated with lower urine pH and volume, and increased urinary excretion

of uric acid, oxalate, sodium and phosphate, indicating that these children may be at higher risk for renal stones in general and uric acid stones in particular. Additionally, insulin resistance causes impaired renal ammonia production. While some studies found an association between body mass index and pediatric nephrolithiasis, others believe that additional lithogenic risk factors are necessary in order to develop a stone [5, 11, 91].

Management

Dietary Considerations

High intake of fluid, vegetables and fruit, and salt restriction should be recommended in all patients with nephrolithiasis regardless of cause [92]. Aggressive fluid intake at 1.5 times "maintenance" is aimed to prevent tubular precipitation of renal stone promoters. Targeted fluid goal can be calculated using either body surface area (minimum 2 L/m^2) or desired age-related daily urinary volume: infants ≥750 mL; children below 5 years of age ≥1000 mL; children 5–10 years of age ≥1500 mL; children >10 years of age ≥2000 mL. Water should be encouraged because other beverages may contribute to increased calorie intake, an undesired effect in overweight children. Additionally, the fructose found in sugary drinks could further enhance the stone risk by increasing urinary excretion of calcium and oxalate.

High intake of fruit and vegetables is desirable in all patients because they represent a good source of potassium (which facilitates urinary citrate excretion) and phytates (which increase calcium salt solubility). Sodium should be restricted to <3 mEq/kg/day. Patients should be advised to avoid adding salt or sodium-rich seasoning to food during preparation or consumption. Families should be educated to read food labels, and to choose food with low sodium High-salt foods such as processed and canned food, fast food, pickles and olives, salt crackers and pizza should be avoided.

Restriction of calcium and protein intake is not recommended in children because they are both needed for growth and bone health, and should be consumed at 100% of the daily allowance. Moreover, calcium binds to free oxalate in the digestive tract and prevents hyperoxaluria. If not consumed with meals, calcium supplements may increase the risk of calcium oxalate stones. Vitamin D should be monitored and supplemented if low.

Urinary alkalinization to achieve a pH >7.0 can be achieved with oral potassium citrate or high lemon extract intake ("Lemon Protocol") and is useful in patients with dRTA, hypercalciuria, hyperoxaluria, hyperuricosuria, hypocitraturia and cystinuria. This can be achieved with a diet rich in whole grains and with cranberry extract. However, high intake of lemon or cranberry may not be well tolerated by children leading to non-compliance.

The renal dietitian has a key role in identifying the nutritive risk factors for nephrolithiasis, which is important for both treatment and prevention of stone recurrence. Patients should be asked to complete a dietary diary at the same time as the 24-h urine collection. The diary should include the type and amount of each consumed food and drink. Disease-specific dietary considerations are addressed in Table 46.6.

Surgical Treatment

Surgical management is indicated for larger, obstructing and infected stones, and in those who develop sepsis, acute kidney injury and have pain refractory to analgesics. A retrospective pediatric study of 129 patients found it to be extremely rare for stones >5 mm to pass spontaneously [23]. Despite revolutionary advances in pediatric endourological techniques and the development of small endoscopic instruments such as Miniperc and Microperc, surgical management of stones continues to pose technical challenges and requires a skilled pediatric urologist. A shift in the surgical approach has been noted in recent years. While the majority of stones were treated by open surgery in the past, now this is mainly reserved for patients with

Table 46.6 Dietary and pharmacologic intervention in pediatric nephrolithiasis

Condition	Dietary	Pharmacologic
Hypercalciuria	• High fluid intake • Restricted sodium • High potassium diet • RDA for calcium intake • Moderate animal protein • High fiber and low in oxalate	• Hydrochlorothiazide (1–2 mg/kg per day, older children 25–100 mg/day)-may need potassium supplementation if hypokalemia occurs • Potassium citrate in dRTA (2–4 mEq/kg/day, older children 30–90 mEq/day)
Hypocitraturia	• High fluid intake • RDA for animal protein intake • High lemon intake • High in fruit and vegetables	• Potassium citrate (2–4 mEq/kg/day, older children 30–90 mEq/day)
Hyperoxaluria	• Very high fluid intake (>3 L/1.73/m^2/day) • Moderate oxalate restriction (avoid spinach, nuts) • High magnesium and potassium • Low-fat diet • Avoid excessive vitamin C • RDA for calcium intake	• Potassium citrate (2–4 mEq/kg/day, older children 30–90 mEq/day) • Pyridoxine (8–10 mg/kg per day) for primary hyperoxaluria • Neutral phosphate (25–30 mg/kg/day of elemental phosphate divided into 3–4 doses) • Magnesium
Hyperuricosuria	• High fluid intake • RDA for animal protein intake • Restricted sodium • Avoid red meats	• Potassium citrate (2–4 mEq/kg/day, older children 30–90 mEq/day) • Allopurinol (4–10 mg/kg/day, older children 300 mg per day)[a]
Cystinuria	• Very high fluid intake (day and night) • Low protein diet (avoid excessive consumption of eggs, fish, meat, cheese)	• Potassium citrate (2–4 mEq/kg/day, older children 30–90 mEq/day)[b] • Alpha-mercapto-propionyl-glycine (Thiola) (10–15 mg/kg/day) • D-Penicillamine (30 mg/kg/day divided in 4 doses) • Captopril (0.5–1.5 mg/kg/day divided in 4 doses)

Abbreviations: *RDA* recommended daily allowance, *dRTA* distal renal tubular acidosis
[a] Reserved for children with a known disorder of uric acid metabolism
[b] Dose targeted to achieve a urine pH equal to or above 7.0

associated congenital renal anomalies requiring anatomical repair combined with stone removal (such as ureteropelvic or ureterovesical junction obstruction), for infected and staghorn stones, and for patients with previous multiple abdominal surgeries (augmented bladder with large bladder calculus).

Various minimally invasive surgery (MIS) techniques have been developed and are increasingly being used. These include extracorporeal shock wave lithotripsy (ESWL), ureteroscopy (URS), percutaneous nephrolithotomy (PCNL), and laparoscopic and robotic surgery (pyelolithotomy and nephrolithotomy). Their specific indications, advantages and complications are presented in Table 46.7.

MIS allows faster recovery and less pain than open surgery, but has an increased risk for the need for repeated procedures.

The choice of surgical intervention depends on the child's age and body habitus, stone characteristics (composition, size, and location), number of calculi (single vs. multiple), presence of obstruction or infection, the anatomy of the urinary tract, associated conditions (bladder dysfunction), patient or family preference, and the surgeon's experience and skills. Careful patient selection for the best choice of MIS ensures a better outcome for stone treatment and renal preservation as well as increased safety. Complete stone clearance is crucial for the prevention of stone recurrence, known to be high in children [8, 93, 94]. The remaining stone fragments can facilitate bacteria growth and become a nidus for a new struvite stone. Each MIS technique can cause gross hematuria, UTI or urosepsis, renal subcapsular hematoma, renal parenchymal injuries and injury to surrounding structures.

Table 46.7 Surgical management of pediatric nephrolithiasis

Indications and advantages	Specific complications
Extracorporeal shock wave lithotripsy First line treatment of small renal and proximal ureteral stones<1.5 cm Non-invasive outpatient approach High safety, minimal morbidity Overall success range 81–96%	Ureteric obstruction with stone fragments causing pain Debate on the long-term effect on the kidney development
Ureteroscopy/retrograde intrarenal surgery Best choice for stones located in the lower pole calices (<1.5–2 cm) and for mid- or distal ureteral stones (<1 cm) Overall success rate 47–100% Good visualization due to their fiber optic and video systems Wide range in size scopes 4.5–12 Fr, allowing use in children of all ages and sizes The stone can be directly extracted (using basket or grasping forceps), or can be fragmented (by laser or electrohydraulic or ultrasonic probes)	Shorter operative time and in-hospital stay due to less invasive approach Ureteral injury, which may be prevented by a double stent placement for 2–3 weeks before the treatment to induce passive ureteral dilatation
Percutaneous nephrolithotomy Kidney stones >1.5 cm Staghorn stones Overall success range 67–100%	Longer operative time and in-hospital stay due to more invasive approach Hemorrhage (0.4–23.9% cases) (can be severe requiring blood transfusion) Can be difficult in patients with obesity, hemorrhagic diathesis and renal tumors Renal pelvis perforation
Laparoscopic and robotic surgery (pyelolithotomy and nephrolithotomy) Hard stones difficult to fragment (staghorn calculi, stone in calyceal diverticulum) When simultaneous reconstruction and repair is needed (concurrent ureteropelvic junction obstruction) Stones in ectopic kidney Failed previous endourological procedures; stones containing gas Excellent clearance rate up to 96% due to complete stone removal without fragmentation Reduced need for repeat procedures It can be done retroperitoneally Improving suturing and reconstruction	Shorter operative time and in-hospital stay due to less invasive approach Lower rate of bleeding and sepsis

Extracorporeal Shock Wave Lithotripsy

ESWL is usually successful in children and is becoming the first-line intervention in many centers. The likelihood of success depends on stone size (lower with larger stones), stone location (poorer with calyceal stones located in the lower pole compared to those located in the upper and mid-pole), stone composition (lower with struvite, cystine and calcium oxalate monohydrate), and the number of previous ESWL sessions. Struvite fragments in particular are friable facilitating stone fragment retention. Decreased urine output in CKD may also compromise the stone clearance. Success rates are lower in children with abnormal anatomy of the urinary tract or obesity.

During the procedure, it is important that the shock waves pass into the body and hit the stone with minimal loss of energy. New generation instruments that use a smaller focal area and provide less energy have been developed. The shock waves fragment the stones and the patient passes fragments spontaneously, although some need a stent placed at the time of ESWL. An ESWL method called ramping decreases the risk of complications. It starts with low energy shockwaves, followed by a stepwise increase in the power. This allows the surgeon to find the lowest energy level at which a stone starts to disintegrate, therefore avoiding overtreatment and decreasing the risk of tissue injury and bleeding. Repeated ESWL may be needed for stones >1.5 cm, and nomograms and scoring systems

for prediction of outcomes after ESWL have been developed [93, 95, 96]. Younger children require the insertion of a ureteral stent. One major concern with ESWL use is the potential for damaging the developing kidney. In a small study of 16 children, no significant changes in serum creatinine and kidney morphology were found on ultrasound imaging post ESWL. Subsequent studies showed no significant impairment in kidney growth, loss of kidney function or the development of parenchymal scarring or hypertension after ESWL/PCNL. However, transient enzymuria and elevated urinary β2-microglobulin indicating proximal renal tubular dysfunction can be found post ESWL, but resolves 14 days following the procedure [46] (Fig. 46.3).

Stones >1.5–2 cm are not usually amenable to ESWL, and will require other surgical techniques including PCNL, URS/retrograde intrarenal surgery (RIS) and robotic-assisted pyelolithotomy (RPL) [97]. Nephrectomy may be a consideration if renal function is markedly decreased, in association with a large stone burden and recurrent UTIs. Infected stones require careful antibiotic treatment because bacteria may be released during stone fragmentation following any surgical procedure. Bladder stones are managed endoscopically via the urethra (per-urethral cystolithotripsy), or via percutaneous cystolithotripsy. Open surgery may be needed in patients with reconstructed urinary bladders (Fig. 46.4).

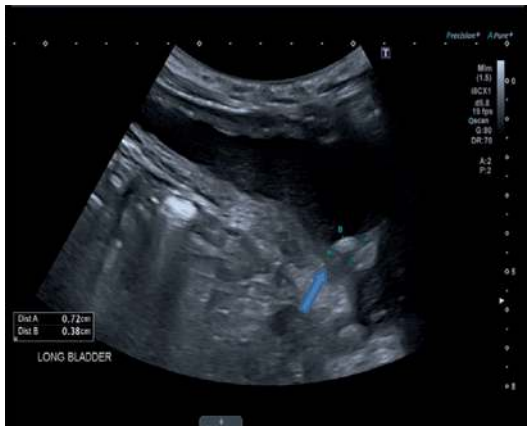


Fig. 46.3 Real time gray scale ultrasound of the urinary bladder in a 20-year-old male with history of neurogenic bladder. Echogenic debris is noted within the bladder. A shadowing calculus that measures approximately 0.7 cm is noted within the dependent portion of the urinary bladder (arrow)

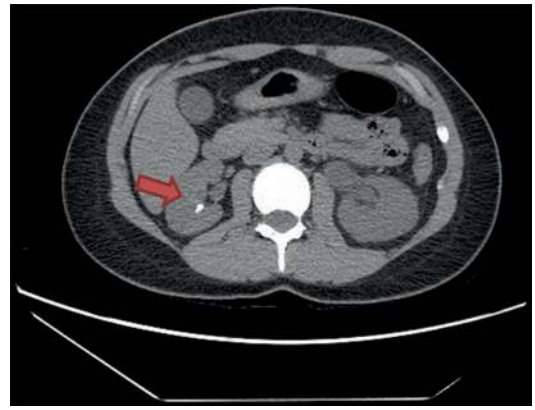


Fig. 46.4 CT imaging of stone: Non-obstructive 5 mm radiopaque calculus is demonstrated at the inferior polar region of the right kidney

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Voiding Disorders in Children

47

Johan Vande Walle and Søren Rittig

Abbreviations

AVP	Arginine vasopressin
CAKU	Congenital abnormalities of kidney and urinary tract
CKD	Chronic kidney disease
FBC	Functional bladder capacity
LUTS	Lower urinary tract symptoms
MNE	Monosymptomatic nocturnal enuresis
MVV	Maximal voided volume
NE	Nocturnal enuresis
OAB	Overactive bladder
PD	Pharmacodynamic
PK	Pharmacokinetic
PLMS	Periodic limb movements during sleep
UTI	Urinary tract infection
VCUG	Voiding cystourethrography
VUR	Vesicoureteral reflux

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Introduction

Wetting either the bed or pants is still one of the most feared events among children, especially when school age is reached, resulting in poor self-esteem and burden for child and parents [1–4]. It is one of the most common chronic disorders of childhood [5, 6]. Despite the high prevalence and negative psychological consequences, enuresis as a disease has not been of much interest to medical doctors, and there is little good clinical research, leading to a deficiency in medical training and absence of the subject in pediatric nephrology courses and handbooks [7–9]. Consequently, in most areas of the world the perception of pathophysiology and treatment are too simplified and guidelines insufficient, all resulting in a variety of non-evidence based approaches [10–16].

Voiding problems are often not benign, self-limiting conditions restricted to delayed acquirement of continence (daytime incontinence) and enuresis (bedwetting), but play a major role in many conditions in the interface between paediatric nephrology and urology. Voiding disorders include a complexity of conditions in children, caused by abnormalities in bladder function and diuresis. Bladder dysfunction is a well-known pathogenic or comorbid factor in urinary tract infections (UTIs) [17–19], in many congenital anomalies of the kidney and urinary tract (CAKUT) (uropathies) [20], constipation [21,

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F. Schaefer, L. A. Greenbaum (eds.), *Pediatric Kidney Disease*,
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22] and neurogenic bladder [22–25]. The prevalence and importance of bladder dysfunction in children with chronic kidney disease (CKD) prior to and after transplantation is underestimated [26–28]. It is associated with hypertension, sleep disturbances, and cognitive dysfunction. Deficient concentrating capacity as well as circadian rhythm abnormalities of renal function, have been documented in patients with enuresis, but are also an essential characteristic of glomerular as well as tubular renal pathophysiology.

The International Children's Continence Society (ICCS) has standardized both the terminology and management of various aspects of incontinence in children, including enuresis, bladder overactivity, dysfunctional voiding, and psychological comorbidities. A number of guidelines have been published to aid those involved in the care of children with lower urinary tract (LUT) symptoms [8, 23, 29–35].

Nocturnal Enuresis

Definition and Epidemiology

Nocturnal enuresis (NE) is defined as intermittent incontinence that occurs exclusively during periods of sleep. Enuresis can be qualified as frequent (≥4 per week) or infrequent (<4 per week) [32, 36]. There is ample evidence that children with NE and concomitant symptoms of LUT dysfunction differ clinically and therapeutically from children without daytime symptoms [32, 34, 36, 37]. There is less evidence that the pathogenesis and genetic predisposition are different [38].

However, since enuresis is a common disorder and many patients can benefit from simple therapies (desmopressin, alarm and urotherapy), a strategy has been developed to identify patients who can be treated with a high success rate in primary care, thereby avoiding unnecessary exposure to secondary and tertiary care [2, 32, 36].

Enuresis without other LUT symptoms (nocturia excluded), and without bladder dysfunction, is defined as *monosymptomatic enuresis*. Children with enuresis and any LUT symptom have *non-monosymptomatic enuresis* (Fig. 47.1). Subtyping of NE in this manner is promoted in all guidelines, and helps direct the patient to the right therapy [33]. The rationale for this approach is the observation that non-monosymptomatic enuresis has a lower response rate to first line treatment with desmopressin, although the evidence is weak. Once daytime LUT symptoms have abated, the enuresis can be reclassified from the non-monosymptomatic to the monosymptomatic subgroup [33, 34] (Fig. 47.1). It is unclear if other LUTS symptoms beyond daytime incontinence have the same negative predictive value for therapy response.

In *primary* NE, the child has never been dry for a period longer than 6 months, whereas in *secondary* NE there has been such a period. Most

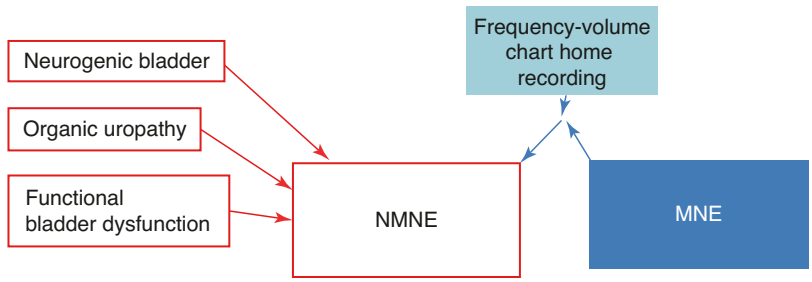

Fig. 47.1 Nocturnal enuresis can be subdivided into monosymptomatic enuresis (in absence of daytime symptoms) and non-monosymptomatic enuresis, when daytime symptoms (LUTS) are present. Children with daytime symptoms likely have underlying bladder dysfunction, and in the differential diagnosis, we should always exclude congenital uropathies and neurogenic bladder dysfunction, before considering it as functional bladder dysfunction

countries regard NE as pathological when it is present after the age of 5 years, but in practice it may be appropriate to delay treatment to 6–7 years of age. Although major importance was previously given to differences between primary and secondary enuresis, where secondary was more related to psychological problems [39, 40], there is an increase in the view that the diagnostic and treatment algorithm do not differ between the two subtypes.

NE is the most frequent disease in children after asthma, with a prevalence of 5–10% at the age of 7 years and of 0.5–1% during adulthood [5, 6, 9, 41–49]. More boys than girls have NE, although this difference tends to diminish after the age of 10 years. The spontaneous cure rate has been estimated to be approximately 15% annually between the age of 5 and 19 years, but is much lower in children with frequent bedwetting, making a 'wait and see' attitude unacceptable [5, 6].

Although only 15–30% of enuretic children were reported to experience daytime incontinence [8, 49] and were labelled non-monosymptomatic according to the old ICCS definition [50], a recent epidemiological, population-based study analyzed children aged 7.5 years and found that 15.5% wet the bed and 2.6% had a frequency of 2 or more wet episodes per week [51]. Of those children with 2 or more wet nights per week, 68.5% were classified as monosymptomatic and 31.5% as non-monosymptomatic. Other studies have estimated that more patients have subtle daytime symptoms of bladder dysfunction such as urgency or decreased or increased voiding frequency and thus qualify for the label non-monosymptomatic NE (NMNE) [34], leaving the subgroup of enuretic children who are truly monosymptomatic to less half of all bedwetting children [52–54]. The majority of guidelines and standardisation papers recommend to differentiate between MNE and NMNE, with the major aim to treat concurrent LUT symptoms before the enuresis. Thus, it has been documented that NMNE patients who have been resistant to desmopressin treatment become desmopressin responders after successful treatment of daytime LUT symptoms [52]. The prevalence of specific subtypes of NE is still unclear, since most of the available epidemiological studies either did not differentiate between MNE and NMNE, or used the previous pre-2006 ICCS definitions. After inclusion of all daytime LUT symptoms as qualifiers for NMNE, 30–50% of previously labeled MNE patients would be classified as NMNE [32, 55].

Pathophysiology

The pathophysiology of enuresis is complex, involving the central nervous system, circadian rhythm regulation (sleep, arousal, hormones), the kidney (diuresis, renal function), and bladder function disorders. It is widely accepted that the central nervous system, and especially dysregulation in control of biorhythms and bladder capacity, plays a major role. Several neurotransmitters and receptors may be involved. There is also a strong correlation with a variety of comorbidities, where it remains to be elucidated if there might be common pathways in pathogenesis and reciprocal effects (constipation, sleep, psychology, neurocognitive functions) [33].

NE in the majority of patients is caused by a mismatch between nocturnal diuresis and functional bladder capacity overnight in the presence of a deficient arousal mechanism [8, 56, 57]. Although some authors have proposed decreased arousability to be a primary mechanism in enuresis, direct evidence for this is rather week since only a minority of children (4.1%) acquire continence by waking them during the night [49]. Multiple mechanisms, discussed below, are involved in the pathogenesis.

Nocturnal Urine Production

In humans, a marked circadian rhythm of urine production is present from early childhood, with a pronounced nocturnal reduction in diuresis to approximately 50% of daytime levels [58–65]. This nyctohemeral rhythm of diuresis in children is controlled by increased nocturnal release of hormones that regulate free water excretion (arginine vasopressin, AVP) [58] as well as solute

excretion (renin, angiotensin II, aldosterone, and atrial natriuretic peptide). These mechanisms are associated with multiple other circadian rhythms: sleep, melatonin, and blood pressure. Circumstances such as light/dark, sleep/awake, activity/rest, body posture, fluid and food intake, and temperature as well as several drugs may interfere with the circadian rhythm of diuresis [66–70]. Interestingly, acute sleep deprivation results in elevated blood pressures and increased solute and water excretion overnight [71].

The initial description by Poulton in the 1950s that children with NE have significantly larger nocturnal urine productions than non-enuretic children [64] has been confirmed by multiple authors [58, 59, 65, 72–74]. Consensus has been obtained that the optimal method of measuring this variable is diaper weighing and measurement of morning voided volume [33]. Nocturnal diuresis has significant night-to-night variation, making nocturnal polyuria only present intermittently [75]. The demonstration that nocturnal urine volume is significantly larger on wet nights compared with dry nights has emphasized that nocturnal polyuria should only be evaluated during nights where enuresis is experienced [76]. Patients with nocturnal polyuria are likely to be desmopressin responders, in absence of associated bladder dysfunction [59, 77–79]. Nocturnal polyuria is defined as nocturnal diuresis >130% of expected bladder capacity for age ((expected bladder capacity = age + 1)*30 mL)

One NE archetype is the patient without an increase in plasma arginine vasopressin (AVP) level during the early night, correlating with the occurrence of increased nocturnal diuresis (nocturnal polyuria) with low urinary osmolality as well as a good response to the AVP analogue desmopressin. Whether this deficit in AVP is always the primary cause or if it is in some cases secondary remains to be elucidated.

There is, however, growing evidence from studies in children who are refractory to desmopressin that the pathogenesis of nocturnal polyuria might be much more complex. Increased solute and sodium excretion overnight has been documented, both in absolute values, as a loss of circadian rhythm in a subpopulation of children with nocturnal polyuria and is probably multifactorial [80–83]. High osmotic load, especially in the evening, is associated with high diuresis rate overnight, but whether dietary intervention would be beneficial in such patients remains to be elucidated [82, 83]. Indirect evidence may have been provided by reports of an association between enuresis and metabolic syndrome and obesity. The increased sodium and osmotic excretion overnight may be caused by increased osmotic load (due to increased caloric intake) as well as abnormal circadian rhythm of osmole excretion [84–86].

Vasoactive hormones also play a potential role in NE pathogenesis, but results have so far not been convincing except for prostaglandins (PGs). Kamperis et al. found an abnormal circadian rhythm of urine PGs. Intervention with a PG inhibitor was, however, not superior to placebo [65, 80, 87]. Increased sodium excretion overnight may be secondary to increased sodium retention during daytime [81], a mechanism described in adults with nocturia. There is certainly a continuum between the pathogenesis of enuresis in adolescence and nocturia in adulthood [88–90]. The role of hypercalciuria has been overestimated, and is secondary to nutritional intake and renal sodium handling [81, 91–93].

Many pharmacokinetic (PK) and pharmacodynamic (PD) studies with desmopressin demonstrate that only 75% of enuresis patients reach >850 mosmol/L on therapeutic desmopressin doses, which is clearly a lower percentage than in control studies. This does not indicate a renal diabetes insipidus diagnosis, but might reflect the lower end of the normal spectrum, where a 20% loss of maximal concentrating capacity results in a 20% higher diuresis rate (the difference between 300 and 360 mL, or possibly between a dry and wet night) [94–96]. Since none of the studied PK/PD enuresis populations received intravenous vasopressin to study their maximal concentrating capacity, the question remains open if a subpopulation of enuresis patients have suboptimal ability to maximally concentrate the urine.

Glomerular dysfunction and specifically an abnormal circadian rhythm of glomerular filtra-

tion rate (GFR) might also play a pathogenic role. In healthy children and young adolescents, GFR decreases overnight by approximately 25%, a phenomenon that is absent in a subgroup of refractory enuresis patients [97]. This observation is in line with the decreased nocturnal dipping of blood pressure in children with nocturnal polyuria. The relationship between hypertension and absent circadian rhythm of blood pressure and nocturnal polyuria is well-known in adults and renal transplant patients [28, 70].

Bladder Function

Bladder function comprises a storage phase and a micturition phase. Micturition in infants and young children, until the age of 2–4 years, is involuntary. The functional bladder volume increases with age, and reaches a maximum at teenage. This maturation requires intact pathways in the central, pontine, spinal, and peripheral nervous systems. The innervation of the bladder is complex, with an interplay between the voluntary motor system and the ortho- and parasympathetic nervous systems.

The pathogenic role of functional abnormalities of the bladder in the pathogenesis of daytime LUTS and NMNE is accepted, but remains unclear in children with MNE. Organic abnormalities and neurogenic bladder are rare in children presenting with MNE, but should always be considered in the presence of daytime symptoms, history of UTI or enuresis refractory to conventional therapy.

In MNE, most evidence points towards a deficient development of nocturnal bladder reservoir function, at least in a subgroup of patients. Nocturnal bladder capacity in normal children is significantly larger than daytime capacity, probably due to inhibitory effects of sleep on the micturition centers. Nocturnal bladder capacity is not easy to measure in enuretic patients, even with diaper weighing, as many enuresis episodes are incomplete voids associated with significant residual urine [98]. However, when estimated as maximal voided volume (MVV) during daytime and excluding the first morning void, daytime bladder capacity is also reduced in many enuretics, even in MNE patients. Clinically, an estimate of daytime bladder capacity is relatively easy to obtain and it has been shown to be of value when selecting a treatment modality in the individual patient. Thus, a MVV that is below 70% of the predicted MVV for age (age × 30 + 30 mL) has been shown to predict a poor response to desmopressin treatment [99–101]. This corroborates very closely the fact that alarm treatment, which that is highly effective in this patient subgroup, increases nocturnal bladder capacity, whereas nocturnal urine production does not change [101, 102].

Comorbidities

Voiding abnormalities are strongly associated with various comorbidities.

Constipation

The coexistence of LUT symptoms and functional constipation and/or faecal incontinence in children is not uncommon, and was previously identified as dysfunctional elimination syndrome (DES) [103–106] and more recently as ‘bladder and bowel dysfunction’ (BBD) [22]. Although the association with dysfunctional voiding, decreased voiding frequency and underactivity is obvious, there is also a clear comorbidity between bladder overactivity (urge), increased voiding frequency, bladder underactivity and constipation [21]. It is a well-established that treatment of defecation problems in children with BBD enhances successful management of LUT disturbances such as daytime urinary incontinence (DUI), enuresis, and UTIs [21, 22, 103]. Treatment of the bowel dysfunction ameliorates the voiding disorder and should be first-line treatment [21].

Psychological/Behavioural Problems: Attention Deficit, Neurocognitive Dysfunction

Psychological comorbidity among children with functional urinary incontinence is high: 20–30% of children with NE, 20–40% of children with DUI, and 30–50% of children with fecal incontinence (FI) [107–110]. Both internalizing and externalizing characteristics are represented [109, 111]. The best documented comorbidity conditions are attention deficit-hyperactivity disorder

(ADHD) and oppositional defiant disorder (ODD). Children with attention deficit disorders have a higher prevalence of enuresis (both MNE and NMNE), whereas the prevalence of ADHD in the enuresis population is up to four times higher than the background population. The association of abnormal prepulse inhibition (Startle reflex) in both ADHD and enuresis patients might suggest a common central nervous pathogenic pathway, but it is far from fully understood [109, 112–114].

Sleep

Regardless of the cause of the mismatch between nocturnal bladder capacity and nocturnal diuresis, enuresis only occurs when the child is unable to wake up; hence, lack of arousal is a prerequisite for NE. This has caused many to conclude that sleep disturbance *per se* is the major pathophysiological factor in enuresis and it is still a general belief in the general population that enuretic children are deep sleepers. However, this hypothesis has been questioned due to the inability to convincingly show abnormalities in sleep EEG patterns together with the observation that a considerable proportion of non-enuretic children also are unable to wake up when polyuria is induced overnight [11, 115–120].

It was documented that enuretic children did not exhibit deep sleep, but rather a disturbed sleep with increased periodic limb movements during sleep (PLMS), cortical arousals, and awakenings [66, 67, 121]. Polysomnography studies documented a significant difference in PLMS index, arousal index, and awakening index compared with healthy control subjects. The presence of sleep fragmentation does not exclude a high sleep pressure or high arousal threshold. The role of sleep fragmentation in children with NE was earlier emphasised using sleep actigraphy [122]. Obstructive sleep airway syndrome causes nocturnal polyuria in both children and adults, eventually resulting in enuresis [123–125]. The prevalence of this NE subtype in patients primarily consulting for NE is not known, but the anti-enuretic response to therapy to address obstructive sleep airway syndrome suggests that in refractory cases this might be considered [126–129].

In conclusion, albeit there is little doubt that sleep and/or arousal plays a role in the pathophysiology of NE, the clinical relevance and possible implications are still unclear and so far sleep investigation is not part of routine evaluation of enuretic children.

Hypertension

One of the first characteristics of arterial hypertension is the loss of nocturnal dipping and patients without nocturnal dipping have a poor cardiovascular prognosis. The association between hypertension and increased nocturnal diuresis is well-documented [28, 70].

Renal Diseases

Many renal diseases are associated with disrupted circadian rhythms of renal water and solute handling [60, 81, 83, 87, 97]. Thus, the kidney disease may have a role in causing the NE. However, such patients may have the same predisposition to NE as other children. Hence, they should have the same workup as any child with NE.

Drug Induced Changes in Circadian Rhythm

Many drugs result in disrupted circadian rhythms. The best known are the steroids, certainly when given twice a day or in high doses. Even a low dose at 08.00 h induces a shift in circadian rhythm, since the normal physiologic cortisol peak is 4 h earlier. Diuretics have of course a major effect on water and sodium excretion, especially long-acting thiazides, or loop-diuretics when they are given twice a day.

Genetics

NE has a strong hereditary component given that approximately 70% of NE patients have a positive family history and that monozygotic twin studies find concordance rates of 80%. Positive linkage was established between enuresis and several gene loci on different chromosomes (4p, 12q, 8q, 13q, and 22q). However, although several candidate genes have been proposed, no specific pathogenic variant has been identified. Moreover, it is unknown whether there is a

genotype-phenotype correlation to specific NE subtypes [38]. Interestingly, a recent genome wide association study in a large Danish population identified common genetic variants associated with NE. The study pinpointed potential NE risk genes, among which *PRDM13*, *SIM1*, and *EDNRB* might affect sleep, urine production, and bladder function, respectively. It also identified for the first time a significant genetic overlap between NE and ADHD [130].

Evaluation

In the child with *primary* NE, evaluation should not be initiated before 5 years of age. In contrast, children with *secondary* NE should be evaluated for an underlying etiology (e.g. diabetes mellitus, constipation, and UTI) when the wetting reappears. The initial approach to primary NE should avoid unnecessary investigations if there are no concerning symptoms (Fig. 47.1).

Evaluation should comprise a detailed history, with a focus on the duration and severity of the night wetting, but also inquire about LUT symptom such as daytime incontinence, increased or decreased daytime voiding frequency, urgency, voiding postponement, holding maneuvers, constant dribbling, and intermittent urinary stream. It is important to ask specific questions regarding LUT and bowel symptoms. A history of uropathy, UTI, and constipation suggests possible bladder dysfunction.

The physical examination should comprise the external genitalia (e.g. congenital anomalies, phimosis), the lumbar region (e.g. deformations, pigmentations, and hair growth), and neurological examination (e.g. ano-cutaneous and cremaster reflexes, lower extremity reflexes, muscle tone, and gait). Laboratory evaluation should include a urinalysis of a morning or spot urine sample, with examination for glucose, leukocytes, nitrite, and albumin. In the majority of children with NE, these investigations will be normal.

A cornerstone in the further evaluation of a child or adolescent with NE is an estimation of their bladder capacity and nocturnal urine production. For this purpose, a frequency-volume chart (registration of the time and volume of all micturitions and fluid intakes during daytime) during two weekends and a recording of nighttime urine production (weight of diaper in the morning (g) − weight of diaper before bedtime (g) = volume of morning micturition (mL)) during 1 week are very useful approaches for obtaining this information. Compliance is usually good when the purpose is explained to the parents. Measurement of MVV (excluding the first morning void) needs to be made over a minimum of 3–4 days for accuracy: weekends or school holidays are ideal, but have the disadvantage that lifestyle is significantly different and often less structured than on schooldays [33, 76].

Treatment

The simplified evaluation process enables the identification of two archetypes of NE that correlate with their pathophysiologic characteristics (Fig. 47.2). The first archetype is children with MNE and no apparent LUTS; the mismatch between nocturnal diuresis and functional bladder capacity is caused by nocturnal polyuria. The majority of these patients will have low urinary osmolality overnight due to low vasopressin levels [18, 19], and will be desmopressin responsive. The second archetype is the "small for age" bladder capacity. These patients will likely be desmopressin resistant, and are expected to have a good response to the enuresis alarm [33]. A combination of both types is not uncommon and such patients generally respond well to combined therapy with desmopressin and an alarm [33]. If patients do not respond to one of these treatments, a more complicated underlying mechanism might be present and referral to a tertiary multidisciplinary centre should be considered.

Thus, a simple "trial and error" treatment strategy for enuresis is no longer recommended as it may even have an adverse psychological effect on the child [2]. A rational therapeutic approach as outlined in the ICCS standardization document [31, 33] leads to higher success rates.

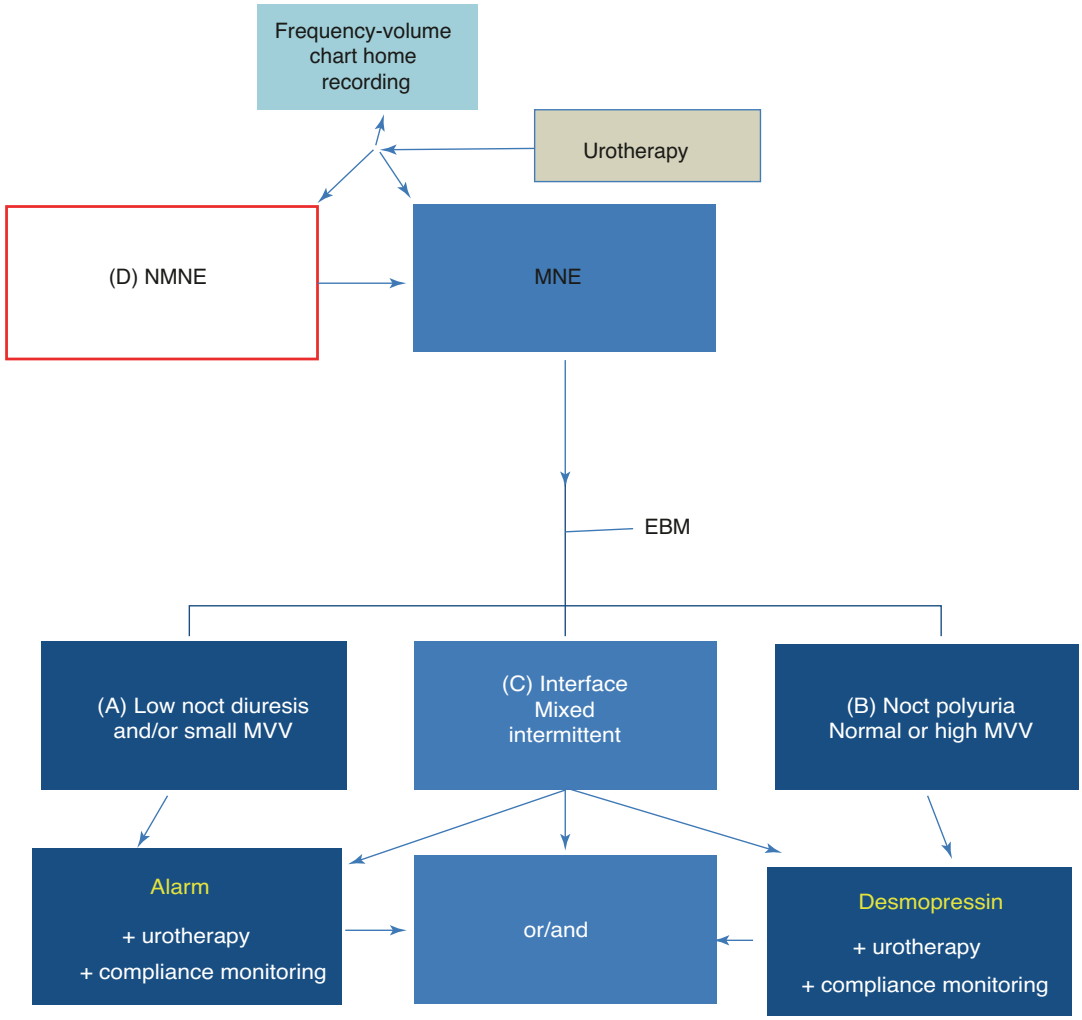

Fig. 47.2 A simple flow chart illustrating that noninvasive screening, identifying the subgroup of patients with monosymptomatic enuresis, may lead to a rationalized therapeutic approach in primary and secondary care avoiding unnecessary invasive investigations. All children should receive urotherapy according to the ICCS guidelines, including normalization of fluid- and nutritional intake, voiding pattern and sleep hygiene. If despite this urotherapy, enuresis persists, according to the ICCS standardization papers, children should only receive EBM therapies: the alarm and desmopressin. Although both therapies reach EBM IA levels, success rates vary between 30% and 60%, with high relapse rates. Targeting the underlying pathophysiological characteristics of the enuresis, and especially the mismatch between nocturnal diuresis volume and functional bladder capacity (MVV) overnight might lead to increased success rates. Patients can be subdivided into two archetypes, children with nocturnal polyuria (B) and children with overactive bladder (A). Children with low diuresis-rate overnight (and high osmolality) and/or small bladder capacity (MVV), are likely desmopressin resistant, and should receive the alarm as primary therapy. Children with nocturnal polyuria (+low osmolality) and normal bladder capacity (MVV) for age will likely benefit from antidiuretic therapy rather than from the alarm. Desmopressin should be first line therapy. For some there is a continuum between the two archetypes (C), where some children have only a mild, often intermittent mismatch, between diuresis and bladder, where each characteristic does not reach the level of abnormality as defined by the ICCS. In these children both therapy options are defendable. In cases, refractory to therapy or in the subgroup where we identify a combination of nocturnal polyuria and overactive bladder, combination therapy should be advocated. (D) NMNE-children with successful therapy of the LUTS symptoms can subsequently be treated as MNE patients

Therapy is often initiated at the age of 6–7 years, although enuresis is considered abnormal in children ≥5 years of age. Reasons for a proactive attitude towards treatment include the distress caused to child and family, difficulty with "sleeping over" on holiday or at friends' houses, social withdrawal, reduced self-esteem [2], disrupted sleep with possible secondary effects on cognitive function and health [119, 131–134], and the burden [3] and costs associated with frequent laundering of bedsheets and clothing [135, 136]. In addition, spontaneous resolution is low in children >5 years with frequent bedwetting; the negative impact on the child and family induces a risk for intolerance of some parents [137]. Whether early treatment reduces the prevalence in adulthood is unclear [5, 6, 88, 138–141].

Treatment [8–11]

Step 1: Demystification

Explanation of the pathophysiological mechanisms that are involved in enuresis is essential. Children and families should be convinced that bedwetting is a common problem, that they should not be embarrassed or feel guilt, and that it is definitely not a primary psychological problem. Patients should understand that the primary cause is a mismatch between nocturnal diuresis volume and the bladder capacity overnight.

Parents and children should be warned that success rates of monotherapy vary from 30% to 60%, leaving up to 1/3 with persistent symptoms after 1 year. This message is obligatory to prevent early frustration during therapy [8, 31, 33].

Step 2: Urotherapy [35, 142, 143] includes all nonspecific advice, including toilet habits, frequency, fluid intake, and nutrition advice. This is a well-accepted first line therapy in children with enuresis, especially in children with associated bladder dysfunction. Recently there is increasing evidence that urotherapy might not be effective at all in treatment of the enuresis component. Increased fluid intake might be beneficial in some patients, but might worsen enuresis in other patients. This data does not support starting with full spectrum urotherapy in all enuresis patients, but rather as an individualized modality in therapy refractory patients [144].

Advice on fluid and nutritional intake:

Many children have both their maximum fluid intake and main meal in the evening, and the high sodium and protein load may result in high osmotic excretion and polyuria overnight, as has been demonstrated in children with desmopressin refractory polyuria. Opinion leaders empirically advise that fluid and nutritional intake should be normalised, with fluid intake spread during the whole day, and avoiding caffeinated beverages and high fluid intake in the evening [8, 31, 33, 145]. This should be accompanied with healthy food intake in the evening, avoiding high protein and sodium load, to reduce osmotic diuresis [82, 83]. However, the evidence level for this strategy is weak.

Sleep advice is suggested due to the close interaction between sleep and several renal, hemodynamic, and endocrine circadian rhythms. Children should be encouraged to develop good sleep habits, including sufficient time for sleeping. Hyperstimulation of the brain (e.g. bright light exposure, computer games, television, music) should be avoided before sleeping, since it might have profound effects on the regulation of circadian rhythms, potentially affecting melatonin secretion and sleep patterns. Children should void immediately before going to bed, and lights should be turned off, once children are in bed. There is ample evidence that the overall sleep quality of children and teenagers is decreasing. There are also issues with timing and duration. The use of mobile devices once in bed is a big challenge, and should be discouraged. Children should not drink during the night [96, 146], and definitely not during therapy. However, the evidence supporting these interventions is weak.

Step 3: Non-medical behavioural management:

If urotherapy and lifestyle interventions are unsuccessful, more intensive treatment modalities are recommended. The alarm and desmo-

pressin are the only two evidence-based management level I, grade A recommended treatments. The choice between the two options should ideally be individualised based upon patient characteristics identified during the evaluation [33]. There is evidence that the response to these treatments is highly dependent on the underlying pathogenic mechanism: The alarm is the treatment of choice in the archetype patient with *small* bladder capacity (i.e. MVV) and *normal* nocturnal urine production on wet nights. Such patients are usually desmopressin resistance. In contrast, desmopressin is the treatment of choice in the patient with *large* nocturnal urine production on wet nights and *normal* bladder capacity. Such patients will likely not respond to the alarm, unless they are developing nocturia. In general, desmopressin and the alarm should not be regarded as competing modalities, but as complementary or even supplementary to each other and targeting different patient subtypes. If, however, initial evaluation of the underlying enuresis mechanism is not possible, or the clinical pattern is unclear, other factors such as family motivation and preferences, availability of close follow-up, cost, and frequency of enuretic episodes, should all be included in the decision process.

Enuresis alarms have a level 1, grade A International Consultation on Incontinence (ICI) recommendation [8, 31, 33, 147–149]. Different types of alarms exist, but the common feature is a sensor placed in the sheets or nightclothes that is triggered when it becomes wet, setting off an auditory or vibration signal and causing the child to awaken, cease voiding, and arise to finish the void at the toilet. Parents are advised to wake their child when the alarm is activated; otherwise, children are prone to ignore it or turn it off and go back to sleep. The first weeks are a burden for the child and parents, and thus appropriate education prior to initiation and close follow-up are essential. The alarm should be worn every night. Response is usually not immediate and treatment should be continued for 2–3 months or until the child is dry for 14 consecutive nights (whichever comes first). Often compared to Pavlov training mechanisms, the true mechanism remains poorly understood. However, successful alarm treatment has been associated with a significant increase in bladder capacity [101, 150], whereas nocturnal urine production and sleep is unchanged. There may be cultural differences in its acceptability, as it may be highly disruptive for the household. The family must be motivated and adhere to the regimen to be successful. Doctors should monitor the child's progress early to address any problems and facilitate adherence. The response rate is high in families who continue treatment for a sufficient period, with relatively low relapse rates, though the lasting cure rate is still <50% [147]. Poor compliance and early withdrawal from treatment are common [14, 151, 152], which may exacerbate parental intolerance. Identifying the motivated patient in the right family is essential, and in cases where the child or the family is reluctant to accept the alarm, desmopressin is the alternative [149]. Alarm therapy has high failure rates when the buzzer is activated more than once a night. The goal is to obtain continence without waking up, although up to 25% develop nocturia. Relapse rates vary from 30% to 60% [147].

Desmopressin (DDAVP) [78, 153–156]

Desmopressin also received a level 1, grade A recommendation from the ICI in 2009 [49]. Desmopressin is a synthetic analogue of arginine vasopressin, the naturally occurring antidiuretic hormone. One of its major actions via the renal V2 receptor is to reduce the volume of urine produced overnight to a normal amount. Since the demonstration of nocturnal polyuria in patients with MNE, and the lack of the normal rise in plasma AVP levels during night, the use of DDAVP became a logical choice [58, 59]. Beyond the major effect on free water reabsorption, DDAVP has been claimed to have additional effects in enuresis, including detrusor muscle activity, central control of micturition, tubular sodium handling [157], and sleep, or arousal

function, but the evidence in unselected enuretic populations is very low. The direct effect on sleep and arousal is controversial since it is unclear if DDAVP crosses the blood-brain barrier. Several formulations are available: rhinal nasal solution, nasal spray, oral tablet and oral lyophilisate (sublingual MELT formulation), all characterized by low bioavailability with large intra-individual variability (0.08–0.16% in adults), but only the oral tablet and lyophilisate formulations are labelled for treatment of NE in children. The low bioavailability is related to the peptide structure compromising oral and intestinal reabsorption. It is available for the treatment of enuresis as a tablet (dosage, 0.2–0.6 mg) or a fast-melting oral lyophilisate (MELT formulation, dosage, 120–360 μg). The latter is a recommended formulation for all children and is preferred for children under 12 years [94, 95, 158, 159]. Unlike other preparations, the MELT formulation is not affected by nasal congestion or gastrointestinal transit and does not require fluid intake. Since tablets usually require 1–200 mL of fluid intake, which is a significant percentage of a 7-year-old's bladder capacity and nocturnal diuresis, the MELT formulation seems more suited to the antidiuretic indication of desmopressin. Good pharmacodynamic data are available for the MELT formulation and its dosing in children with enuresis [94].

The efficacy of desmopressin in the treatment of NE is well documented. In a Cochrane analysis, 17 controlled trials all showed superiority of desmopressin compared to placebo regardless of the route of administration [153]. An estimated 70% of the NE population has full or partial response [95]; the response to desmopressin is highly dependent on factors such as nocturnal urine production and bladder capacity. The few studies that have tried to compare different doses of the drug reveal comparable efficacies [160–162]. Several key issues should be taken in consideration. First, 200–400 μg tablets are considered to be bioequivalent to 120–240 μg of the MELT formulation and represents the therapeutic range for children between 7 and 18 years with MNE. However, higher doses might be needed in larger children [159].

Desmopressin should be taken 1 h before the last void at bedtime to allow timely enhanced concentration of urine to occur. Fluid intake should be reduced from 1 h before desmopressin administration and for 8 subsequent hours to optimize concentrating capacity and treatment response, as well as to reduce the risk of hyponatremia due to water intoxication [94, 95]. Desmopressin is only effective on the day it is taken; full adherence is required to avoid wet nights [151, 152].There are different initial dosing regimens with comparable efficacy such as gradual dose escalation over a few weeks or starting with a high dose followed by gradual tapering to the lowest effective dose. If desmopressin is effective, then treatment can be continued for an additional 3 months; country-specific regulations regarding treatment breaks should be followed. If patients are dry on desmopressin treatment, breaks are recommended to ascertain whether the problem has resolved and therapy is no longer needed. If the child does not achieve complete dryness, or if wetting resumes once treatment is withdrawn, it should be continued or resumed, respectively. There is some evidence that structured withdrawal of medication may reduce relapse rates following its discontinuation [163, 164]. Desmopressin is well-tolerated, but clinicians should be aware that it is a potent antidiuretic and families must be educated regarding the rare possibility of developing hyponatremia due to water intoxication with symptoms including headache, nausea, and vomiting [155]. Self-titration of medication should be avoided.

Adherence to the management plan is crucial, especially for a drug whose action covers only the first night after intake. It is estimated that approximately 30% of non-responders are not taking medication correctly [151, 152]. Non-adherence to recommendations regarding timing of medication, voiding before bedtime, and limiting evening fluids can decrease treatment success [100]. Moreover, compliance is often overestimated by patients and caregivers; therefore, it should be documented in a diary. Regular contacts between caregiver and patient are necessary to maintain compliance. Patients who appear treatment-resistant should be advised of the

importance of full adherence and asked if they have had any difficulty complying with recommendations.

Desmopressin has higher success rates in children with large bladder capacity and nocturnal polyuria [100]. The response rates in the initial studies of >70% decreased to 20–30% in subsequent studies. This lower response rate is likely due to local referral patterns and the use of desmopressin among general practitioners and consequent selection of more challenging patients at tertiary referral centers, and higher incidence of patients with overactive bladder (OAB) since the prevalence of occult OAB patients with small bladder volume and low nocturnal diuresis volume was high in these populations [54].

There are several unanswered questions regarding desmopressin therapy. It is still unclear whether desmopressin treatment leads to better long-term outcome than the spontaneous cure rate. The long-term cure rate of 15–30% annually with desmopressin in unselected populations is higher than the spontaneous cure rate [79, 154, 165], but depends on the severity of the study population. Long-term follow-up showed persistent LUT symptoms in the patients with NMNE and not in the MNE patients [88]. The suggestion that tapering of the dose and the structured withdrawal program should be beneficial is still unproven [163, 164].

It is obvious that many renal patients (CKD, and tubulopathies) with NE have impaired response to desmopressin, and do not reach maximal concentrating capacity, but this does not exclude at least some antidiuretic effect overnight, not normalizing but at least optimizing antidiuresis [166].

Tricyclic antidepressants were among the first drugs widely used in NE [167–172].They act on adrenergic and serotonergic receptors in the central nervous system. Although the exact mechanism of action remains unclear, imipramine reduces both urinary sodium and the overall urinary osmolar excretion, decreasing the nocturnal urine output in children with nocturnal polyuria. Imipramine has potential to modulate sleep and increase arousability and it has a parasympatholytic effect on the detrusor and an alpha-mimetic activity on the sphincter. Side effects are the major drawback, ranging from postural hypotension, mouth dryness, and constipation to hepatotoxicity and cardiotoxicity in large doses [173]. Hence, imipramine is currently not recommended as a first line enuresis treatment but, has been suggested in treatment of refractory patients. Some recommend a pre-treatment ECG to exclude long QT-syndrome, but there is no evidence of benefit in children, and few MD's follow this strategy,

Anti-muscarinic Therapy

Anti-muscarinic therapy (e.g. oxybutynin, tolterodine, propiverine, solifenacin) has not been proven effective mono-therapy for patients with MNE, but may have a role in combination with first line modalities [174, 175]. These drugs are widely prescribed in children with NMNE for the daytime symptoms and are suggested to have beneficial effect on NE [175], but prospective there is no supportive evidence from randomized clinical trials. They might be indicated as add-on therapy in the subgroup of patients with MNE and small for age bladder capacity.

Cognitive Training and Psychotherapy

Cognitive training and psychotherapy was more widely used before desmopressin was available, and when physicians were less involved in treatment. Cognitive training and psychotherapy may still have value as an adjuvant to conventional therapy, although the evidence is weak. Most of studies were performed in patient groups defined by criteria from the Diagnostic and Statistical Manual of Mental Disorders, fourth edition, which does not differentiate between MNE and NMNE, and with a higher incidence of psychopathology [33].

Alternative Treatments

There are multiple alternative treatments for NE, but little or no evidence. There is no evidence supporting the efficacy of hypnosis [13, 176, 177], chiropractic [178–180], and reflexology. There is weak evidence demonstrating benefit from traditional acupuncture [181, 182]. There is insufficient evidence supporting acupuncture

variations such as manual acupuncture, acupressure, and electro- and laser-acupuncture [183]. Transcutaneous electrical nerve stimulation (TENS) may have benefit, but mainly in a NMNE population [184, 185].

Treatment Refractory MNE (Fig. 47.3)

The major reasons that patients with MNE are non or partial responders to first line treatment modalities are that (1) the initial evaluation was not sufficient and the patient was a NMNE patient, (2) non-adherence to the treatment prescribed (3) a complex phenotype with more than one underlying mechanism (e.g. both nocturnal polyuria and low bladder capacity) and (4) rare types of enuresis with underlying mechanisms not influenced by treatment (e.g. polyuria caused by large osmotic excretion) (Fig. 47.2).

In such patients, a repeat, extended evaluation should be performed, including detailed history of LUTS with particular emphasis on daytime OAB symptoms and constipation (classification of constipation according to the Rome IV criteria) [269]. The patient should keep a bladder diary with recordings of daytime intake and output and nighttime urine volume (diaper

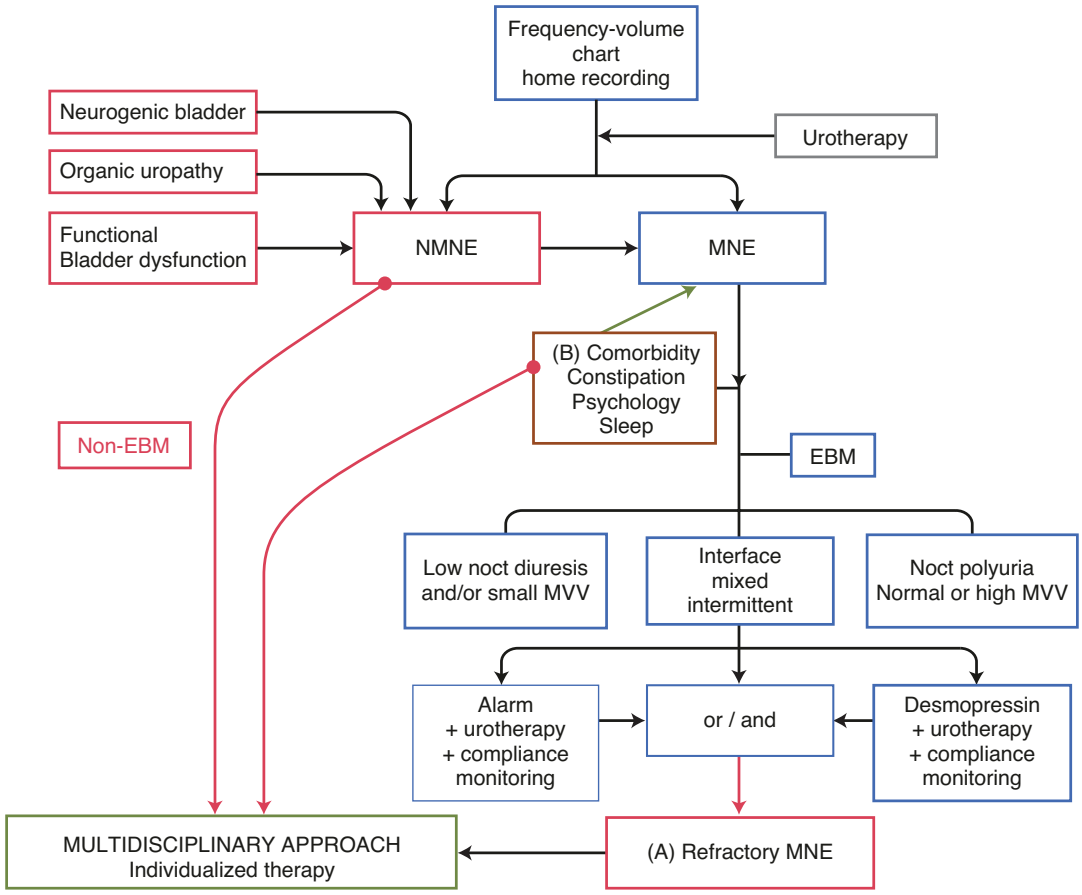

Fig. 47.3 Illustrates further the flow chart when children are refractory to initial therapy. (A) In these patients EBM therapy is insufficient and a multidisciplinary diagnostic and therapeutic approach should be offered, resulting in individualized therapy regimens. This multidisciplinary therapeutic approach should involve experts in pediatric nephrology and urology, as well as targeting psychological characteristics, compliance issues and comorbidities. Since the majority of comorbidities associated with therapy-resistance are easy to identify by simple questionnaires, we advocate not only to search for them in refractory cases, but also in the early stages of evaluation (B)

weighing). Advice the patient to keep the diary during a day with standardized fluid intake (1.5 L/1.73 m^2/day), since low fluid intake might mask frequency and daytime incontinence. The night-time recording can be repeated during 1 week of desmopressin treatment in order to verify whether the patient has the intended reduction in urine production. Spontaneous low fluid intake suggests a defence mechanism, and may mask OAB symptoms. These symptoms are often not mentioned by parents, and thus deserve repeated questioning or documentation in a bladder diary. Uroflowmetry, bladder ultrasonography and rectal diameter measurement can give additional information on the voiding pattern, bladder emptying, bladder wall thickness (OAB), and constipation.

In desmopressin refractory patients without bladder dysfunction, alternative explanations for the lack of efficacy may include (1) anti-enuretic effect = number of wet nights, (2) anti-diuretic effect (= nocturnal diuresis rate), and (3) concentrating capacity (= urinary osmolality). Partial response to desmopressin in MNE (anti-enuretic effect) is related to persistent nocturnal polyuria on wet nights [75] (Fig. 47.4).

Poor compliance should be excluded [152], including not taking the drug (record in drug-diary). Determine the number of filled prescriptions to monitor compliance. Other causes for suboptimal effect of desmopressin are:

1. The child forgets to void and empty the bladder before sleeping thereby increasing the risk of exceeding maximal bladder capacity during the night.
2. Fluid intake overnight or even the hour before desmopressin administration reduces both the maximum and duration of the anti-diuretic effect.
3. Intermittent polyuria might also be related to the PK/PD characteristics of desmopressin [94, 95, 159], but the phenomenon might be intermittent, varying from night to night. Factors which influence PK/PD include;
   (a) The recommended formulations (tablet, MELT formulation), but also the nasal spray, have poor bioavailability, ranging 0.2–2%, but with a large intra-individual variability. Only for the MELT formulation are dose-response and PK/PD data available in children. The MELT

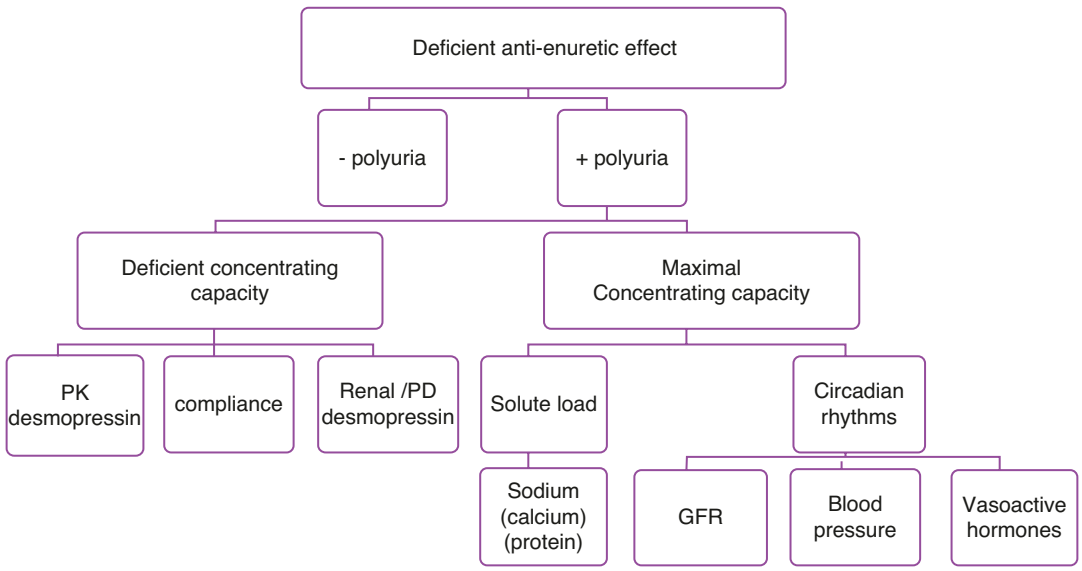

Fig. 47.4 Patients with desmopressin resistant monosymptomatic enuresis can be subtyped according to persistence or not of nocturnal polyuria and ability to reach maximal concentrating capacity. An individualized treatment choice is possible when by considering the different pathophysiologic mechanisms involved

formulation had superior PK and PD profile, better compliance, and some indices of higher response rates than the tablet, although there have been no randomized trials comparing the anti-enuretic effect of the two preparations. In treatment resistant patients on tablets, a switch to the MELT formulation should be considered.

(b) The child takes desmopressin just before sleeping time. Since the time to reach maximum concentrating capacity and anti-diuretic effect is 1–3 h, the drug should be taken at least 1 h before the last void at bedtime [94].

(c) Even in the therapeutic range of 120–240 μg, there are large standard deviations in maximal concentrating capacity and antidiuresis, as well as duration of action. Better understanding of this PK/PD, can lead to more personalized medicine, with individualized dose schemes. The PK/PD data demonstrate that at least 25% of patients might benefit from higher doses; these patients can be identified by a PD test in an ambulatory setting, especially the older patient (24 h concentration-profile). Increasing the dose should only be done following this test, typically done in expert centers, due to the risk of toxicity.

In desmopressin refractory nocturnal polyuria with low urinary osmolality, nephrogenic diabetes insipidus (NDI) should be excluded. The X-linked NDI in boys does not usually present as enuresis, but female carriers might have a more subtle phenotype with enuresis as the major symptom. Many renal diseases (CKD, tubulopathies, renal dysplasia, uropathy) may present with enuresis. Hypertension, especially nighttime hypertension, coincides with nocturnal polyuria, and should be considered in refractory patients.

Desmopressin resistant nocturnal polyuria might be associated with high urinary osmolality overnight. This can be caused by an increased solute load only in the evening or during all 24 h [82, 83]. Sodium is the major osmotic agent [80, 81, 83, 87, 186]. Although nutritional intake plays a major role, abnormalities of several circadian rhythms like prostaglandins [65, 80, 87], GFR [187], blood pressure [70] and sleep pattern [66, 67] may also have a significant impact. Extrapolation to primary care enuresis patients remains premature, but these findings suggest future treatment options. Some pilot studies had promising results such as sodium restricted diet, diuretics (furosemide [186]), nonsteroidal antiinflammatory drugs (NSAIDs), and melatonin [127, 188–190]. There was a focus on the role of calcium [91], and the efficacy of a calcium restricted diet [92]. However, hypercalciuria might be a secondary phenomenon, unrelated to diet.

Therapy resistance may be related to the *presence of comorbidities* [33], and addressing them, when possible, might increase the response rate. Constipation and FI should be treated before treating MNE, but are often underestimated and underreported. Psychological comorbidities, both internalizing as well as externalizing, are more frequent in NE patients. Attention deficit disorder and autism seem to have common central nervous pathways with enuresis. Renal dysfunction, hypertension, diabetes mellitus and sleep disturbances may worsen NE, and should be treated. NE may also be exacerbated by drug interfering with circadian rhythms, including diuretics, steroids, cyclosporine A, and neurotropic drugs.

In conclusion, children with MNE the two first-line treatment options are desmopressin and the enuresis alarm, as monotherapy or in combination. Outcomes are less favorable if therapy is not guided by patient characteristics, with initial success rates of monotherapy potentially below 30% and a relapse rate of up to 50%.

In summary, the optimal strategy is as follows:

1. The initial treatment choice should be guided by the family's level of motivation and their preference.
2. Based on the information from diaries, one can identify subtypes of MNE that should

allow further fine-tuning of treatment according to the child's characteristics and family motivation.

1. Children with a normal urine output during the night and normal bladder capacity can be given either the alarm or desmopressin.
2. Children with smaller than expected bladder capacity for age will likely be desmopressin-resistant and more responsive to the alarm.
3. Children with nocturnal polyuria and normal bladder capacity will be more sensitive to desmopressin.
4. Children with both nocturnal polyuria and reduced bladder capacity may have a successful outcome with the combination of alarm and desmopressin.

Other Forms of Urinary Incontinence (Fig. 47.5)

The classification of daytime LUT conditions is more complex than enuresis because of the heterogeneity of symptoms of LUT dysfunction and the considerable overlap between the conditions. Although there is a significant overlap with enuresis, not all LUT patients have enuresis, and not all enuresis patients have apparent LUT symptoms. Additionally, borderline cases are common and the rationale for grouping of symptom complexes into specific LUT dysfunction conditions is often not fully evidence-based. The ICCS has standardized the terminology and approach [31].

Epidemiology

Most children are toilet trained by 2 years of age, although there are important country and cultural differences. Early interventions might speed up toilet training, but have not been shown to decrease the prevalence of any LUTS at 5–7 years [191, 192]. Daytime symptoms from the LUT are very common in childhood, especially urgency and frequency (approximately 5–20% of 7-year olds), rapidly decreasing during the following years. As outlined above, the majority of these children have non monosymptomatic enuresis. Urinary incontinence is also relatively common, with a prevalence of approximately 3% of 7-year olds [49]. Voiding complaints, such as dysuria and interrupted stream, are far less frequent. Five years of age is the usual cutoff for reporting symptoms and LUT conditions. The prevalence of the various subtypes of LUTs, especially OAB, versus dysfunctional voiding is not known.

Definitions and Characteristics

Assessment

To classify daytime LUT dysfunction conditions, we recommend assessment and documentation be based on the following parameters:

1. Incontinence (presence or absence, and symptom frequency)
2. Voiding frequency
3. Urgency (presence or absence)
4. Voided volumes
5. Fluid intake

This is more important than subgrouping the children into the various recognized conditions listed below.

There are several archetypes, but with large overlap. Rather than forcing children into an archetype as was done historically (*non-neuropathic* bladder-sphincter dysfunction, *urge incontinence*, *dysfunctional voiding*, *lazy bladder syndrome, Hinman bladder) it is advised to start with a detailed clinical history and using a diary and uroflowmetry to describe the characteristics of the bladder during the filling phase (storage) and the emptying phase*. Urgency, holding manoeuvres and daytime incontinence are frequent with an overactive bladder, but infrequent in an underactive bladder.

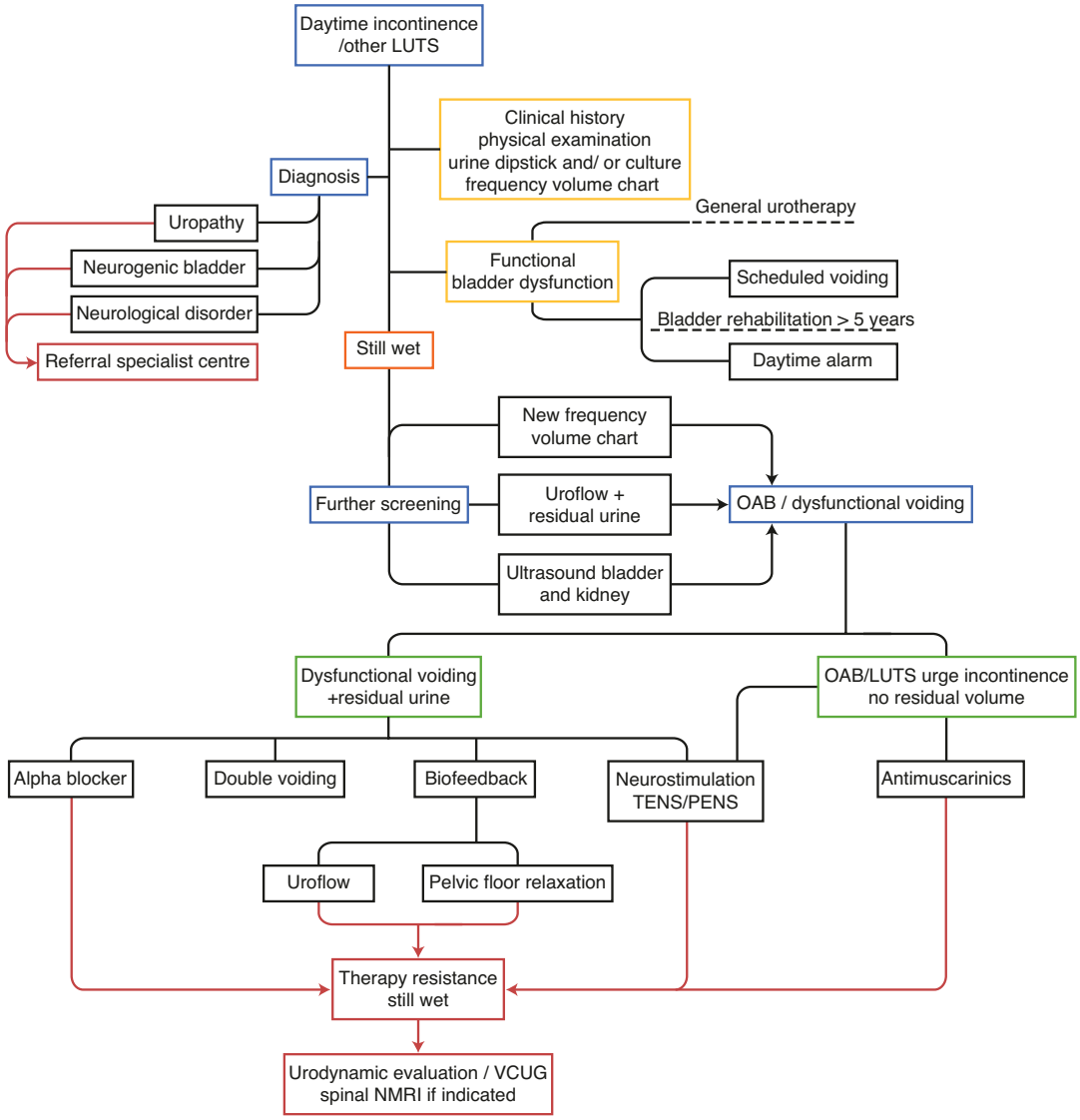

Fig. 47.5 Flow chart illustrating the evaluation and treatment of daytime incontinence/LUTS in children. The initial evaluation in children with LUTS starts at the age of 4–5 years. Clinical evaluation should focus not only on the bladder symptoms, but also on comorbidities such as constipation, neurologic disorders, neurologic bladder, and anatomical abnormalities uropathies. If these are excluded, we can conclude to a functional bladder dysfunction. Initial therapeutic approach, consists of treatment of constipation, and subsequent urotherapy. Bladder rehabilitation regimens and daytime alarm might be add on therapies. If this therapy fails, additional screening should be performed, including frequency-voiding chart during normalized fluid intake, uroflowmetry with US evaluation of residual volume, and ultrasound of kidney and the bladder. This non-invasive approach may lead to identification of the two archetypes, OAB and dysfunctional voiding, although there is a certain overlap between the two. In children with OAB, antimuscarinics are the treatment of choice, although neurostimulation offers an alternative treatment option. Patients with dysfunctional voiding benefit from a variety of urotherapy regimens, including pelvic floor relaxation- and uroflow biofeedback. In refractory cases, video-urodynamic investigation is mandatory. In cases where the videourodynamics give further indication for a neurologic disorder, spinal NMRI should be performed

Comorbidities: Dysfunctional voiding (previously called bladder-sphincter dysfunction), such as OAB with defence mechanisms, is associated with recurrent UTI, asymptomatic bacteriuria, vesicoureteral reflux and constipation and/or faecal incontinence (30–50% of children with urge incontinence). Furthermore, urinary incontinence is highly associated with increase in body mass index, constipation and/or faecal incontinence (10–15% of children with urge incontinence), neuropsychiatric conditions (e.g. ADHD, oppositional defiant disorder) and intellectual disabilities.

Urinary Incontinence Classification

Several structural congenital abnormalities (e.g. ectopic ureter, congenital urethral valve), and neurogenic bladder (e.g. spina bifida, sacral agenesia) often present with therapy refractory LUTS. Urinary incontinence can be divided into separate subtypes based upon the underlying mechanism [193]. The two subtypes that pose the greatest threat to the upper urinary tract and therefore should be identified and treated as early as possible are *neuropathic* bladder sphincter dysfunction and *structural* incontinence (organic). These two groups comprise only a few percent of incontinent children as most have no underlying neurological or anatomic cause. In *non-neuropathic* bladder-sphincter dysfunction, it is possible to further subtype the type of bladder dysfunction based upon clinical history, voiding pattern, uroflowmetry and residual urine. Continuous incontinence is often due to an organic disorder.

Bladder and bowel dysfunction (BBD): BBD is a condition of combined bladder and bowel disturbances that encompasses LUT and bowel function. *Severe BBD* is LUT and bowel dysfunction that is characteristic of the dysfunction seen in children with neurologic conditions, yet have no identifiable neurologic abnormality. This is almost synonymous with the historical term Hinman syndrome.

Overactive Bladder

OAB manifests as urinary urgency, usually accompanied by frequency and nocturia, with or without daytime incontinence, in the absence of UTI or other alternative etiology. Some patients may have minimal symptoms because they limit fluid intake during the day in an effort to decrease symptoms.

OAB is the most frequent cause of LUTS, with daytime incontinence, urgency, frequency, and small voided volumes as symptoms. Parents often notice efforts to prevent daytime enuresis, which they interpret as delaying micturition ("postponement"), but these children have high micturition frequency, in contrast with the typical postponers, who have low frequency of large voided volumes. Children with OAB usually have *detrusor overactivity*, but this should not be assumed without cystometric evaluation (see above). *Urgency incontinence* is involuntary loss of urine associated with urgency and is applicable to many children with OAB.

Voiding Postponement

Children who habitually postpone micturition using holding maneuvers have *voiding postponement*, which is diagnosed by clinical history. It is often associated with a low micturition frequency, and a feeling of urgency and possibly incontinence, due to a full bladder. Some children restrict fluid intake to reduce incontinence. The rationale for delineating this entity lies in the observation that these children often suffer from psychological comorbidity or behavioral disturbances such as oppositional defiant disorder (ODD).

Underactive Bladder

Children with underactive bladder need to strain with increased intra-abdominal pressure to void. The children may have low voiding frequency despite adequate hydration, but may also have frequency due to incomplete emptying with prompt refilling of the bladder. These children often produce an interrupted uroflow pattern, and have *detrusor underactivity* during invasive urodynamics. Flow patterns may also be plateau-shaped; pressure–flow studies will distinguish it from bladder outlet obstruction. The prevalence is higher in females and increases with age.

Dysfunctional Voiding

The child with *dysfunctional voiding* habitually contracts the urethral sphincter or pelvic floor during voiding and demonstrates a staccato pattern with or without an interrupted flow on repeated uroflowmetry when concomitantly recording electromyography (EMG) activity. There is a clear correlation with UTI and constipation, and in rare cases high intravesical pressure during voiding might lead to VUR and renal damage with or without UTI.

Bladder Outlet Obstruction

Bladder outlet obstruction has to some extent comparable symptoms as dysfunctional voiding, but it can only be diagnosed during urodynamic investigation.

Stress Incontinence

Stress incontinence is the involuntary leakage of small amounts of urine when intraabdominal pressure is increased. It is rare in children, in contrast to adult females, and can only be diagnosed during urodynamic investigation. This rare type of incontinence is occasionally seen in female elite athletes [194]. The underlying mechanisms are not fully understood, but may involve a hypermobile pelvic floor.

Vaginal Reflux

Vaginal reflux is frequent in young girls after they complete toilet training. They experience daytime incontinence in moderate amounts shortly after normal voiding and have no underlying mechanism other than obvious vaginal entrapment of urine. This is not associated with other LUT symptoms or enuresis. It is worsened by voiding with the legs closed, leading to urine entrapment inside the introitus. It may be seen in girls with labial adhesions and chronic, recurrent vaginitis.

Giggle Incontinence

Giggle incontinence is a rare condition in which complete voiding occurs specifically during or immediately after laughing in girls. Bladder function is normal when the child is not laughing. Giggle incontinence is not linked to any other disturbance of LUT function. It is often familial, and has a proven hereditary predisposition. It should be differentiated from giggling incontinence as a symptom, where the leakage is secondary to contraction of an overactive bladder, induced not exclusively by laughing but also by physical activity and coughing.

Extraordinary Daytime Urinary Frequency

Extraordinary daytime urinary frequency is seen in toilet-trained children who void very often and with very small volumes during the daytime only. The daytime voiding frequency is at least once per hour and average voided volumes are less than 50% of expected bladder capacity age for age (usually much smaller). Nocturia is absent, and it is not usually associated with incontinence.

Bladder Neck Dysfunction

Bladder neck dysfunction is impaired or delayed opening of the bladder neck, resulting in impaired flow despite an adequate or elevated detrusor contraction [195]. The prolonged opening time, which is the time between the start of a voiding detrusor contraction and the start of urine flow, can be seen on videourodynamics. Alternatively, bladder neck dysfunction can be diagnosed non-invasively with a uroflow/EMG when there is a prolonged EMG lag time, which is the time interval between the start of pelvic floor relaxation and the actual start of flow.

Evaluation

If a wetting child has daytime symptoms, initial evaluation should be started from the age of 4 to 5 years due to the importance of excluding neurological and structural causes. The history is very important, including the psychomotor development, the type of incontinence (e.g. constant dribbling indicating a structural cause), recurrent UTI, constipation, and FI. Information about the family situation, motivation and behavioral problems should be included in a structured history. The physical examination should focus on the

same areas as in a child with NE (above); urinalysis is also indicated. If the initial evaluation raises suspicion of an underlying disease, e.g. a neurological disorder or anatomical anomaly, the child should be referred to a secondary or tertiary referral center. The cornerstone in the initial evaluation of an incontinent child is a frequency-volume chart, as described for enuresis, with documentation of all incontinence episodes as well as the time and volume of all intake and voids during 2 days, typically during a weekend. This gives very valuable information about the severity of symptoms, drinking habits, voiding pattern and bladder capacity. Occasionally, just filling out a frequency-volume chart enables the parents to adjust an inappropriate voiding or drinking pattern, resulting in a continent child before the next clinic visit.

In a child with recurrent UTI, prophylactic antibiotics should be instituted and an ultrasound of the urinary tract obtained. If there is suspicion of constipation, and in all children with soiling or FI, this should be treated aggressively. If, however, no underlying disease is suspected, further evaluation is not necessary at this stage and treatment can be reduced to general advice about good voiding habits and awareness about signs of constipation and UTI. In the motivated child, bladder rehabilitation with scheduled voids can be commenced. If the incontinence symptoms persist after 5–6 years of age, the initial evaluation should be supplemented with an ultrasound examination of the urinary tract, uroflowmetry and measurement of postvoid residual urine by ultrasound. These investigations usually require referral to a specialist. These simple urodynamic evaluations characterize the voiding and bladder emptying and will enable differentiate between types of incontinence, e.g. urge syndrome and dysfunctional voiding, and may raise suspicion of a urinary tract obstruction. More invasive evaluations such as conventional transurethral cystometry, natural fill ambulatory continuous bladder pressure monitoring, and voiding cystourethrography are only indicated if there is suspicion of neuropathic or structural incontinence (abnormal uroflow, residual urine) or if the patient does not respond to the initial therapy.

Treatment

Overactive Bladder (Formerly Urge Incontinence)

1. Two to three percent of 7-year-olds have incontinence with concomitant signs of an overactive bladder, and up to one third of 7-year-olds have urgency with increased voiding frequency. This syndrome has undergone a conceptual change over the last decade. Previously, there was a clear-cut urodynamic definition of the "unstable" bladder, with bladder contractions during the filling phase of a cystometry; it has expanded to a diagnosis based on clinical history and diary.

   There is evidence that cystometry at screening adds no value to the initial therapeutic approach. Moreover, the majority of patients with OAB on cystometry had the typical clinical pattern of OAB (urgency, frequency, small voided volume). Hence, a clinical diagnosis and a cystometry diagnosis of OAB are viewed as equivalent on initial evaluation. OAB probably does not have a single pathogenic explanation. Rather, mechanisms include hypersensitivity of the bladder wall (efferent), low compliance of the bladder wall, and an imbalance between ortho and parasympathetic tone (documented in refractory patients by the therapeutic action of anticholinergics and beta-3-adrenoceptor mimetics).

   Because the clinical picture of this incontinence is so typical, the diagnosis can be made with confidence by a structured approach as described above and no further urodynamic testing is necessary unless the child fails to respond to initial therapy. The urodynamic definition, however, has been widened to include bladder and urethral dysfunction. Clinically, urge syndrome is characterized by frequent attacks of a need to void, countered by holding manoeuvres such as squatting, eventually resulting in usually small leaks of urine.
2. Treatment:
   (a) Urotherapy [143, 196]:
   Treatment of urge incontinence begins with elimination of concomitant

constipation and UTI followed by behavioural treatment (bladder rehabilitation).

- Standard urotherapy [143]
- Standard urotherapy (ICCS, ICI level 3C) will cure at least 50% of children and can be started by primary care physicians. The cornerstone of urotherapy is to establish the child's awareness about his/her particular voiding habits. Different specific strategies are utilized, but most include a normalisation of fluid intake and elimination of caffeine intake. The evidence is weak [145, 197]. Standard urotherapy also includes an initial increase in the number of voids, followed by a gradual decrease to normal frequency [35]. Based on a randomized study, it is helpful to use a programmable watch that gives a signal to the child when it is time to void. Inappropriate posture during voiding, if present, should be corrected. At referral centres, the clinical team should include a specialized team of doctors, specialist nurses (urotherapists), psychologists and eventually physiotherapists [198].
- Although the majority of urotherapy regimens concentrate mainly on the quality and quantity of fluid intake, elevated nutritional intake of sodium and protein will result in higher sodium and acid load in the urine [82, 83]. High urinary acidity and osmolality might trigger bladder overactivity [199, 200]. Furthermore, in patients with CKD, (who may have a deficit in concentrating capacity, a high osmotic load) will result in higher diuresis rate and therefore more OAB symptoms [201, 202].

(b) Anticholinergics/antimuscarinics

If urotherapy for a couple of months does not eliminate the symptoms, antimuscarinics should be initiated [203, 204].

There are only a few medications with substantiated effect in children, and some have significant side effects, especially in children with ADHD. More recently developed antimuscarinics seem to have less influence on cognitive function and are therefore better tolerated [205, 206]. Antimuscarinic therapy should not be instituted in a child with significant post-void residual urine and many centres monitor bladder emptying 4–5 weeks after initiation of treatment.

- Oxybutynin hydrochloride
  - Oxybutynin hydrochloride, a tertiary amine, is a moderately potent anticholinergic agent with strong musculotropic relaxant and local anaesthetic activity. In animal studies, it has weak anticholinergic activities, but strong spasmolytic effects [13, 207, 208]. It is worldwide the most prescribed drug for OAB.
  - The side effects are those of all antimuscarinic agents and include inhibition of salivary secretion (dry mouth), blockade of the ciliary muscle of the lens (disturbed accommodation, blurred vision), facial flushing during exercise, tachycardia, drowsiness and inhibition of gut motility, leading to constipation. Central nervous system side-effects, especially in children with ADHD, are quite important, including pronounced attention deficit and behavioural problems, and are related to ability of oxybutynin and its metabolites to pass the central blood barrier.
  - The half-life of the drug is low, necessitating at least three doses a day. One dose in the morning for daytime symptoms or one dose in the evening for OAB, is ineffective, based on this PK characteristics. Oxybutynin XL is absorbed in the large intestine, thereby bypassing the first pass liver metabolism

[209]. However, the tablets must be swallowed intact, and thus cannot be used in many young children. Transdermal administration has been proposed as an alternative, but data in children are absent [210]. Intravesical administration has been promoted, but is only feasible in neurogenic bladders with intermittent catheterisation [211–213].
  - Although widely prescribed, the therapeutic evidence in children as monotherapy is rather weak (Evidence 3C), certainly for the enuresis symptom, though it is mentioned in all guidelines [196, 214]. Many studies report the use of combination therapy (desmopressin, imipramine, urotherapy, neurostimulation) [175, 215–218].
- Tolterodine
  - Tolterodine is a selective antimuscarinic drug, which has a more pronounced antimuscarinic effect on the urinary bladder than on salivary gland, and there is less penetration through the blood-brain barrier, potentially decreasing side-effect [219]. This was supported by EEG measurements [203].
  - In adults, tolterodine effectively decreased the symptoms of detrusor overactivity and caused fewer side-effects than oxybutynin. Studies in neurogenic bladder were promising, but this was not confirmed in pediatric OAB studies. There were three explanations given for this failure: (a) the chosen primary endpoints in the study design, (b) pediatric OAB has never been proven to have the same pathophysiology as in adults, and (c) the large difference in metabolism of the drug, dependent of genetic differences in cytochrome P450 (CYP)-metabolism, resulting in underdosing in 1/3 of children. Therefore, the drug was never approved in children [220–222].
  - The extended-release form was developed for the adult indication, evaluated in two pediatric studies, but without clinically significant benefit [223, 224].
  - Although the evidence level 3C is in line with other available drugs, the large intra-individual PK variation related to the CYP-polymorphisms for metabolism of the drug suggests that it should not be first line therapy [220, 225, 226].
- Propiverine hydrochloride is an antimuscarinic drug with proven efficacy and better tolerability than oxybutynin in children with OAB. This was documented in a phase 3 trial. Both ICI and ICCS classifies this drug as evidence level 1 B/C, but there were never confirmatory data with propiverine as monotherapy [227–229].
- Solifenacin is a recently developed antimuscarinic with some interesting characteristics [206, 230–233]. Given once daily, it significantly decreased urgency episodes in a pooled analysis of four pivotal trials including more than 2800 adults. PK/PD data in adults demonstrated a long half-life, enabling once daily administration with a potential of increasing therapy compliance [234]. Additional studies showed promising results [205, 232]. The side effect profile, with a high affinity for the M3 muscarinic receptor, is more favourable than that of some nonspecific antimuscarinics. In children with neurogenic bladder and OAB, studies documented a good safety profile and a half-life almost comparable to adults [205, 235, 236].
- Preliminary PK data show that metabolism of the drug is even higher than expected from adult data. Recent PK/PD data in children with neurogenic bladder and OAB are in line with the

PK predicted data based on adult studies. The clinical effect seems to be better in neurogenic bladder than in OAB in the paediatric population, although the evidence is limited. The drug has a very acid taste, which makes crushing the pills to individualize the dose to the size of the child challenging. Moreover, the drug powder is very irritative, especially to the eye, if the pills are cut or crushed.

- Several drugs have been proposed off-label as alternatives to anticholinergics such as calcium channel blockers, NSAIDs, alpha-adrenergic blockers, and beta-3-adrenoceptor mimetics. However, none have enough evidence to recommend as first line OAB therapy in children.
- Calcium channel blockers and alpha-adrenergic blockers have common side effects, including hypotension, facial flushing, headache, dizziness, constipation, and nausea.
- Fesoterodine [237] has documented antimuscarinic PD-effect on OAB in adults, and comparable PK characteristics in children [238], but more side-effects than solifenacin, without improved efficacy [203, 225, 239–241]. Darifenacin is approved for adults, but without convincing data in children [203, 242].
- If one anticholinergic is not effective or has intolerable side effects, it is reasonable to switch to another given different PK and side effect profiles. However, combination therapy of two anti-muscarinics is not recommended since there are no clinical data regarding efficacy or side effects.

(c) Tricyclic antidepressants are useful agents for facilitating urine storage, by both decreasing detrusor contractility and increasing outlet resistance. They are no longer recommended as first line treatment for MNE. The mechanism of action NE remains unclear, but might involve anticholinergic effects on the bladder, effects on sleep patterns and reduced nocturnal polyuria (through reduced natriuresis) [169]. Many expert consensus papers consider imipramine as a third-line therapy for OAB after urotherapy and anticholinergics for LUTS. Imipramine has weak anticholinergic effects as well as adrenergic effects on bladder muscle. It may exert a local anaesthetic-like action on the bladder. Clinically, imipramine is effective by decreasing bladder contractility and increasing outlet resistance. Stimulation of the beta-receptor by peripheral blockade of noradrenaline reuptake could account for the decrease in bladder contractility, and stimulation of the alpha-receptors in the smooth muscle of the bladder base and proximal urethra increases bladder outlet resistance [203, 215, 243]. Some favour reboxetine because of efficacy and safety reasons, although convincing data are lacking [244, 245].

(d) The beta3-adrenoreceptor agonist mirabegron is the first of a new type of drugs targeting the orthosympathetic pathway [145, 246, 247]. In controlled randomised trials in adults mirabegron had a positive effect on OAB symptoms [145, 234]. A few limited studies have supported this effect in children [234, 248–251]. The tablets cannot be crushed or chewed because of major changes in bioavailability, and this limits their use in smaller children [231].

(e) Electro neuromodulation

Electro neuromodulation using cutaneous stimulation of either the sacral or peripheral nerves is a treatment modality in children, gaining interest over the last decade despite some controversy regarding efficacy. The devices and techniques are very heterogeneous, using skin surface or percutaneous electrodes (TENS or percutaneous electrical nerve stimulation), but also anal or intravesical

electrodes. The devices have varying frequencies, waveforms and intensities, but there is no evidence regarding the optimal choice for each subtype of patients. The major proposed indication is in OAB patients refractory to antimuscarinic drug therapy, either alone or in combination with the drug. If percutaneous needle sacral stimulation is beneficial, an implantable pacemaker is a possible option in very treatment-resistant patients.

(f) Botulinum toxin
There is increasing evidence to support the use of botulinum A-toxin in the detrusor for treatment-refractory OAB (EB 3C). It was approved by the Food and Drug Administration in 2021 for neurogenic bladder detrusor overactivity. The safety and efficacy is well-documented. Small, single center studies show promising results in children with OAB, but prospective controlled studies are ongoing.

Dysfunctional Voiding (Staccato Voiding)

Voiding dysfunction in children is rare. Approximately 1% of 7-year-olds will have a uroflowmetry clearly deviating from normal [55, 252]. The predominant pattern is that of staccato voiding, which consists of frequent interruptions of detrusor initiated voiding. Dysfunctional voiding has many names, including non-neuropathic bladder-sphincter dyscoordination and overactive urethra another, but ICCS recommends the term 'dysfunctional voiding' [36]. The symptoms are to some extent similar to those of the urge syndrome, although recurrent UTI, constipation and soiling are more prevalent in this patient group. It is generally agreed that diagnosis requires a consistent pattern of three consecutive characteristic uroflowmetry results. The aetiology of dysfunctional voiding is not fully elucidated. The simplest theory is that it can be a long-term effect caused by voiding pains following a UTI, where the child learns to protect the urethra from the full urine stream by contracting the sphincter and continues to do so after the urethral pain has disappeared. This may lead to overtraining of the pelvic floor and the urethral sphincter during holding. It is also hypothesized that it is a sign of a delayed maturation of the interaction between the detrusor and the pelvic floor or an increased nociceptive receptor response in the proximal part of urethra.

Besides elimination of UTI and constipation, initial treatment consists of bladder rehabilitation with timed voids and double voiding twice a day. If insufficient, uroflow-biofeedback training may provide additional pelvic floor relaxation [143]. A treatment trial with an alpha-adrenergic blocker can be considered in refractory cases, although the evidence is weak [252, 253]. This approach is based on the fact that the physiological internal sphincter is controlled by alpha-adrenergic receptors in the smooth musculature of the bladder neck and proximal urethra. Although the theoretical expected effect would be best in those patients with bladder neck obstruction, good results are described in patients with detrusor sphincter dyssynergia, suggesting that there may also be an effect on striated sphincter tone [254–257]. To date, alpha-adrenergic blockers have no approved indication in children. Terazosin, alfuzosin and other similar new alpha-adrenergic blockers are more selective for the urinary tract. The side effects are orthostatic hypotension, tachycardia and a possible first-dose phenomenon with faintness, dizziness, palpitations and syncope. Effectiveness can be monitored with a combination uroflowmetry and residual urine measurement. Special techniques have been used in therapy-resistant cases, like training in voiding schools, as well as intermittent catheterisation.

Underactive Bladder

This entity is characterized by few (≤3) daily voids with unusually large volumes, poor bladder emptying, and a very high prevalence of UTI, constipation, and vesicoureteral reflux; it is more frequent in girls [258, 259]. The child typically strains during micturition and leakage occurs secondary to overflow incontinence. The

aetiology is multifactorial but one hypothesis suggests that it is the result of sustained bladder-sphincter dyscoordination. In some children, the syndrome is associated with a prior history of previous infravesical obstruction. In order to establish the correct diagnosis, it is often necessary to perform a urodynamic examination to exclude an associated underlying neuropathy. The cystometry shows large bladder capacity, most often a weak detrusor, a negative bethanechol test and no signs of obstruction on the pressure-flow examination. The treatment is similar to that described for dysfunctional voiding except that a larger proportion of patients require a period of clean intermittent catheterisation [270]. Structured training programs offer a non-invasive alternative.

Neuropathic Incontinence

Although incontinence secondary to neuropathy is rare, it is associated with significant risks of serious long-term injury to the urinary tract as well as decreased renal function [260, 261]. This chapter will not provide details of the different types of incontinence due to neuropathic causes, but emphasize the importance of close and thorough long-term monitoring of bladder and renal function in such patients. The variability of urodynamic abnormalities and their ability to change over time in children with congenital neurological defects such as myelomeningocele illustrates the need for life-long specialist follow-up [9, 29]. Treatment of severe neuropathic incontinence usually requires a large interdisciplinary team, including pediatric nephrology, urology, neurology, neurosurgery, orthopedics, psychology, and physiotherapy. Furthermore, all incontinent children should be screened for neurological defects as outlined above. If a suspicion of neuropathy is raised, an MRI of the spinal canal should be performed together with urodynamic evaluation, cystourethrogram, and ultrasound of the urinary tract. Conservative treatment includes antimuscarinics, either peroral or intravesical, clean intermittent catheterization, laxatives, and prophylactic antibiotics. Surgical treatments include bladder augmentation, cutaneous vesicostomy, anti-reflux surgery, and continence preserving surgery. Intravesical injection of botulinum toxin represents a newer method of detrusor relaxant therapy.

Structural Incontinence

Anatomical abnormalities can also be associated with significant voiding symptoms and are potentially damaging forms of incontinence [262]. Although not all are visible by physical examination, it is mandatory to inspect the genital region of the incontinent child for a possible cause of the incontinence.

Sphincter by-pass: Dribbling incontinence should always raise suspicion of a structural defect in the urinary tract, such as an ectopic ureter, often arising from the upper segment of a duplex kidney. Other forms of structural incontinence with sphincter by-pass are the exstrophy complex and epispadia, where diagnosis is much less complicated than the subsequent complex treatment.

Urinary tract obstruction: Voiding dysfunction and incontinence resulting from congenital malformations that cause LUT obstruction is much more common in boys than girls. The classical example is a posterior urethral valve, which most often arises from the colliculus and forms a membrane that obstructs urinary flow. In the severe forms, the diagnosis may be detected by prenatal ultrasound showing a distended bladder, bilateral hydronephrosis, and hydroureter. In milder cases, the diagnosis is often obtained by urodynamic evaluation, either by reduced flow rate on a uroflowmetry or by detrusor hyperactivity during filling and high pressures during voiding on invasive urodynamic investigation in a boy with treatment resistant day-time incontinence. Valve patients have long-lasting bladder dysfunction that often requires treatment and should be followed to adulthood [7]. Another problem in these patients is polyuria caused by post-obstructive renal tubular damage. Especially

at night, this can result in bladder retention and a nocturnal catheter may be necessary. Other forms of LUT obstructions exist ranging from bladder neck obstruction, syringocele, meatal stenosis, and in rare cases a stenosis caused by phimosis.

Other Types of Incontinence

Giggle Incontinence

Giggle incontinence typically occurs during prepuberty and more commonly in girls [259, 263]. The aetiology is unknown [264]. Physical and urodynamic examination are most often normal. It has been postulated that the giggling, via central nervous system centres, triggers a reflex relaxation of the urethral sphincter, which starts a bladder contraction and micturition. The syndrome is often very disturbing for the affected child, and it is of little comfort to the child that the condition generally improves over time. Many different treatment modalities have been tried, but no controlled trials exist. No treatment is documented as effective, and most are ineffective. Sympathicomimetic agents, such as methylphenidate, and imipramine, together with biofeedback training for better pelvic floor control and for control of the sphincter are some of the treatment options. Others have tried anticholinergics. Only 50% of patients benefit from medication [263, 265–268].

Vaginal Voiding

In a substantial number of girls, the hymen is funnel-shaped or the labia are partly fused, and during voiding part of the stream will enter the vagina so that the urine will dribble after the patient has left the toilet. In addition, some girls, especially obese ones, tend to sit on the toilet with the thighs close together, which will obstruct normal urine flow and direct some urine into the vagina during micturition. Symptoms are diagnostic, with dribbling just after leaving the toilet and the treatment is simple in most cases: the child changes to a backward position on the toilet, with one leg on each side of the toilet so that she will be forced to spread her legs widely.

Summary

NE and voiding dysfunction are very common disorders of childhood and adolescence that despite their benign nature are often chronic and cause significant negative effects on the child's well-being. Furthermore, although the large majority of patients have a non-organic etiology, some have an underlying structural or neurogenic anomaly that causes not only resistance to standard therapy but also poses a threat to renal function. With a structured approach based mainly on a thorough history and physical examination and a frequency-volume chart, it is possible to identify subjects at risk for having underlying pathology and to obtain the correct diagnosis in the majority of cases. Only a minority of patients need invasive urodynamic investigation and imaging. In patients with NE, knowledge about functional bladder capacity and nocturnal urine volume facilitate the choice of treatment modality. The initial conservative treatment of daytime incontinence is based primarily upon bladder rehabilitation and only some patients need pharmacological treatment, biofeedback, or more aggressive treatment. Although there have been advances in our understanding of the mechanisms behind non-neuropathic bladder-sphincter dysfunction, there are still many unanswered questions and good evidence-based treatment modalities are still needed.

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Part X

Hypertension

48 Hypertension: Epidemiology, Evaluation, and Blood Pressure Monitoring

Ian Macumber and Andrew M. South

Abbreviations

2016 ESH guidelines	2016 European Society of Hypertension guidelines for the management of high blood pressure in children and adults
2017 CPG	2017 American Academy of Pediatrics Clinical Practice Guideline for Screening and Management of High Blood Pressure in Children and Adolescents
ABPM	Ambulatory blood pressure monitoring
BSA	Body surface area
CKD	Chronic kidney disease
CTA	Computed tomographic angiography
Fourth Report	2004 Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents
HTN	Hypertension
LVH	Left ventricular hypertrophy
LVMI	Left ventricular mass index
MRA	Magnetic resonance angiography

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Introduction and Importance

Hypertension (HTN) remains an important but underappreciated medical condition in youth. In the short term, acute and chronic HTN rarely are associated with increased morbidity and mortality during childhood, though specific sub-populations of youth with secondary HTN (e.g., patients with kidney failure on dialysis) have an increased incidence of morbidity and mortality during childhood. Youth-onset HTN predicts persistent adult HTN and cardiovascular disease [1] and is associated with target organ damage both in childhood and adulthood [2]. While long-term outcome data remain limited [3], it is paramount that children

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and adolescents continue to be appropriately screened for HTN and that patients with HTN are accurately diagnosed, evaluated, and treated by health care providers with sufficient expertise in pediatric HTN [4]. In this chapter, we will discuss blood pressure screening recommendations and high-risk populations, compare the different methods of blood pressure assessment, and review diagnosis and evaluation recommendations for children and adolescents with HTN.

Morbidity and Mortality During Childhood

Cardiac Complications

Subclinical cardiovascular disease is detectable in children and young adults with HTN. Left ventricular hypertrophy (LVH), defined by an elevated left ventricular mass index (LVMI) relative to body size, is detectable in approximately 40% of children and young adults with HTN [5–7]. Children with HTN had impaired ventricular relaxation and diastolic dysfunction compared to healthy peers with normal blood pressure [8]. Higher blood pressure was associated with fatty streaks and fibrous plaques in the coronary arteries and aorta of deceased children and young adults in the Bogalusa Heart Study [9]. Congestive heart failure may develop, presenting with peripheral or pulmonary edema, dyspnea, chest pain, or a gallop rhythm. Some or all of these symptoms can occur in up to 29% of patients with acute severe HTN [10]. Cardiomegaly may be detected on imaging, though chest radiographs have poor sensitivity and specificity and should not be used to screen for cardiomegaly in this setting [11]. LVH was found in 43% of pediatrics patients presenting with hypertensive emergency [12], suggesting a high prevalence of chronic HTN.

Neuropathy/Retinopathy

Neurologic manifestations are the most common symptoms and include headache and dizziness, with seizures occurring in up to 20% of patients [13]. Facial nerve palsy secondary to acute severe HTN is most commonly seen in children and can occur in 5% of cases [14]. Additional neurologic signs include vomiting, mental status changes and lethargy (i.e., encephalopathy), and visual disturbances including cortical blindness. The constellation of mental status changes, headache, visual disturbances, and seizures, coupled with radiographic findings, constitutes reversible posterior leukoencephalopathy syndrome. Increased blood pressure over time was strongly associated with development of reversible posterior leukoencephalopathy syndrome in children and adolescents at high risk [15]. Visual changes may be a sign of hypertensive retinopathy and can be due to ischemic neuropathy, retinal infarcts, optic disk edema, cortical blindness, or increased intracranial pressure. A careful physical exam can reveal papilledema or retinal hemorrhages, which indicate target organ damage in the eyes, and it is important to note that lack of visual changes does not exclude hypertensive retinopathy [16].

Cognitive Dysfunction

Increasing evidence suggests that HTN affects neurocognition during childhood and into adulthood. Multiple studies have shown that children with HTN perform worse in tests of neurocognitive function compared to normotensive controls, though of note these individuals still test in the normal range [17–19]. Recent evidence suggests that uric acid may play a role in HTN-associated neurocognitive changes [20]. Neurocognitive test results improve with successful HTN treatment [21, 22].

Kidney Disease

Kidney disease—both acute and chronic—remains one of the most common etiologies of acute severe HTN in children and adolescents [13]. Less robust evidence suggests that acute severe HTN may contribute to kidney dysfunction as well. In adults, hypertensive crisis has been associated with elevated levels of urine neutrophil gelatinase-associated lipocalin [23], an early urinary marker of acute kidney injury. The data in children are less clear, though hyponatremic hypertensive syndrome—severe HTN and natriuresis thought to be due to increased renin-angiotensin-aldosterone system activity—has been described in children with acute severe HTN [24, 25], particularly those with unilateral renal artery stenosis.

Risk Across Life Course

Numerous clinical and epidemiological studies with long-term follow up have consistently demonstrated that, across diverse populations, childhood blood pressure tracks with blood pressure throughout adulthood and that youth-onset HTN increases the risk of HTN, cardiovascular disease, and kidney disease, including associated mortality, in later adulthood [1, 26, 27]. Data from the Fels Longitudinal Study demonstrated that even a single childhood systolic blood pressure measurement that exceeded age- and sex-specific criteria (at approximately the 50th percentile) increased the odds of HTN and metabolic syndrome at ≥30 years of age [28]. This association increased exponentially with increasing numbers of blood pressure measurements [28, 29]. Individuals with higher blood pressure trajectories during childhood were more likely to have HTN as adults, a relationship that was more pronounced during adolescence in individuals who were Black and in females [30–32]. In the Childhood Determinants of Adult Health Study, children with high blood pressure had a 35% increased risk of having high blood pressure as adults compared to those with normal childhood blood pressure [27]. Higher blood pressure trajectories were also associated with the presence of more cardiovascular disease risk factors at age 38 years [32].

Pediatric blood pressure consistently has been shown to be associated with several intermediate markers of cardiovascular disease risk. Individuals with higher blood pressure during both childhood and adulthood had an increased risk of higher pulse wave velocity and higher carotid intima-media thickness (a marker of subclinical arteriosclerosis) as older adults [33–39]. Further, individuals who decreased their number of cardiovascular disease risk factors from childhood into adulthood, including resolution of prior pediatric high blood pressure, had a decreased risk of higher pulse wave velocity and higher carotid intima-media thickness compared to individuals whose risk factors did not improve into adulthood [40, 41]. Moreover, higher childhood blood pressure (12–18 years) and greater blood pressure trajectories in young adulthood (18–30 years) were associated with higher coronary artery calcification scores 25 years later [42, 43].

Higher blood pressure trajectories during childhood and adolescence were associated with greater left ventricular mass index and LVH in later adulthood [36, 44]. Cumulative exposure to higher blood pressure in childhood and young adulthood—defined by the total and incremental areas under the curve—were significantly associated with concentric LVH, eccentric LVH, and left ventricular systolic and diastolic dysfunction in later adulthood [45–47]. In the Bogalusa Heart Study, higher blood pressure beginning in childhood was associated with greater premature mortality due to coronary artery disease (mean age 45 years) [48]. In large population-based studies from Sweden, higher blood pressure in adolescents and young adults was associated with an increased risk of coronary heart disease, myocardial infarction, stroke, and death from cardiovascular causes [49, 50]. In the Harvard Alumni Health Study, high blood pressure at young adult age was associated with increased risk of death from coronary heart disease and cardiovascular disease in later adulthood (mean age 46 years) [51]. Compared to normal or elevated blood pressure, HTN in young adults was associated with shortened life expectancy of at least 2.2 years and accounted for 58.5% excess deaths from cardiovascular disease [52].

Blood pressure during childhood is also associated with the development of kidney disease later in life. In the Hanzhong Adolescent Hypertension Cohort, worse blood pressure trajectory starting in childhood (mean age 12 years) was associated with chronic kidney disease (CKD) in adults 30 years later, defined as either an estimated glomerular filtration rate 30–60 mL/min/1.73 m^2 or albuminuria [53]. In a study of over 2.6 million healthy adolescents in Israel (mean age 17 years), adolescents with HTN had double the risk of kidney failure and triple the risk of stroke mortality after a median of 20 years of follow up [54, 55].

As summarized by the 2020 U.S. Preventive Services Task Force, "there is adequate evidence about the longitudinal association between high blood pressure in children and adolescents and high blood pressure and other intermediate outcomes in adults" [3]. Despite this wealth of evi-

dence, long-term interventional trials are lacking due in large part to the difficulty to perform such studies. Hence, it remains unknown whether identifying or treating youth-onset HTN improves long-term outcomes.

Definition and Epidemiology

In adults, normative blood pressure thresholds and definitions for hypertensive disorders are based on robust outcomes data, including cardiovascular and all-cause mortality and morbidity [56]. However in children there is a lack of clinical outcome data, and pediatric blood pressure thresholds and definitions are instead based on epidemiological data from normative blood pressure distributions obtained from approximately 50,000 healthy children [57]. Normative distributions are based upon age, sex, and height, as these factors strongly influence blood pressure values.

In 2017, the American Academy of Pediatrics released a clinical practice guideline (CPG) for the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents [4]. According to this guideline, normative blood pressure values should be based on age, sex, and height-based percentile distributions for children aged 1–13 years (tables can be found in [4]). These normative values are based on reference populations from which children with overweight or obesity were excluded since these individuals have higher blood pressure than their normal-weight peers [58–61]. Thresholds based on absolute blood pressure values were instituted for adolescents 13 years and older to integrate with the 2017 American College of Cardiology/American Heart Association (AHA) Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults [56].

Definition and Classification

Children and Adolescents Aged 1–17 Years of Age

The 2017 CPG instituted several changes in how it defined hypertensive disorders (Table 48.1). "Prehypertension" was replaced with "elevated blood pressure" to be consistent with adult guidelines and to emphasize the importance of lifestyle measures in preventing HTN development. Stage 2 HTN in children <13 years of age is defined as "≥95th percentile plus 12 mmHg". In adolescents 13 years of age and older, the definitions are now based only on absolute blood pressure values to be harmonious with the 2017 adult guidelines.

Table 48.1 Blood pressure classification by casual (office) measurement. Adapted from [4]

Classification	Age <13 Years[a]	Age ≥13 Years (mmHg)
Normal BP	<90th %ile or <120/80 mmHg	<120/80
High BP	≥90th %ile or ≥120/80 mmHg	≥120/80
Elevated BP	≥90th to <95th %ile or 120–129/<80 mmHg	120–129/<80
HTN	≥95th %ile or ≥130/80 mmHg	≥130/80
Stage 1 HTN	≥95th to <95th %ile + 12 mmHg or 130–139/80–89 mmHg	130–139/80–89
Stage 2 HTN	≥95th %ile + 12 mmHg or ≥140/90 mmHg	≥140/90

BP blood pressure

[a] Whichever is lower between the percentile-based or absolute blood pressure

Infants Under a Year of Age

The normative blood pressure tables in the 2017 CPG start at age 1 year [4]. For infants under a year of age, the definition of HTN is less clear due to a paucity of normative data and increased difficulty in obtaining accurate and consistent blood pressure measurements. The 2017 CPG recommended using data compiled in Dionne et al. 2012 to define high blood pressure values in neonates up to 44 weeks postmenstrual age [62] and the published blood pressure curves provided in the 1987 Second Task Force for infants aged 1–12 months of age [63]. However, important caveats remain and warrant further study, including validation in larger and more diverse populations and normative values for infants born preterm, small for gestational age, or who have additional early-life risk factors.

Hypertensive Emergency

Acute severe HTN—also termed hypertensive emergency, a type of hypertensive crisis—describes any episode of acute, symptomatic HTN with severely high blood pressure and is associated with acute target organ damage, including acute neurologic injury, acute kidney injury, and hypertensive cardiomyopathy/congestive heart failure [4]. There exist a wide variety of symptoms—and symptom severity—that may occur with acute severe HTN. There is no established, evidenced-based definition for what constitutes severely high blood pressure. According to the 2017 CPG, clinicians should be concerned when blood pressure is 30 mmHg or more above the 95th percentile for age, sex, and height [4]. Among pediatric patients presenting to the emergency department with hypertensive crisis, 98–100% had blood pressure at or above the stage 2 HTN threshold, and patients with hypertensive emergency had higher systolic and diastolic blood pressure ratios (blood pressure indexed to the stage 2 HTN threshold) compared to those with hypertensive urgency (i.e., severely high blood pressure without symptoms or evidence of target organ damage) [10, 64, 65]. Blood pressure equal to the stage 2 HTN threshold strongly predicted development of reversible posterior leukoencephalopathy syndrome in children and adolescents at high risk [15]. Future studies are warranted to better define acute severe HTN in children, including at which blood pressure thresholds predict organ or life-threatening injury, and to develop more specific criteria to define "symptomatic" HTN with adequate predictive capabilities.

Incidence and Prevalence

True HTN incidence in the general pediatric population has not been well described historically or after publication of the 2017 CPG. In the United States, a Houston, Texas school-based screening study estimated the yearly incidence of HTN in adolescents to be approximately 0.5% [66]. In that study, HTN incidence was significantly higher in at-risk groups, including 1.4% per year in adolescents who had a prior elevated blood pressure reading that normalized at follow up and 6.6% per year in adolescents with persistent elevated blood pressure [66]. Future investigation is needed to better define the true HTN incidence in the general pediatric population and select high-risk groups.

Prior to the 2017 CPG, multiple studies estimated youth-onset HTN prevalence to be 2–5% [67–69], while a recent meta-analysis reported the global prevalence to be 4% [70]. HTN is more common in certain pediatric populations: up to 50% of youth with CKD [71] and 24% of youth with obesity [72]. Several studies have assessed the change in prevalence of pediatric hypertensive disorders due to the 2017 CPG compared to the Fourth Report [59, 61, 73–76]. Due in part to the slightly lower threshold to define HTN in the 2017 CPG, high blood pressure prevalence increased in 6–17-year-olds in a large study in China compared to the Fourth Report [74]. A large, retrospective cohort study found prevalence of HTN to be 4.9% and of elevated blood pressure to be 4.3% [77]. Bell et al. found that high blood pressure prevalence (defined as elevated blood pressure plus HTN [78]) increased using the 2017 CPG criteria, while HTN prevalence decreased [59]. This was due, in part, to a large proportion of adolescent participants aged 13–14 years who were categorized using absolute thresholds rather than percentiles.

Screening Recommendations

There is a plethora of data demonstrating that youth-onset HTN increases the risk of morbidity and mortality in childhood (see section "Morbidity and Mortality During Childhood") and persists into adulthood and increases the future risk of cardiovascular disease, kidney disease, and related mortality across the life course (see section "Risk Across Life Course"). However, there remains insufficient evidence regarding which blood pressure thresholds predict future risk, the appropriate age to start blood pressure screening, or if screening is even efficacious [3]. Despite this, numerous organizations

have recommended routine HTN screening of asymptomatic children and adolescents [79–82].

The 2017 CPG was incorporated into the recommendations of the AHA, Hypertension Canada, and the American Academy of Family Physicians [83–85]. According to these guidelines, annual blood pressure measurements should start at age 3 years—ideally at annual health supervision visits. Children with known risk factors such as obesity, CKD, history of aortic arch obstruction or coarctation (regardless of repair status), diabetes mellitus, or who are taking medication known to increase blood pressure should have screening blood pressure measurement at every health care encounter [4]. The 2016 European Society of Hypertension guidelines for the management of high blood pressure in children and adolescents confirmed that routine blood pressure screening should start at age 3 years but clarified that, once normal blood pressure is established, further screening should occur every 2 years [86].

Children <3 years of age should continue to have screening blood pressure measurements at every health care encounter if they have certain high-risk conditions (Table 48.2) [4]. Unfortunately, several studies have shown that these children often are not screened appropriately [87]. It is important to note that for individuals who are otherwise healthy, a diagnosis of HTN should not be based on blood pressures measured during for acute illnesses or other complaints that could be associated with inaccurate blood pressure measurement. There remains no direct evidence that high blood pressure screening in children and adolescents aged 3–17 years improves health outcomes [3]. Thus, more evidence is needed to support routine blood pressure screening and to establish recommendations that are more specific.

Table 48.2 At-risk children in whom blood pressure screening should occur before 3 years of age. Adapted from [4]

Evidence of elevated intracranial pressure
Prematurity <32 weeks' completed gestation, small for gestational age, very low birth weight
Neonatal complications that required Neonatal Intensive Care Unit admission
Umbilical artery lines in the neonatal period
Congenital heart disease irrespective of repair status
Recurrent urinary tract infections
Hematuria or proteinuria
Known kidney disease, including congenital anomalies of the kidney and urinary tract
Family history of congenital kidney disease
Solid organ transplant or stem cell transplant recipient
History of malignancy, including active or in remission
Taking medications known to raise blood pressure
Systemic medical conditions associated with HTN: sickle cell disease, William syndrome, Marfan syndrome, neurofibromatosis, tuberous sclerosis complex, etc.

Emerging At-Risk Populations

Certain existing risk factors for HTN in youth such as antenatal and postnatal exposures require further investigation to clarify the precise nature of the risk conferred. Emerging evidence suggests novel risk factors may exist but that require further investigation.

Antenatal and early-life exposures are increasingly associated with higher blood pressure and risk of developing HTN during childhood and across the life course [88, 89]. Lower **birth weight** and greater relative weight gain in infancy have been associated with higher blood pressure trajectories during childhood [90]. Individuals born preterm with very low birth weight had higher blood pressure compared to term-born peers as adolescents (age 14–15 years) and young adults (age 18–23 years) [91, 92]. Young adults born extremely preterm with extremely low birth weight had higher ambulatory blood pressure at age 25 years and a greater trajectory of ambulatory blood pressure from 18 to 25 years compared to term-born peers [93]. Lower birth weight was associated with an increased risk of coronary artery disease in adulthood [94]. Individuals born preterm or small for gestational age demonstrated altered heart structure and function as young and middle-aged adults [95–97]. However, specific thresholds for prematurity and birth weight as well as the relative contributions of each exposure that confer risk for subsequent HTN, cardiovascular disease, and CKD remain unknown [89, 98]. It is unclear if being born prematurely

or small for gestational age per se are the relevant risk factors or if it is the antecedent maternal or fetal condition or exposures that led to premature birth and fetal size that actually confers the risk, such as maternal preeclampsia [99].

In addition, neonatal conditions such as **bronchopulmonary dysplasia** and postnatal exposures such as **acute kidney injury** during the neonatal period may increase the risk of HTN in the neonatal period and throughout childhood and adulthood, however these data remain limited and warrant further study [100–103]. The underlying mechanisms responsible for this increased risk of HTN remain incompletely defined but may include programming of key hormonal pathways such as the renin-angiotensin-aldosterone system, uric acid, klotho, and alterations to the structure and function of various tissues, including the kidneys, heart, vasculature, and brain [91, 92, 96, 104–109]. See Chap. 50 for a more detailed discussion of the mechanisms underlying development and etiology of youth-onset HTN.

There is increasing evidence that **unmet social needs**, health disparities, and adverse childhood experiences are associated with adverse health outcomes, including higher blood pressure, HTN, and cardiovascular disease [110–113]. Data from 7125 participants aged 8–17 years in the National Health and Nutrition Examination Survey revealed that household and child food insecurity were associated with high blood pressure [78]. The Georgia Stress and Heart study found that participants who experienced multiple childhood traumatic events had a greater blood pressure rise from childhood to after age 30 than those who did not [114]. Among participants in the Coronary Artery Risk Development in Young Adults study, low childhood socioeconomic status predicted increased blood pressure over a 10-year period directly and indirectly through childhood family environment, negative emotionality, and health behavior [115]. Thus, further investigation is needed to define these exposures and develop appropriate screening approaches and interventions to mitigate adverse health risks [116].

Blood Pressure Measurement

Normative blood pressure data in children are based on manual pressures taken in the upper portion of the right upper extremity in large cross-sectional studies [57]. For this reason, a definitive diagnosis of HTN continues to rely on manual blood pressures in the right upper extremity [4]. However, oscillometric blood pressure devices are commonly used in the clinical setting due to their ease of use and can be an appropriate screening approach, as long as any high blood pressure readings are confirmed with manual, auscultatory measurement [4]. Oscillometric devices provide some advantages compared to manual blood pressures. They require less training and are more user-friendly. The machines can be programmed to automatically take repeated measurements over time—useful in the inpatient setting—and they tend to provide consistent intra-individual results once the patient has acclimated to the cuff. Readings can be stored and retrieved from the device at a later time.

Despite these advantages, oscillometric devices have significant shortcomings. Importantly, they measure mean arterial pressure (MAP) rather than directly measuring systolic and diastolic blood pressures. The devices use proprietary algorithms to convert the MAP to systolic and diastolic blood pressure values that have not been independently evaluated or validated, leading to variability between brands and concerns about measurement bias in clinical research. Reputable resources to determine if a device has been properly validated are accessible on the internet and are summarized here [117, 118].

As a whole, oscillometric devices tend to overestimate systolic blood pressure when compared to auscultatory measurement, while diastolic measurement is often inconsistent and both over and underestimated [119, 120]. Generally, manual auscultated blood pressure more accurately predicts target organ damage in children [121]. Importantly, few oscillometric devices have been validated in children, and studies have found that certain devices differ in their accuracy between adults and children [122, 123]. U.S. Food

and Drug Administration device approval only indicates that the device is as good as previous devices already on the market, and less than 20% of all devices have undergone validation testing for accuracy.

For these reasons, oscillometric blood pressure readings obtained with devices—validated in children—are acceptable to use as a screening method but should not be used alone to diagnose hypertensive disorders in children [4]. High blood pressures obtained via the oscillometric method should be confirmed by auscultation [4, 124].

Technical Considerations

Appropriate measurement technique is critical for accurate blood pressure assessment and HTN diagnosis. Errors in technique are especially common in children and include incorrect positioning, improper cuff bladder size, inadequate equipment, or poor provider technique.

Best practice mandates that the patient should sit calmly in a quiet room for a minimum of 5 min prior to blood pressure measurement [4, 85]. The patient's back should be supported and their feet flat on the floor and uncrossed. Unless there is a contraindication, blood pressure should be measured in the upper portion of the right upper extremity. In addition to normative blood pressures being defined in the right upper extremity, this approach also avoids falsely normal blood pressures in the setting of coarctation of the aorta. Repeated blood pressure measurements should be taken in the same extremity at each visit.

Incorrect cuff size is a common source of error, especially in children; cuffs that are too small can provide a falsely high reading, while cuffs that are too large can provide a falsely low reading [125]. Health care providers must ensure that they have the full range of cuff sizes, including thigh cuffs. The provider should measure the mid-arm circumference, located at the midpoint between the acromion and the olecranon. The cuff's bladder length should cover 75–100% of the arm circumference, without overlapping itself, and the width should be 37–50% of the arm circumference. The cuff should be placed on bare skin—avoiding tightly rolled clothing proximally—and should fit snuggly but not too tightly approximately 2 cm above the antecubital fossa. The middle of the bladder cuff should be placed over the brachial artery proximal to the antecubital fossa; most cuffs have a line or marker to assist with lining the cuff up with the artery. The extremity should be supported at heart level (i.e., the midpoint of the sternum) by the provider or a table. The patient should remain calm and still during measurement, and both the provider and the patient should avoid talking.

For manual measurements, the provider should first palpate and locate the brachial artery, which is often just proximal and medial to the antecubital fossa. They should palpate the radial artery pulse as they inflate the cuff, up to 20–30 mmHg above the point at which the pulse is no longer palpable and avoiding over-inflation. Either the diaphragm or bell of the stethoscope should be placed over the brachial artery below the lower edge of the cuff, ensuring that the diaphragm or bell is not under the cuff itself. The provider should ensure a steady and appropriate rate of cuff deflation, typically 2–3 mmHg per second. The first audible sound (phase I Korotkoff) and last audible sound (phase V Korotkoff) should be noted as the systolic and diastolic blood pressures to the nearest even number (i.e., by 2 mmHg). If the Korotkoff sounds are heard at 0 mmHg, the point at which the sound is muffled (phase IV Korotkoff) should be taken as the diastolic blood pressure.

If the patient's initial blood pressure is high, it should be repeated at least two additional times separated by 1–2 min and the subsequent readings should be averaged. If the initial blood pressures were taken manually, this averaged value is used to classify the patient's blood pressure category. If measured via an oscillometric device, then two manual blood pressure measurements should be taken and averaged [4].

Lower Extremity Measurement

For blood pressure screening and HTN diagnosis, blood pressure measurement in the lower extremities should generally be avoided as blood pres-

sures measured in the lower extremities have been shown to correlate poorly and unreliably with blood pressures measured in the upper extremities and centrally [126–129]. However, there may be times that upper extremity blood pressures are medically contraindicated or four-extremity blood pressures are indicated (e.g., screening for coarctation of the aorta). For lower extremity measurements, the patient should be in the prone position and the appropriately sized cuff should be placed on the middle of the upper portion of the lower extremity. Cuff size recommendations are the same as for the upper extremity, and the stethoscope's diaphragm or bell is placed over the popliteal artery in the popliteal fossa [4].

Measurement in Infants

Infants provide a challenge to accurate and reliable blood pressures measurement [62]. Oscillometric readings are recommended, at least until the child's upper extremity is large enough to appropriately fit the smallest auscultatory cuff [4]. Ideally, the cuff should be left undisturbed for 15 min after placement and the measurement should not be taken within 90 min of a feed or medical intervention. Once the patient is in a relatively calm state, the blood pressure should be taken three times at 2-min intervals with the patient in the prone or supine position [62]. While these guidelines provide a standardized approach to measurement of infantile blood pressures, they are cumbersome and may be difficult to follow in practice, especially in an outpatient setting.

Importance of Repeated Measurements

As stated previously, high initial blood pressures need to be repeated at least two times at the same visit and the values averaged. Ample evidence has shown that misclassification of high blood pressure status occurs when based on only a single blood pressure reading [130, 131]. Studies in adult patients have consistently found a mean decrease in systolic blood pressure on repeat blood pressure measurements that can reclassify many patients to a lower blood pressure category [132–134]. In a cohort study of over 800,000 children in the United States, among children with an initially high blood pressure value 71% had normal blood pressure value when rechecked at the same visit; 51% had a mean blood pressure below the 95th percentile when the two blood pressure values were averaged [130].

Repeating blood pressure measurements at subsequent visits is also critical in order to appropriately classify children. Large, population-based studies have found that 63–66% of children with initial blood pressure measurements meeting criteria for HTN will have normal blood pressures when rechecked at subsequent visits [135, 136]. Unfortunately, many children do not have appropriate follow-up visits for repeat blood pressure checks within the recommended time period. A large, retrospective study found that among 6108 individuals that had initial blood pressure values at the HTN threshold by office measurement, only 45% had a repeat measurement obtained within 6 months of the original blood pressure measurement [135].

Blood Pressure Follow-Up and Diagnosis Protocol

No additional action needs to be taken if a patient's averaged manual blood pressure is normal (Fig. 48.1). The patient should continue to have their blood pressure measured at least yearly, depending on their specific factors. However, if a screening blood pressure value is high, specific follow-up recommendations to repeat and monitor blood pressure should be followed [4]. All patients with a confirmed high blood pressure measurement should undergo lifestyle counseling (see Chap. XX).

Elevated Blood Pressure

For patients whose initial blood pressure is in the 'elevated' category, blood pressure should be checked again in 6 months. If the blood pressure remains in the elevated category at the 6-month follow-up visit, a more thorough examination should be conducted, including blood pressure

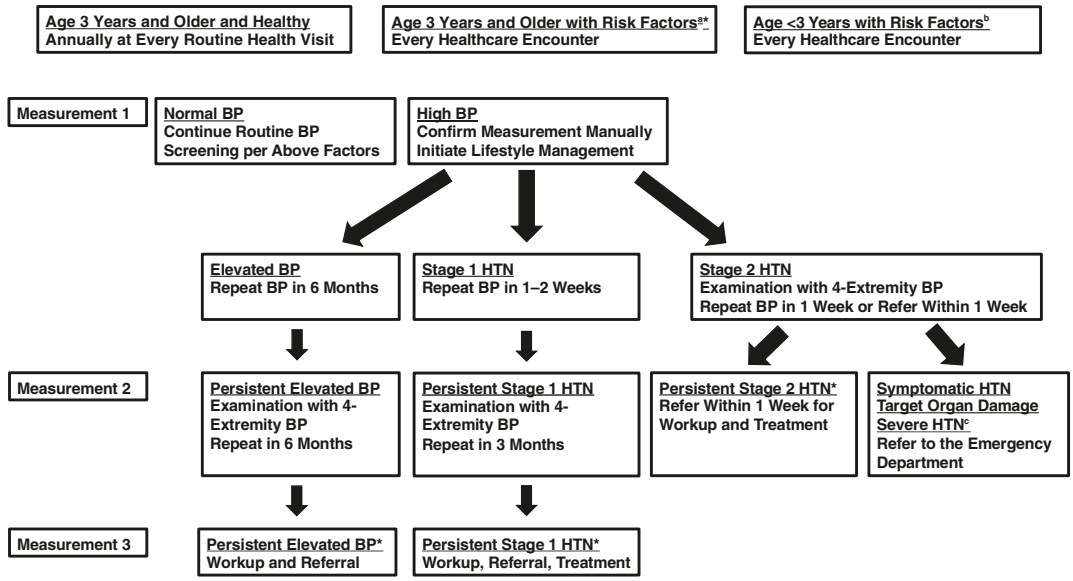

Fig. 48.1 Blood pressure screening and follow-up protocol. Recommendations based on the 2017 CPG [4]. Flow diagram based on classification of each BP measurement; a hypertension disorder diagnosis requires at least three BP measurements—obtained with appropriate methods—from different days. If BP changes classification on repeated measurement, follow flow diagram for most recent values. ABPM can be used to more accurately assess follow-up BP. [a]Obesity, chronic kidney disease, history of aortic arch obstruction or coarctation (regardless of repair status), diabetes mellitus, or taking medication known to increase BP; [b]see Table 48.2; [c]systolic or diastolic BP >30 mmHg above the 95th percentile for age, sex, and height if age <13 years or >180/120 mmHg if age ≥13 years; *indicates consideration for obtaining ABPM. ABPM, ambulatory blood pressure monitoring; *BP* blood pressure; *HTN* hypertension

measurements in both upper extremities and at least one lower extremity. The patient should be evaluated again in 6 months for another blood pressure assessment [4]. If at that point the blood pressure remains in the elevated blood pressure category—now persistent for at least 1 year—the patient should undergo ambulatory blood pressure monitoring (ABPM) under the direction of a qualified provider if available (see section "Ambulatory Blood Pressure Monitoring"), an initial workup (see section "Evaluation"), and/or referral to a HTN specialist. If at any point during this time the blood pressure normalizes, the patient can resume the standard screening regimen based on their individual risk. However, if their blood pressure worsens to the HTN category, follow-up measurements should occur more frequently as discussed below.

Stage 1 Hypertension

Patients with an initial blood pressure in the stage 1 HTN category should have their blood pressure rechecked in 1–2 weeks and, if still high, should include both upper extremities and at least one lower extremity. If stage 1 HTN persists, blood pressure should be rechecked in 3 months. If at the third blood pressure check, the patient has persistent stage 1 HTN, they should be referred to a HTN specialist for ABPM, further evaluation, and treatment. As before, if the blood pressure improves or worsens, the provider should follow the appropriate follow-up protocol.

Stage 2 Hypertension

Patients with an initial blood pressure value in the stage 2 HTN category require more urgent follow up and evaluation. The blood pressure should be measured in the bilateral upper extremities and at least one lower extremity at the first visit. A repeat blood pressure should be obtained within 1 week, or the patient should be referred to a HTN specialist to be seen within 1 week. If the second measurement remains in the stage 2 HTN category, the patient should be referred to and

seen by a HTN specialist within 1 week, in order to initiate a thorough evaluation, ABPM, and to determine if the patient warrants treatment.

Importantly, patients whose blood pressure at any point is at the stage 2 HTN category and who are symptomatic, have signs of target organ damage, or whose blood pressure is >30 mmHg above the 95th percentile for age, sex, and height (or >180/120 mmHg for adolescents), should be sent right away to the emergency department.

Ambulatory Blood Pressure Monitoring

ABPM has become a critical tool in the diagnosis and management of HTN. It more accurately predicts intermediate cardiovascular outcome measures [137] and development of future HTN [138] compared to other blood pressure measurement methods, may better distinguish primary from secondary causes of HTN [139], and is the only modality to formally evaluate for white coat HTN or masked HTN. It also has greater intra-individual reproducibility compared to in-office or out-of-office blood pressure measurement [140, 141]. The 2016 European Society of Hypertension (2016 ESH) guidelines and the 2017 CPG recognize the increasing importance of ABPM and provide specific recommendations for its use in children and adolescents at risk of HTN and as a screening tool in specific patient populations [4, 86].

Technical Considerations

The AHA has published detailed guidelines for ABPM in children and adolescents [2, 142]. The following is a brief summary, and the latest version of the AHA guidelines should be referred to for more detail.

To ensure that the data are properly collected and analyzed, ABPM should be administered by clinical staff who have sufficient training and expertise. Use of ABPM devices validated in children is essential [117, 123]. The cuff should be placed on the upper portion of the non-dominant upper extremity to minimize disruption of daily activities, unless medically contraindicated. However, if there is a significant discrepancy in office blood pressure measurement (e.g., approximately >10 mmHg) between the two upper extremities, the cuff should be placed on the upper extremity with the higher blood pressure. Proper cuff size is determined using the same guidelines that are used for office blood pressure measurement [4].

Prior to having the ABPM placed, the patient and family should receive education about the study. Patients should be instructed to keep their arm still during blood pressure measurements whenever possible [2]. Patients should record major activities in a provided diary, including the patient's sleep and awake times, episodes of physical activity, stress, or pain, and medication doses. The device should only be taken off during bathing or swimming. The monitor should be programmed to record blood pressures every 15–20 min while awake and every 20–30 min while asleep.

An ABPM study is considered adequate for interpretation if there is at least one valid reading every hour and at least 40–50 valid readings in the 24-h period [2]. Interpretation should be based on the recorded sleep and awake times as opposed to the preset times to decrease the risk of misclassification [143]. When interpreting the results, it is important to determine how well the patient tolerated wearing the cuff. Reported intolerance during both awake and sleep are common [144, 145], and may influence the results of the study [146]. Some monitors use actigraphy to measure patient activity at night, and this may be a more accurate method to determine the patient's sleep time and quality [147]. The provider should screen all recorded blood pressures to identify and exclude outlier values that are above the preset parameters for values that are likely out of the normal physiologic range; most software packages do this automatically.

Classifications Incorporating Ambulatory Blood Pressure Monitoring

ABPM classifications recommended by the AHA and the 2017 CPG are primarily based on blood pressure percentiles for age, sex, and height that are distinct from those based on manual blood pressure

measurement. However, important caveats remain regarding the available normative ABPM data in children. The generally accepted normative ABPM data in use are from a study of the German Working Group in 949 healthy individuals aged 5–20 years [148]. While this is widely used for ABPM interpretation, there are legitimate concerns regarding the data. The study was comprised of Caucasian children from Mid-Europe, and it is not clear how generalizable the data are to other populations. There have been few robust attempts thus far to address this issue [149], and it remains one of the most pressing issues for pediatric ABPM. There is also surprisingly little variability in the mean diastolic blood pressures among children of different heights, unlike that seen with casual blood pressures. The data also do not account for children under 120 cm in height, which may be problematic in young children with chronic conditions such as CKD in whom short stature is common.

As opposed to adult ABPM, which classifies blood pressure according to mean ambulatory blood pressure values, pediatric ABPM classification is based upon ambulatory blood pressure mean values (Table 48.3). The device software calculates and reports mean ambulatory blood pressure values for the 24-h period and for the awake and asleep periods. Mean values at or above the 95th percentile for sex and either age or height confirm a diagnosis of ambulatory HTN (Table 48.4). Adolescent patients who are tall—whose 95th percentile ambulatory blood pressure values are higher than the absolute values used in adults—are often categorized according to the adult criteria. Prior recommendations included the blood pressure load (the proportion of ABPM readings that are at or above the 95th percentile) in the diagnostic classification [2].

Table 48.3 Ambulatory blood pressure parameters

Variable	Definition	Abnormal threshold
Mean BP	Mean BP over each time period	≥95th %ile
% dipping	[Mean awake − mean sleep BP]/[mean awake BP] × 100	<10%
BP index	Mean BP/95th %ile per time period	≥1

BP blood pressure

Table 48.4 Ambulatory blood pressure classification. Adapted from [261]

Category	Casual (office) BP Age <13 years[a] Age ≥13 years	Mean ambulatory BP Age <13 years[a] Age ≥13 years
Normal BP	<95th %ile or <130/80 mmHg <130/80 mmHg	<95th %ile <130/80 wake, <110/65 sleep, <125/75 24 h
White coat hypertension	≥95th %ile or ≥130/80 mmHg ≥130/80 mmHg	<95th %ile <130/80 wake, <110/65 sleep, <125/75 24 h
Masked hypertension	<95th %ile or <130/80 mmHg <130/80 mmHg	≥95th %ile ≥130/80 wake, ≥110/65 sleep, ≥125/75 24 h
Ambulatory hypertension	≥95th or ≥130/80 mmHg ≥130/80 mmHg	≥95th %ile ≥130/80 wake, ≥110/65 sleep, ≥125/75 24 h

BP blood pressures

[a] Whichever is lower between the percentile of absolute blood pressure

However, more recent data suggest that blood pressure load does not provide prognostic value in children [150, 151], and it is no longer recommended to be included in the classification.

White Coat Hypertension

White coat HTN is a condition in which the patient's office blood pressure is in the HTN category but ambulatory blood pressure is normal (Table 48.4). White coat HTN is common in children, occurring in up to 30–50% of patients who are referred for HTN evaluation [152, 153].

Traditionally thought to be a benign condition, increasing evidence supports that white coat HTN may be associated with progression to sustained HTN [152, 154]. Although the prevalence of LVH between children who are normotensive and children with white coat HTN is not significantly different, there is evidence that patients with white coat HTN have higher LVMI [153]. The presence of obesity complicates the interpretation of these and similar data, as children with white coat HTN are more likely to have obesity, and obesity is associated with higher LVMI [155]. Studies of associations with other intermediate outcomes, such as increased carotid intima-media thickness,

have provided mixed results [152, 156]. In adults, white coat HTN is associated with increased risks of cardiovascular and all-cause mortality compared to patients with normal blood pressure [157, 158], although controversy exists as to whether this increased mortality is due to white coat HTN or is due to confounding bias from common comorbidities [159].

Given the uncertainty regarding the clinical implications of white coat HTN, the optimal approach to management of these patients remains undefined. The 2017 CPG recommend that children with white coat HTN have repeat ABPM in 1–2 years to assess for improvement or progression to ambulatory HTN [4]. Further investigation is warranted to better delineate these relationships and the risk white coat HTN confers and to develop evidence-based management strategies.

Masked Hypertension

Patients whose office blood pressure is normal but who have ambulatory HTN on ABPM are classified as having masked HTN (Table 48.4). Due to the fact that patients with normal blood pressures in the office, masked HTN is generally only diagnosed in high-risk patients that undergo ABPM screening. This ascertainment bias makes it difficult to accurately estimate masked HTN prevalence in children and adolescents; several studies have estimated an 8–10% prevalence [160–162]. Masked HTN is more strongly associated with target organ damage than white coat HTN [156]. It is more common in children with secondary HTN and may help in determining HTN etiology.

Isolated nocturnal HTN, a specific form of masked HTN in which ambulatory blood pressure is only in the HTN range during sleep and office blood pressure is normal, has been strongly associated with cardiovascular outcomes in adults [163, 164]. The few studies in children have shown associations with obesity, CKD, insulin resistance, and proteinuria [165–168].

Indications for Ambulatory Blood Pressure Monitoring

While ABPM is recommended for all children and adolescents who are being evaluated for HTN, certain patient populations who are at high-risk for HTN (including those with masked HTN) may benefit from more frequent ABPM screening (Table 48.5) [4]. In particular, the 2017 CPG recommends annual ABPM screening in patients with CKD and patients with coarctation of the aorta starting no later than 12 years post-repair.

Table 48.5 Indications for ambulatory blood pressure monitoring and associated hypertensive disorders

Clinical condition	Hypertensive disorder
Chronic kidney disease	Nocturnal HTN, masked HTN [71]
Kidney failure on dialysis	Masked HTN, worse HTN on non-dialysis days [262]
Solid-organ transplant recipient	Nocturnal HTN, masked HTN [263, 264]
Solitary kidney	Ambulatory HTN, non-dipping [265]
Type 1 or type 2 diabetes mellitus	Nocturnal HTN, masked HTN [266, 267]
Coarctation of the aorta	Masked HTN, post-repair HTN [268]
Sickle cell disease	Non-dipping [269]
History of prematurity	Ambulatory HTN, non-dipping [102, 270]

Evaluation

Once a diagnosis of HTN is established, careful and thorough evaluation, including a complete history and physical exam, is necessary to determine the etiology, detect target organ damage, and identify co-morbid conditions that may increase the risk of cardiovascular disease. In addition, emerging evidence suggests that children and adolescents with HTN may have specific cardiovascular phenotypes that stratify their risk of future cardiovascular disease. The history and physical exam in particular can detect causes of secondary HTN and can help avoid expensive and unnecessary testing.

History

With the widespread use of electronic health records, it is important to verify a patient's history recorded in their chart with what they and

their caregiver report. This includes reconciling records from other practices and hospital systems. The history should evaluate for risk factors for systemic disease that may cause secondary HTN as well as risk factors for primary HTN (see Table 48.6 and Chap. XX). These include, but are not limited to, acute kidney injury or CKD (gross hematuria, edema, poor growth), rheumatologic diseases (fever, weight loss, rashes, arthritis), hyper- or hypothyroidism/endocrine disease, including pheochromocytomas/paragangliomas, (weight changes, sweating, flushing, hair changes, palpitations, tachycardia, anxiety), and sleep-disordered breathing (snoring, daytime somnolence). A careful review of medications, supplements, and recreational substance use is important. Most patients with HTN are asymptomatic, but some patients describe nonspecific symptoms such as fatigue and sleep disturbances [169]. It is vitally important to detect symptoms associated with severe HTN, including headaches, visual changes, mental status changes, seizures, chest pain, shortness of breath, and edema (Table 48.7). In infants, severe, symptomatic HTN may present as irritability, poor feeding, or poor weight gain.

Table 48.6 Key components of a history relevant to pediatric hypertension. Adapted from [271]

History	Risk factor
Birth history	Gestational age and birth weight Preterm birth (<37 weeks' completed gestational age) Small for gestational age
	Fetal risk factors Intrauterine growth restriction Abnormal fetal ultrasound including oligohydramnios
	Maternal risk factors Chronic hypertension/gestational hypertension, preeclampsia, maternal diabetes/gestational diabetes, maternal medications (including antenatal corticosteroids), maternal recreational drugs, maternal tobacco or alcohol
	Birth risk factors: Cesarean section Asphyxia Resuscitation
	Postnatal risk factors: Neonatal Intensive Care Unit admission Umbilical catheters Intubation with mechanical ventilation
Medical history	Kidney disease Congenital anomalies of the kidney and urinary tract (including hydronephrosis and vesicoureteral reflux), solitary kidney Urinary tract infection, including pyelonephritis Acute kidney injury (including in neonatal period) Chronic kidney disease, kidney failure on dialysis
	Prior hypertension (including in neonatal period)
	Congenital and inherited conditions Congenital heart disease, including coarctation of the aorta Genetic variants and syndromes
	Bronchopulmonary dysplasia, chronic lung disease, asthma
	Growth history and trajectories, overweight or obesity
	Systemic disease Rheumatologic (systemic lupus erythematosus) Endocrinologic (diabetes mellitus of any type, hyperthyroidism, hypothyroidism) Hematologic (sickle cell disease)
	Malignancy (remission or active) Chemotherapy and which regimen Complications

Table 48.6 (continued)

History	Risk factor
	Transplantation: Solid organ, stem cell Medication regimen including immunosuppression Complications
	Mental/behavioral health Attention deficit hyperactivity disorder Anxiety Depression Post-traumatic stress disorder
	Sleep-disordered breathing
	Dyslipidemia, insulin resistance, hyperglycemia
Surgical history	Repaired congenital heart disease or coarctation of the aorta or vascular malformations
	Gender identity surgery
Medications/drugs	Decongestants (pseudoephedrine, phenylpropanolamine)
	Corticosteroids (short-term or chronic)
	Non-steroidal anti-inflammatory drugs
	Stimulants Methylphenidate Methylxanthines (caffeine, theophylline, aminophylline)
	Recreational drugs (marijuana, amphetamines, cocaine)
	Immunosuppressants (tacrolimus, cyclosporine)
	Tricyclic antidepressants
	Oral contraceptives
	Nicotine
Family history[a]	Hypertension (and cause)
	Overweight or obesity, dyslipidemia, diabetes mellitus of any type
	Cardiovascular disease[b] Coronary heart disease (myocardial infarction, angina pectoris, heart failure, and coronary death) Heart surgery Sudden cardiac death Cerebrovascular disease (stroke, transient ischemic attack) Peripheral artery disease Aortic atherosclerosis Thoracic or abdominal aortic aneurysm
	Chronic kidney disease, kidney failure on dialysis
	Congenital or inherited disease, including genetic variants and syndromes
	Systemic disease: rheumatologic, endocrinologic, hematologic, etc.
Nutritional history	Sodium intake
	Fruit and vegetable intake
	Fruit juice and sugar-sweetened beverages
	Caffeine, black licorice
Physical activity history	Organized sports or activities
	Access to physical activity resources (playground, gym, physical education in school)
Social history	More than one home and whose homes
	Who lives at home
	Who is (are) the primary caregiver(s)
	School environment (nurse available to check blood pressure)
	Unmet social needs Food insecurity Transportation insecurity Financial insecurity

[a] Immediate biological family members, including parents, siblings, grandparents, aunts, and uncles
[b] Especially in men before 55 years and women before 65 years

Table 48.7 Key signs and symptoms associated with hypertensive crisis

Symptoms	Physical exam findings
Visual changes	Papilledema
Headaches	Retinal hemorrhages or exudates
Mental status changes	Facial palsy
Shortness of breath	Tachypnea, crackles
Chest pains	Cardiac findings: gallop, new murmur
Gross hematuria	Peripheral edema
Seizures	Abdominal mass

Increasing and substantial evidence has demonstrated that a thorough antenatal and neonatal history should be obtained (see section "Screening Recommendations"), including maternal and fetal conditions, abnormal fetal ultrasounds (including cystic kidneys, hydronephrosis, oligohydramnios), gestational age and birth weight (including intrauterine/fetal growth restriction, small for gestational age), delivery mode and indication, and maternal medication exposure. A detailed review of any hospitalizations and medical conditions in the neonatal period is important, including admission to the Neonatal Intensive Care Unit, umbilical artery/vein catheter placement, intubation and mechanical ventilation, bronchopulmonary dysplasia, asphyxia, head or body cooling, antimicrobial exposure, nonsteroidal anti-inflammatory drug exposure, urinary tract infections, acute kidney injury, and HTN in the neonatal period.

Family history may give clues to the risk of primary HTN or select heritable forms of secondary HTN or CKD. Questions should center around family members who have HTN and at what age they were diagnosed, as well as for other conditions that are associated with HTN, such as CKD, diabetes, obesity, and sleep-disordered breathing. A specific surgical history should be obtained, with emphasis on surgeries involving the heart, intra-abdominal and retroperitoneal organs, or vasculature.

A general nutrition history should be obtained, focusing on aspects of the diet known to be associated with HTN. There is strong evidence in adults linking high dietary sodium intake with increased risk of HTN, and while the evidence in children is not as robust, numerous associations have been described, particularly in children with overweight and obesity [170]. Potassium intake is an important nutritional component, as higher potassium intake has been associated with lower blood pressures in adults [171]. This is thought to be related to the interplay between sodium and potassium, with potassium depletion increasing blood pressure sensitivity to sodium intake [172]. In children and adolescents, the relationship between potassium and blood pressure is less clear. Some studies have found an association between higher long-term potassium intake and lower risk of HTN, particularly in adolescent females [173, 174].

A comprehensive social history should be carefully obtained, as one can acquire important information about risk factors for HTN as well as barriers that may hinder the patient's ability to access and adhere to lifestyle counseling and medication. Providers should understand the patient's home and school environment, including who are the primary caregivers and if a school nurse is available to check blood pressure. Providers should assess a patient's current physical activity status, including organized sports and activities, physical education in school, and access to parks, playgrounds, and gyms. Increasing evidence in children and adults demonstrates that health disparities and unmet social needs—including limited access to health care, food, and transportation as well as financial insecurity—contribute to poor health outcomes in the short and long term [78, 175–182]. Importantly, these risk factors and barriers preferentially affect under-represented populations [183]. Providers should be cognizant of how systemic and implicit bias can affect patients' health, including HTN [184]. Further investigation into these important relationships is strongly needed, including mitigating barriers to equitable health care and social needs.

Physical Exam

The physical exam will be normal in the majority of children and adolescents with HTN. Blood pressures should be measured at all four extremi-

Table 48.8 Physical exam findings associated with secondary hypertension. Adapted from [4]

System	Exam finding	Associated condition
Growth	Overweight, obesity, central adiposity	Cushing syndrome, hypothyroidism
	Low body mass index for age and sex	Hyperthyroidism, neuroblastoma, pheochromocytoma, paraganglioma
	Short stature and poor growth velocity	Systemic disease, including chronic kidney disease, kidney failure on dialysis, inherited condition/syndrome
Dermatologic	Café-au-lait spots	Neurofibromatosis
	Neurofibromas	Neurofibromatosis
	Ash-leaf spots	Tuberous sclerosis complex
	Malar rash	Systemic lupus erythematosus
	Striae, acne	Cushing syndrome
	Flushing, diaphoresis	Pheochromocytoma, paraganglioma, neuroblastoma
	Acanthosis nigricans	Insulin resistance, type 2 diabetes mellitus, Cushing syndrome
	Spaced nipples	Turner syndrome
HEENT	Round/moon facies	Cushing syndrome
	Proptosis	Hyperthyroidism
	Adenotonsillar hypertrophy	Sleep-disordered breathing
	Elfin facies	William syndrome
	Thyromegaly	Hyperthyroidism
	Webbed neck	Turner syndrome
Cardiovascular	Tachycardia	Hyperthyroidism, neuroblastoma, pheochromocytoma/paraganglioma
	Decreased femoral pulses	Coarctation of the aorta
	Friction rub	Pericarditis (systemic lupus erythematosus, kidney failure)
	Murmur	Coarctation of the aorta
Abdomen	Mass	Wilm's tumor, pheochromocytoma/paraganglioma, neuroblastoma
	Palpable kidney	Polycystic kidney disease, hydronephrosis
	Hepatosplenomegaly	Infantile polycystic disease
	Bruit	Renovascular disease
	Edema	Kidney/renovascular disease, liver disease, heart disease, protein-losing enteropathy
Back/Flank	Flank tenderness	Pyelonephritis, obstruction, acute nephritis
Genitourinary	Ambiguous genitalia	Congenital adrenal hyperplasia
Extremities	Disparity in BPs	Aortic coarctation
	Edema	Kidney disease
	Joint swelling/stiffness	Systemic lupus erythematosus, collagen vascular disease
	Muscle weakness	Hyperaldosteronism, monogenic hypertension
	Rickets	Chronic kidney disease, X-linked hypophosphatemia

ties to assess for coarctation of the aorta and mid-aortic syndrome. Classically, coarctation is associated with systolic HTN in the upper extremities, low blood pressure in the lower extremities, and delayed or diminished femoral pulses (also called the brachial-femoral delay) [185]. Certain physical exam findings are associated with various causes of secondary HTN and may help with diagnosis (Table 48.8). In addition, the patient should be evaluated for potential signs of HTN-induced target organ damage (Table 48.7).

Secondary Causes and Target Organ Damage

Laboratory Workup

Laboratory evaluation is performed to investigate for secondary causes of HTN and to assess for HTN-induced target organ damage, most commonly in the heart and kidneys (Table 48.9). Up to 20% of all children with HTN have CKD [4] so generally all pediatric patients with HTN should have a chemistry panel to evaluate kidney function and electrolytes. Further laboratory

Table 48.9 Recommended laboratory evaluation in patients with hypertension. Adapted from [4]

Standard assessment	Secondary assessment
Blood	*Blood*
Metabolic panel, including phosphorus, AST, ALT	Uric acid
Complete blood count with differential	Vitamin D 25-hydroxy and vitamin D-1,25-dihydroxy
Lipid profile	Complements 3 and 4
Hemoglobin A1c	Antistreptolysin O antibody
Plasma renin activity and serum aldosterone	Anti-DNase B antibody
Thyroid stimulating hormone and free thyroxine	Antinuclear antibody
	Antineutrophil cytoplasmic antibodies with proteinase 3 and myeloperoxidase antibodies
	Serum cortisol and precursors
	Plasma fractionated metanephrine
Urine	*Urine*
Urinalysis	Drug screen
Albumin, protein, and creatinine	24-h stone profile
Sodium and potassium	24-h fractionated metanephrines and catecholamines
Calcium	Vanillylmandelic acid and homovanillic acid

ALT alanine aminotransferase, *AST* aspartate aminotransferase

Table 48.10 Laboratory abnormalities in monogenic forms of hypertension. Adapted from [271]

Condition	Pota	PRA	Aldosterone	ARR
Glucocorticoid remediable aldosteronism/Familial hyperaldosteronism type I	N or ↓	↓	N or ↑	↑
Familial hyperaldosteronism type II–IV	N or ↓	↑	↑	↑
Liddle syndrome	N or ↓	↓	↓	–
Gordon syndrome	N or ↑	↓	N or ↑	↑
Apparent mineralocorticoid excess	N or ↓	↓	↓	–
Congenital adrenal hyperplasia	N or ↓	↓	↓	–
Familial glucocorticoid resistance	N or ↓	↓	↓	–

ARR aldosterone-to-renin ratio, *PRA* plasma renin activity

evaluation will depend on the specific history, physical exam, and screening study results, but may include thyroid studies, plasma renin activity, serum aldosterone levels, neurohormones, and genetic testing.

Serum electrolytes are commonly normal in children with HTN, but abnormal results may assist in identifying secondary causes of HTN including primary or secondary hyperaldosteronism or monogenic HTN [186]. These can often be distinguished from each other via serum potassium levels, plasma renin activity, and serum aldosterone levels; genetic testing is usually necessary to confirm the diagnosis (Table 48.10, Chap. XX). It is important to note that serum potassium can be normal in aldosterone-mediated HTN and that these diagnoses should be considered if an adherent patient's blood pressure is poorly controlled on multiple anti-hypertensive medications that are optimally dosed [187].

A urinalysis with urine microscopy should be performed on all children and adolescents who have HTN to investigate for secondary causes, and it may play a role in identifying target organ damage. Microscopic hematuria, defined as at least six red blood cells per high-powered field, may indicate glomerular disease—including acute glomerulonephritis of various etiologies—particularly if it confirms dysmorphic red blood cells or red blood cell casts. Proteinuria is concerning for acute kidney injury or CKD and can be a sign of target organ damage to the kidneys. A spot urine sample to quantify urine protein is highly predictive of 24-h concentrations [188] and, if positive (>0.2 mg protein/mg creatinine), should be repeated on a first-morning urine sample to differentiate orthostatic proteinuria—a common and benign condition wherein the first-morning sample is normal [189]—from pathologic proteinuria that is persistently positive.

Albuminuria

Albuminuria is defined as >30 mg albumin/g creatinine in a spot urine sample. In adults it is considered a marker of HTN-induced target organ damage to the kidneys and predicts cardiovascular disease [190]. However, there is inadequate data in children to confirm a causal relationship between albuminuria and HTN [191], and the 2017 CPG does not recommended screening for albuminuria in youth with primary HTN. Thus, the role of urine albumin testing as a marker of HTN-induced kidney-specific target organ damage warrants further study.

Renin-Angiotensin-Aldosterone System

Of note, there may be increasing utility in measuring components of the renin-angiotensin-aldosterone system in circulation and in the urine in patients with HTN. Classically, plasma renin activity and serum aldosterone have been measured to assist in screening for renal artery stenosis and secondary and primary aldosteronism (which can occur in up to 15% of adults with HTN). In adults, a suppressed plasma renin activity, elevated serum aldosterone concentration, and aldosterone-to-renin ratio >20–30 (in [ng/dL]/[ng angiotensin I/mL/h])—even in the absence of hypokalemia—increase the likelihood of an aldosterone-mediated form of HTN [192–197]. Specific diagnostic criteria to screen for and diagnose primary aldosteronism in children do not exist, though some studies suggest an ARR >10 may have utility [198].

Baseline plasma renin activity and serum aldosterone are correlated with HTN severity, and higher values predict greater improvements in blood pressure and LVMI in response to treatment in youth with primary HTN [199]. Among 47 pediatric patients with HTN, higher values of baseline urine sodium-to-potassium ratio (a proxy measure of increased dietary sodium intake relative to potassium) were associated with lower plasma renin activity and higher aldosterone-to-renin ratio values [200]. These findings suggest that these measures of the renin-angiotensin-aldosterone system may distinguish emerging HTN phenotypes such as salt-sensitive blood pressure and renin-angiotensin-aldosterone system activation vs. suppression-mediated HTN.

However, renin and aldosterone measurements have limited clinical value in part because they only represent proximal and distal aspects of the renin-angiotensin-aldosterone system. It is crucially important to consider both pathways of the renin-angiotensin-aldosterone system: angiotensin-converting enzyme/angiotensin II and angiotensin-converting enzyme 2/angiotensin-(1–7) [201]. Emerging evidence supports the value of fully assessing both pathways of the renin-angiotensin-aldosterone system in individuals with HTN. Adults with untreated primary HTN had lower urinary angiotensin-(1–7) concentrations and 24-h excretion rates compared to control participants with normal blood pressure [202]. Children with primary HTN had higher plasma angiotensin-(1–7) levels compared to peers with normal blood pressure [203]. Adolescents born preterm with very low birth weight, who are at increased risk for HTN, had a higher ratio of angiotensin II to angiotensin-(1–7) in plasma compared to term-born peers, an association that was greater in individuals with obesity and in females [105, 204]. In addition, lower urinary angiotensin-(1–7) concentrations corrected for urine creatinine were associated with higher blood pressure in adolescents and adults and predicted higher blood pressure approximately 5 years later [106, 202]. However, accurate and reproducible measurement of the renin-angiotensin-aldosterone system depends upon adhering to rigorous methods [205, 206], and reliable normative data have not been fully defined for children or adults. Further research into the role of the renin-angiotensin-aldosterone system in pediatric HTN is ongoing.

Uric Acid

The utility of obtaining serum uric acid concentrations remains controversial. Observational studies consistently demonstrate that uric acid levels are associated with HTN in cross-section and predict development of HTN, CKD, and cardiovascular mortality over time, and that lowering uric acid (e.g., with allopurinol) reduces the risk of these outcomes [109, 207–216]. However,

data from clinical trials to lower uric acid levels remain conflicting [217, 218]. Evidence suggests that younger patients with shorter duration of HTN and better cardiovascular health may benefit from pharmacologic uric acid reduction [219, 220]. The reasons for these conflicting data are complex and include inadequate study design and variable patient populations/experimental disease models. Further, uric acid pathophysiology remains poorly described. The renin-angiotensin-aldosterone system likely mediates, in part, uric acid's deleterious effects, and uric acid can have anti-oxidant as well as pro-oxidant properties depending upon which tissue one investigates [109, 221–223]. The 2017 CPG concluded that there remains insufficient evidence to support or refute routine uric acid measurement when evaluating and managing youth with high blood pressure. This area remains an important area of further investigation, including the role of pharmacologic uric acid lowering.

Kidney Imaging

A **kidney ultrasound** should be considered when evaluating a patient for HTN, especially in those under 6 years of age or those who have an abnormal urinalysis or chemistry panel, have proteinuria, or who have a history of kidney abnormalities, acute kidney injury, urinary tract infections, or a family history of congenital anomalies of the kidney and urinary tract [4]. Ultrasonography can detect secondary HTN associated with CKD and aid in the diagnosis of renal artery stenosis; findings of note can include hypodysplasia, size discrepancy between both kidneys, solitary kidney or horseshoe kidney, cystic kidney disease, hydronephrosis, masses, increased echogenicity relative to the liver, and decreased corticomedullary differentiation.

For patients with a history of recurrent urinary tract infections (especially pyelonephritis), dimercaptosuccinic acid scintigraphy has historically been deemed helpful to identify scarring of the affected kidney, though it has well documented limitations [224]. To evaluate for scarring, it is recommended to wait at least 6 months after the most recent infection to allow for resolution of acute inflammation to avoid false-positive results [225]. HTN development may be delayed for years in patients who develop kidney scarring from urinary tract infections and vesicoureteral reflux [226].

Renovascular Imaging

Doppler ultrasonography can be used to screen for possible renal artery stenosis in normal-weight youth at least 8 years of age who are suspected of having renovascular HTN and can cooperate with the procedure [4]. Doppler studies are often the first-line imaging modality of choice to evaluate for renovascular HTN. However, sensitivity (64–90%) and specificity (68–70%) are low to detect or rule out renal artery stenosis and can be highly variable between sonographers and across institutions, particularly in patients with obesity and in younger children less than 8 years of age, and require extensive observer experience [227–229].

Further investigation is required when the Doppler study is normal but suspicion for renovascular disease remains high. **Computed tomographic angiography** (CTA) and **magnetic resonance angiography** (MRA) are commonly used non-invasive imaging modalities that have significantly higher sensitivity and specificity to detect renal artery stenosis compared to Doppler ultrasonography. Data in children are limited, but recent studies have estimated 88–100% sensitivity and 81–100% specificity for CTA [229–231]. For MRA, data are even more scarce, but one study estimated 81% sensitivity and 63% specificity [231]. Both CTA and MRA have additional advantages and limitations. CTA is less expensive and quicker to perform but involves significant radiation and contrast exposure, an important consideration in young patients. MRA is a longer, more expensive test that often requires sedation or general anesthesia in young children or patients with behavioral or developmental conditions that limit their ability to remain still during the test and involves exposure to contrast such as gadolinium.

The gold standard for diagnosis of renovascular HTN remains angiography [231]. Angiography may be therapeutic as well as diagnostic, but requires anesthesia, involves exposure to contrast and radiation, and is an invasive, higher risk pro-

cedure compared to CTA and MRA. A detailed description of the recommended diagnostic workflow for suspected renovascular hypertension is provided in Chap. XX.

Cardiac Imaging

Echocardiography is a useful and recommended test to diagnose cardiac causes of HTN—such as coarctation of the aorta—and HTN-induced target organ damage in the heart. The 2017 CPG recommends that echocardiography be performed when considering pharmacologic treatment of HTN [4], but many programs obtain echocardiograms for the majority of patients referred for HTN due to the fact that it is difficult to predict which patients have target organ damage. Recent evidence suggests that echocardiographic changes including LVH occur in youth with elevated blood pressure.

HTN can induce several deleterious changes to heart mass, structure, and function, including LVH, left ventricular remodeling, left atrial dilation, and, rarely, left ventricular systolic or diastolic dysfunction. Target organ damage in the heart occurs in approximately 40% of youth with HTN [5–7]. LVH is the most prominent evidence of target organ damage in children with HTN, and LVH should be defined by standardized left ventricular mass due to its correlation with body size [232]. Methods by which to calculate left ventricular mass are discussed in detail in Chap. 61.

Echocardiography should be repeated every 6–12 months to monitor for interval changes, including in patients with difficult-to-control HTN, concentric LVH, or reduced left ventricular ejection fraction. In patients with an initially normal echocardiogram, yearly repeated echocardiograms can be considered, especially for patients with stage 2 HTN, secondary HTN, or stable stage 1 HTN that is not well controlled.

While **electrocardiography** is low-cost and easy to perform, it has poor sensitivity and specificity for identifying LVH in children with HTN [233] and is therefore not recommended for use in the evaluation of pediatric HTN [4, 86].

Additional Considerations

Patients with a concern for HTN-related retinal findings should be referred to a pediatric ophthalmologist. The role of routine screening ophthalmologic exams by pediatric ophthalmologists in all patients with HTN remains unknown and warrants further study; however, availability of a pediatric ophthalmologist is a concern. Patients with HTN who have additional indications should undergo evaluation by a pediatric nephrologist for kidney biopsy; indications include presence of persistent proteinuria, hematuria, and acute kidney injury in addition to HTN. Patients with concern for or confirmed secondary causes of HTN should be referred to the relevant specialists for co-management with the HTN specialist (e.g., endocrinologist, cardiologist).

Co-morbidities

Patients with obesity or other risk factors for insulin resistance and diabetes mellitus should have a hemoglobin A1c assessed to screen for diabetes and liver enzymes (aspartate aminotransferase, alanine aminotransferase) to screen for non-alcoholic fatty liver disease, which is associated with HTN in children [4, 166, 234, 235]. A lipid profile should be obtained in all patients to evaluate for dyslipidemia; initial testing may be fasting or non-fasting [4]. Patients with obesity who are at higher risk should have a fasting lipid profile obtained.

Patients who have signs or symptoms of sleep-disordered breathing should be referred for polysomnography [4]. Potential symptoms include snoring, daytime somnolence, or reports of poor sleep quality. Adenotonsillar hypertrophy is a common cause of obstructive sleep apnea in children, and affected patients should be referred even in the absence of obvious symptoms. Unfortunately, access to centers with sufficient expertise in pediatric polysomnography and sleep medicine specialists is commonly limited and an area of pressing need in pediatrics.

Cardiovascular Phenotyping

There is increasing evidence that youth with HTN likely have a spectrum of cardiovascular abnormalities that may affect their HTN trajectory in the short term as well as their long-term

risk of cardiovascular disease across the life course [236, 237]. Newer, non-invasive imaging modalities are under investigation to assess cardiovascular target organ damage, including arterial structure and function, and to provide valuable normative data in children [238]. Measures of central arterial stiffness include pulse wave velocity and augmentation index. Pulse wave velocity estimates the speed at which the wave produced by ventricular systole propagates through the arterial circulation, while augmentation index is an indirect measure of central aortic pressure augmentation [239, 240]. Higher pulse wave velocity and augmentation index can indicate greater arterial stiffness associated with HTN; device-specific normative data exist for youth with and without HTN [241–244]. Carotid intima-media thickness—measured by high-spacial ultrasonography—quantifies the combined thickness of the intimal and medial layer of the carotid artery and is an established marker of subclinical arteriosclerosis [245]. Pediatric normative data are available [246]. Several pediatric studies have shown an association between HTN and higher carotid intima-media thickness [247], including in children with CKD [248, 249] (see Chap. 61 for a detailed review). Additional though less utilized studies include arterial flow-mediated dilation and distensibility [237].

Finally, abnormal autonomic function—characterized by abnormal heart rate or blood pressure variability and baroreflex sensitivity—associates with HTN in children and adults and predicts cardiovascular disease [108, 250–256]. At this time, however, these modalities are not yet recommended for routine use in the diagnosis or management of pediatric HTN [4, 86] and are not widely available in clinical practice, even to HTN specialists. More research is needed, including establishment of normative data and evidence-based cut-offs for abnormal values in diverse patient and healthy control populations.

Follow Up for Patients with Hypertension

All patients with HTN require close follow up with their HTN specialist and primary care provider to monitor their blood pressure and target organ damage response to lifestyle counseling and pharmacologic treatment. Youth treated with an anti-hypertensive medication should be followed up every 4–6 weeks until blood pressure is controlled and then every 2–6 months thereafter, while patients treated only with lifestyle counseling should be followed every 3–6 months. Follow-up periods and modality (HTN specialist clinic, primary care provider clinic, home, school) should be tailored to each individual patient and the resources available to them. ABPM has increasing importance in monitoring patients' response to treatment, especially in patients with CKD or kidney transplant recipients (see section "Ambulatory Blood Pressure Monitoring"). Specific recommendations for long-term follow up and transition of care when patients become adults remain undefined and are a major topic for further research.

Out-of-Office Measurement

Home

Home blood pressure monitoring with an oscillometric device or via manual measurement is not recommended to diagnose HTN in children, due in part to lack of normative data and heterogeneous technique [140], but may be a useful approach to blood pressure follow up to complement office and ABPM measurement [4]. Much like ABPM, home blood pressure measurement has the advantage of occurring in a familiar, calm environment that partially mitigates falsely high blood pressure readings and white coat HTN. Home blood pressure measurement often demonstrates better reproducibility compared to office-based blood pressure measurement [141].

School

Blood pressure measurements taken at school can be useful to monitor children with HTN. However, data on the accuracy of these measurements is limited, and universal protocols have yet to be developed. In addition, it is difficult to evaluate the skill and technique of the person taking the blood pressure. For these reasons, school blood pressure measurements are not recommended for use in the diagnosis of HTN [4].

As with office-based blood pressure measurement using oscillometric devices, it is crucial to use only those devices that are validated in children. Additional considerations include inter-device variation and difficulty in obtaining the correct cuff size [257].

Transition of Care

Health care transition from adolescence into adulthood is an underappreciated but crucially important aspect of care [258]. Exact transition recommendations and risk profiles for long-term outcomes for children with HTN remain undefined. Adolescents with HTN—and arguably those with other hypertension disorders such as elevated blood pressure and white coat HTN—regardless of whether they are receiving pharmacologic treatment should transition their care from a pediatric to an adult health care provider as they enter adulthood [4]. The specific age for transition can vary widely across centers and should be tailored to each individual patient based on their circumstances. The pediatric specialist managing their HTN should transfer all relevant information regarding etiology, target organ damage, co-morbidity evaluation and management history. Generally, adult primary care providers have sufficient experience to manage the majority of young adults with HTN, though certain populations may require adult specialist care, including patients with CKD or secondary HTN. It is extremely important, however, that the patients themselves and all adult providers caring for these transitioned patients be educated about the importance of pediatric HTN, especially the long-term cardiovascular risk conferred.

Conclusions and Future Directions

Pediatric HTN remains an important but underappreciated medical condition that has short and long-term implications for health during childhood and across the life course. Much progress has been made in recent years to better understand pediatric HTN risk factors, pathophysiology, detection, diagnosis, and related target organ damage. It remains critically important that primary care providers and HTN specialists—pediatric and adult providers alike—remain up-to-date on clinical care of youth with HTN and recognize its importance to long-term cardiovascular health. However, there are many areas that warrant further investigation to improve patients' cardiovascular health.

There is a pressing need for updated normative blood pressure data in youth of all ages and from diverse, international populations that can demonstrate intra-individual change over time as those individuals age and enter adulthood. This will allow for the transition of HTN definitions from more traditional, statistical/epidemiological-based normative blood pressure values to cardiovascular outcome-based approaches. Similarly, there is a need for robustly validated normative ABPM data from diverse populations that, ideally, will include cardiovascular outcome measures across the life course. This is especially true for populations with short stature or chronic medical conditions such as CKD. It remains unknown if ABPM data obtained during childhood is associated with increased risk of morbidity or mortality in later adulthood. There is no consensus regarding the appropriate blood pressure cutoffs for ABPM to confirm HTN, particularly in adolescents who are tall. The 2016 ESH guidelines recommend using adult ABPM values in cases in which pediatric thresholds exceed adult thresholds [86]. However, even the adult thresholds are not consistent, as different adult guidelines utilize different values to diagnose HTN [56, 259]. Clarification regarding the optimal thresholds is necessary for children and adolescents, as these competing guidelines can result in significantly different blood pressure classifications in pediatric patients [260]. Finally, rigorous validation and comparison of oscillometric and ABPM devices is a pressing need.

It is critically important to better understand how established and emerging early-life risk factors contribute to pediatric HTN and its short and long-term complications, including antenatal and neonatal exposures as well as social and health disparities. The long-term effects of the COVID-19 pandemic should be considered, including indirect effects from social/structural

changes and direct effects via severe infection and multisystem inflammatory syndrome in children. This new knowledge will allow for more targeted and appropriate blood pressure screening guidelines.

High-quality studies are required to determine if routine blood pressure screening practices can improve short and long-term outcomes. Further investigation is required to determine the optimal approach to defining cardiovascular phenotypes (e.g., pulse wave velocity, carotid intima-media thickness), the utility of biomarkers such as angiotensin-(1–7) and other components of the renin-angiotensin-aldosterone system, and how these studies could improve clinical care. Finally, definitive guidelines and best practices for the transition of care for youth with HTN who are entering adulthood is crucially important to answer the above questions.

Pediatric HTN remains a common and important medical condition with long-term health implications. Patients and health care providers alike should be aware of the importance of screening for, diagnosing, and evaluating patients who have HTN and the risk pediatric HTN confers on long-term cardiovascular health across the life course.

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Renovascular Hypertension in Children

49

Agnes Trautmann and Kjell Tullus

Introduction

Renovascular disease is a rare but important cause of childhood hypertension. It accounts for 10% of secondary childhood hypertension [1–3]. Anatomic stenosis of the renal artery results in renal hypoperfusion with consequent release of renin and activation of the renin-angiotensin-aldosterone system (RAAS) developing a renin-mediated hypertension. Renal artery stenosis is important to accurately diagnose, as it is potentially amenable to curative treatment with several endovascular and surgical techniques in more than half of affected children.

Many children with renovascular disease have additional complex abnormalities of other major blood vessels including aorta, cerebral, intestinal or iliac arteries. Usually, children with complex renovascular disease require treatment provided by a specialised multidisciplinary team of paediatric nephrologists, cardiologists, neurologists, interventional radiologists, vascular and neurovascular surgeons.

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Clinical Presentation

Renovascular hypertension is diagnosed at all ages with equally many cases found in all age groups.

Clinical Symptoms

The initial clinical presentation varies from asymptomatic to severe, life threatening symptoms including congestive heart failure or cerebral symptoms. The spectrum of cerebral symptoms is broad with headaches, visual disturbances, signs of acute hypertensive encephalopathy or cerebrovascular incident with convulsions, strokes with focal neurological deficits like facial palsy and hemiplegia. Young infants can also present with more unspecific symptoms and failure to thrive.

At time of diagnosis blood pressure is usually markedly increased, with predominantly stage 2 hypertension (blood pressure > 99th percentile + 5 mmHg). A systolic blood pressure of ≥160–180 mmHg is not uncommon. Target organ damage, in particular hypertensive left ventricular hypertrophy, may already be seen at initial presentation, depending on the stage and duration of hypertension.

Renovascular hypertension should also be suspected if there is inadequate blood pressure control with two or more antihypertensive drugs in the absence of any other identifiable cause.

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Several rare genetic syndromes are associated with childhood renovascular hypertension, with neurofibromatosis type 1 (NF type 1) and Williams' syndrome being the most common. Infants and children with newly diagnosed renovascular hypertension should be carefully examined for syndromal signs (e.g. NF type 1: café au lait macules, axillary/inguinal freckles, neurofibromas, Lisch nodules; Williams' syndrome: facial dysmorphic features like "elfin" faces, cardiovascular disease). Although only a small fraction of children with these genetic syndromes will develop renovascular hypertension, blood pressure should be regularly monitored in these conditions.

Children presenting with non-specific clinical signs of inflammation and suspected large vessel vasculitis, in particular Takayasu's arteritis (TA), are also at risk of developing renovascular hypertension.

Children with new-onset hypertension post-renal transplantation should undergo screening for renovascular disease. An abdominal bruit in transplanted children reflecting the turbulent blood flow at the stenotic anastomosis. A bruit can also be heard over renal arteries in native kidneys especially in slim children. Clinical signs that may point to renovascular hypertension are summarized in Table 49.1.

Table 49.1 Suspicious signs for renovascular hypertension

Suspicious sign	Specification
Blood pressure level	Very high blood pressure (e.g. stage 2 hypertension)
Secondary symptoms of hypertension	Cerebral symptoms
	Cardiac failure
	Facial palsy
Control of hypertension	Difficult, not controlled with ≥2 or more drugs
Syndromal disease associated with vascular disease	Neurofibromatosis type 1 (NF 1)
	Williams' syndrome
	Tuberous sclerosis
Signs of vasculitis	Takayasu's vasculitis
Previous vascular insult	Renal artery thrombosis
	Umbilical artery catheterisation
	Previous trauma or radiation
Transplanted kidneys	New onset or worsening of hypertension post-transplant
Clinical examination	Bruit heard over renal artery or arteries
Laboratory diagnostic	Raised peripheral plasma renin
	Moderate hypokalemia

Laboratory Evaluation

Suspicious, but non-specific laboratory signs for renovascular hypertension include moderate hypokalemia in combination with hypochloremic metabolic alkalosis compatible with secondary hyperaldosteronism and a highly activated renin-angiotensin-aldosterone system. Typically, age-specific plasma renin, aldosterone and plasma-renin-activity (PRA) are increased. However, elevated plasma renin levels and PRA are not specific for renovascular hypertension as they do not allow a differentiation between renovascular and renoparenchymal disease. It is important to note that normal renin levels and PRA values do not exclude renovascular disease. Up to 15% of children with renal artery stenosis, confirmed by DSA, can have normal initial PRA values [4]. Therefore further investigations for renovascular artery stenosis should be performed in case of clinical suspicion (Table 49.1) even in the presence of normal age-specific PRA values.

Laboratory evaluation of renal function is also essential, as renal artery stenosis, especially if bilateral, can compromise renal function due to hypoperfusion.

Vascular Involvement

Renovascular disease in children is often complex with a wide spectrum of renovascular and other vascular involvement. All types of major renal arteries (main, branch and accessory arteries) as well as intrarenal small vessels (segmental, lobar and accessory arteries) can be affected. Bilateral renal artery disease is more common than unilateral disease. Twenty-five to thirty percent of patients are diagnosed with mid aortic

syndrome (MAS) combined with bilateral or unilateral disease [5, 6] (Fig. 49.3). MAS is often associated with stenotic lesions in the intestinal arteries, mainly the coeliac trunk and the superior mesenteric artery (SMA) which in many cases both are occluded. The inferior mesenteric artery (IMA) is much less involved and collaterals from the IMA maintain the intestinal blood flow [7]. Importantly, abdominal angina is nearly never seen in children and there is virtually never a need for treatment of the coeliac axis nor the SMA. At least 20% of children also show cerebrovascular involvement, which influences the extent of lowering the blood pressure by treatment without risking cerebral hypoperfusion.

Causes of Renovascular Disease in Childhood

Epidemiology

Renovascular disease is associated with a variety of diseases and pathologies (summarized in Table 49.2). The reported distribution of RVD aetiologies in children varies widely in different parts of the world. However, cross-cohort comparisons are hampered by limited information on the diagnostic criteria used.

In Europe and North America, fibromuscular dysplasia (FMD) accounts for 53–88%, neurofibromatosis type 1 for 10–25%, and Williams' syndrome for 5–10%, of cases (Table 49.2) [8–13]. Whereas Takayasu's arteritis is very rare in Western countries, this disorder has been reported in 73–89% of Indian and South African cohorts [14–18].

Table 49.2 Summary of causes of renovascular hypertension

Cause	Specified diagnoses
Fibromuscular dysplasia	Medial fibroplasia
Syndromal diseases	Neurofibromatosis type 1 (*NF1* gene)
	Williams' syndrome (deletion of chromosome 7q11.23, *Elastin* gene)
	Tuberous sclerosis (*TSC1*/*TSC2* gene)
	Alagille syndrome (*JAG1* gene)
	Marfan's syndrome (*Fibrillin 1* gene)
	Other syndromes associated with vasculopathies and mid-aortic syndrome
Vasculitis	Takayasu's disease
	Polyarterits nodosa
	Other systemic vasculitides
Extrinsic compression	Neuroblastoma
	Wilms' tumor
	Pheochromocytoma
	Other abdominal and perirenal tumors
Other rare causes	Abdominal radiotherapy
	Previous renal artery thrombosis
	Umbilical artery catheterisation with thrombosis
	Trauma with renal artery disruption
	Congenital rubella syndrome
Renal transplantation	Transplant renal artery stenosis

Fibromuscular Dysplasia (FMD)

FMD is a non-atherosclerotic, non-inflammatory vascular disorder affecting medium-size arteries. It occurs as unifocal or multifocal disease. The aetiology of FMD is unknown.

FMD can be classified into different types depending on the mainly involved part of the blood vessel (intima, media, adventitia) [19], with medial fibroplasia being the most common type. In childhood, FMD primarily affects the mid and distal renal artery leading to renovascular hypertension [20, 21].

The diagnosis of FMD is based on angiographic appearance and is mainly a diagnosis of exclusion when other conditions have been ruled out. A minority of children display the typical "string of beads" appearance on angiography [22] which is regarded as typical for FMD (Fig. 49.1).

Takayasu Arteritis (TA)

A similar angiographic appearance for renal artery stenosis has been described for vasculiti-

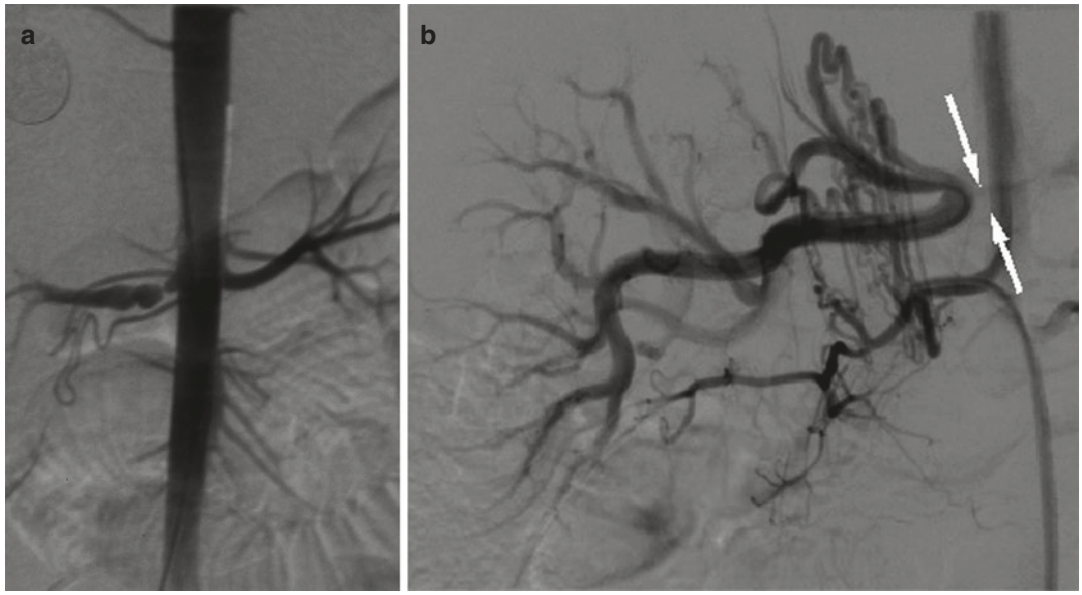

Fig. 49.1 (**a**) Fibromuscular dysplasia: Renal artery stenosis with "string bead" appearance in digital subtraction angiography (DSA) (image kindly provided by Great Ormond Street Hospital, London). (**b**) Obstructed right renal artery in digital subtraction angiography (DSA) (image kindly provided by Great Ormond Street Hospital, London)

des. The by far most frequent vasculitis is Takayasu's arteritis (TA). Rarely, polyarteritis nodosa, and other systemic vasculitides are associated with renovascular hypertension (Table 49.2). TA affects the aorta and its major branches and is mainly a disease of young women in their second and third decades of life. The age of onset is usually between 15 and 30 years [23]. TA has also been reported in young children presenting with malignant hypertension due to renal artery stenosis and cardiac failure [24–27]. However, the rate of misclassification of FMD as TA is suspected to be high in young children.

The aetiology of TA is still undefined. The vascular pathology is characterized by segmental and patchy granulomatous inflammation of all three layers of the aorta and major branches. Finally this inflammation leads to arterial stenosis, thrombosis and aneurysms. Thickened and acutely inflamed vessel walls can be often detected on MRA, CTA and/or PET scan [28] (Fig. 49.2).

The clinical features of TA can be divided into an early, acute and systemic phase, characterized by non-specific clinical features such as low-grade fever, malaise, night sweats, weight loss, arthralgia and fatigue, and a late occlusive phase [29]. Additionally, anaemia and a marked elevation of the erythrocyte sedimentation rate (ESR) can be found in most patients during the systemic phase. The early systemic phase is difficult to diagnose because only half of all patients show these symptoms. The late occlusive phase is characterized by progression from inflammatory into obliterative changes in the aorta and its main branches. In this late occlusive phase, the characteristic features of TA appear, in children predominantly hypertension secondary to renal artery stenosis, cardiac failure and neurological symptoms secondary to hypertension or ischemia [29]. Presentation with vascular bruits, absent or diminished pulses and claudication is less common in children.

The 2006 by EULAR/PRES (European League against Rheumatism/Paediatric Rheumatology European Society) defined consensus criteria for classifying TA are summarized in Table 49.3 [30, 31]. The classification criteria for TA imply that

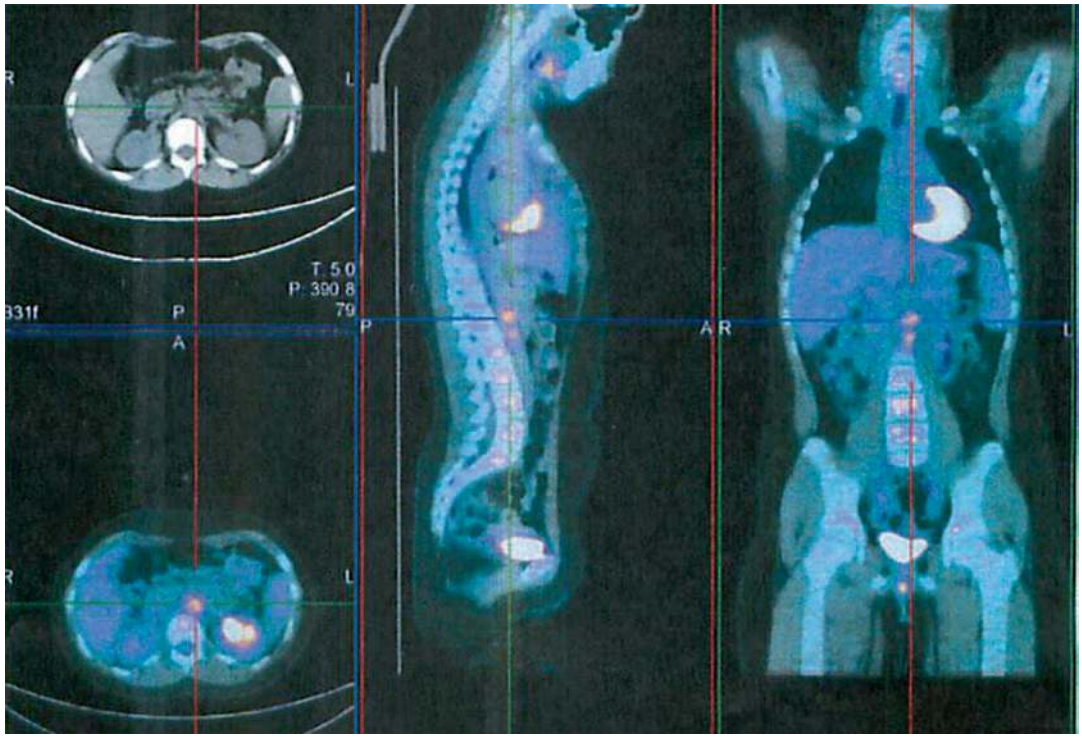

Fig. 49.2 Takayasu's arteritis: PET Scan with lightning of aorta reflecting thickened and inflamed arterial wall (images kindly provided by Great Ormond Street Hospital, London)

Table 49.3 Classification definition for Takayasu's arteritis according to EULAR/PRINTO/PRES (European League against Rheumatism/Paediatric Rheumatology International Trials Organisation/Paediatric Rheumatology European Society) Ankara 2008 consensus criteria (adapted from [31])

Requirement	Criterion	Specification
Mandatory	Angiographic abnormality	Angiographic abnormalities (conventional, CTA or MRA) of the aorta or its main branches and pulmonary arteries showing aneurysm/dilatation
+1 of the 5 following criteria	Hypertension	Systolic/diastolic blood pressure >95th percentile for height
	Pulse deficit or claudication	Lost/decreased/unequal peripheral artery pulse(s) Claudication: focal muscle pain induced by physical activity
	Four limbs blood pressure discrepancy	Discrepancy of four limb systolic blood pressure of >10 mmHg difference in any limb
	Bruits	Audible murmurs or palpable thrills above large arteries
	Acute phase reactant	Erythrocyte sedimentation rate (ESR) >20 mm per first hour or CRP any value above normal (according to local laboratory)

the diagnosis of any vasculitis was already made in the child. In such cases, the classification criteria have shown high sensitivity and specificity when being validated [31].

However, the difficulty is to diagnose vasculitis itself in children, especially if there is a lack of systemic inflammatory signs during the acute systemic phase as well as during the late occlusive phase of TA. Then the criteria are unspecific in diagnosing TA and overlap with features of FMD.

The evaluation of blood vessel wall thickness and inflammation by MR/CT angiography [32] and/or PET scan [28, 33] may help identifying

TA. A markedly thickened blood vessel wall reflecting oedema and inflammation strongly supports the diagnosis of TA (Fig. 49.2).

Differentiation Between Fibromuscular Dysplasia (FMD) and Takayasu's Arteritis (TA)

The differential diagnosis between FMD and TA seems to be difficult in many children. Children with obvious systemic inflammatory symptoms and raised inflammatory parameters (ESR, CRP) are likely to have TA. However, only a minority of children present in the inflammatory early phase of the disease. Inflammatory symptoms are missing during the late occlusive phase of TA. In the late phase of TA the mandatory criteria of angiographic abnormalities with hypertension are the same criteria as used to diagnose FMD. The typical "string of beads" pattern is not regularly seen on angiography in children with FMD.

In that case the evaluation of a thickened, inflamed vessel wall can help in the differential diagnosis, although systematic evaluations of this sign in MRA, CTA or PET scans have not been performed so far.

The ascertainment of the correct differential diagnosis is important for the indication for immunosuppressive treatment in the systemic phase of TA in order to control the active vasculitic process and in view of the timing of interventional or surgical procedures.

Genetic and Syndromic Causes

Several rare genetic syndromes are associated with childhood renovascular disease, with neurofibromatosis type 1 (NF type 1) and Williams syndrome being the most common (Table 49.2). All other associations of syndromes with renovascular disease are rare; these include tuberous sclerosis [34], Alagille syndrome [35, 36] and Marfan's syndrome with predominantly proximal aortic wall abnormalities [37].

To identify new monogenic causes for renovascular hypertension, two studies performed whole-exome sequencing in children with so far unexplained renovascular disease and mid-aortic syndrome [38, 39]. Both studies detected abnormalities in genes causing NF type 1, Williams syndrome, and Alagille syndrome that apparently caused a mild clinical phenotype. However, no new monogenic causes of renovascular disease were identified.

Neurofibromatosis Type 1 (NF Type 1)

The spectrum of NF type 1 associated vasculopathy is broad: Renal arteries are most frequently involved; the stenoses are usually located near the renal ostium. Unilateral and bilateral renal artery stenosis can occur in combination with mid-aortic syndrome by narrowing of the abdominal aorta and stenosis of its major branches (Fig. 49.3). Other common vascular manifestations in NF type 1 are abdominal aortic coarctation, internal carotid aneurysms and cervical vertebral arteriovenous malformations [40–42].

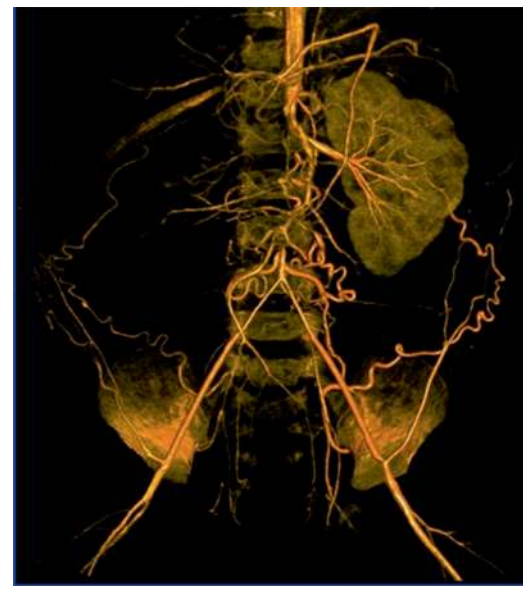

Fig. 49.3 Mid-aortic syndrome with occluded infrarenal aorta and right renal artery stenosis in two-dimensional ('volume-rendered') representation of a three-dimensional computed tomography dataset (image kindly provided by Great Ormond Street Hospital, London)

The real total incidence of secondary hypertension in NF type 1 and age-specific incidences remain unclear [43]; one study reported an incidence of 16%, with most cases involving renal artery stenosis followed by aortic coarctation and pheochromocytoma [44].

In general, blood pressure monitoring every 6–12 months is therefore recommended in all children with NF type 1 [43, 45].

Williams Syndrome

Williams syndrome is associated with cardiovascular abnormalities and a generalized arteriopathy focused on the aorta [46, 47] with consecutive development of mid-aortic syndrome. However, isolated stenoses of the renal and other arteries occur.

Since hypertension is a common feature of Williams syndrome, blood pressure screening is recommended. The reported prevalence ranges between 5% and 70% (summarized in [46]). In most children with Williams syndrome the aetiology of hypertension remains unclear; only in a minority of cases it can be attributed to renal artery stenosis, mid-aortic syndrome or aortic coarctation. It has been argued that arterial vascular stiffness due to defective elastin, resulting in decreased arterial elasticity, vascular smooth muscle cell proliferation and increased intima-media thickness, may contribute to hypertension in this condition [48].

Extrinsic Compression of Renal Artery

Extrinsic compression of the renal artery can mimick renal artery occlusive disease with renal hypoperfusion, activation of the renin-angiotensin-aldosterone-system and development of renin-mediated hypertension. This has been reported in different types of tumours, in particular Wilms tumour [49–51], neuroblastoma [52–54] and pheochromocytoma [55–58]. Mid-aortic syndrome and renal artery stenosis have also been reported after treatment of large tumours (Wilms tumour and neuroblastoma), usually associated with radio- and chemotherapy and postoperative fibrosis causing severe stenosis [59, 60] but also in patients without preceding radiotherapy [61].

Other Rare Causes

Umbilical artery catheterisation during the neonatal period can cause vascular endothelial disruption, thromboembolism with a completely or partially occluded aorta and renal hypoperfusion, renal artery thrombosis, or thromboembolism into the small renal vasculature with renal infarction [62–67].

Renal artery trauma can lead to renovascular hypertension due to surgical injury, disruption of the renal artery or from random accident.

Congenital rubella syndrome can also be associated with renovascular disease [65, 68, 69]. Due to the general recommendation for rubella vaccination, this association is very rarely seen nowadays.

Transplant Renal Artery Stenosis

Data from paediatric cohorts after renal transplantation with newly developed renal artery stenosis are very limited. To date, the prevalence of allograft renal artery stenosis is reported with 4–9% [70, 71]. Clinical presentation usually includes new onset of severe hypertension or significant worsening of pre-existing hypertension during the first few months after renal transplantation.

Imaging

Diagnosing renovascular disease in children is challenging due to the complex anatomical distribution of vascular involvement, the small size of renal arteries affected by age and size of the child and by the degree of arterial branching. Despite the development of non-invasive imaging, selective renal arteriography (digital subtraction angiography, DSA) is still the gold standard imaging

method in diagnosing renovascular disease. This might change with further technical developments in the future.

Non-invasive Imaging

For screening, several non-invasive imaging techniques are used in children although its role is still unclear due to the lack of high-quality evidence. The use of non-invasive imaging in children is based on small studies, clinical expert opinion and studies in adults. So far, non-invasive imaging methods cannot reliably diagnose renovascular disease in children, with at least 10–40% false-negative results as well as false-positive results. However, non-invasive imaging can be helpful to rule out extraparenchymal processes, to diagnose renoparenchymal disease, to plan surgery and to follow-up post-surgery.

Kidney Ultrasound and Doppler Studies

Kidney ultrasound combined with renal and abdominal vessel Doppler ultrasound is an important baseline investigation. It can exclude other renal pathologies (renoparenchymal disease, renal scarring), detect extrarenal processes, in particular tumours (e.g. neuroblastoma, Wilms' tumour) and show significant discrepancies of kidney volume and length (≥1 cm) as a possible indirect sign of renovascular hypertension.

Renal Doppler ultrasound can directly visualise a stenosis or be suggestive of renovascular disease when a *parvus et tardus* waveform pattern (Fig. 49.4) or pathologic age-dependent flow parameters (peak systolic flow >2 m/s [72, 73], acceleration time >80 ms, renal artery to aortic flow velocity ratio >3 and difference in resistive index) can be identified. The entire renal artery should be followed when possible from its origin in the abdominal aorta to the renal hilum and a screening for accessory renal arteries and the abdominal aorta is essential [74]. However, renal Doppler ultrasound in children requires advanced technical skills and experience and cooperative children to reliably suspect renovascular disease by Doppler ultrasound.

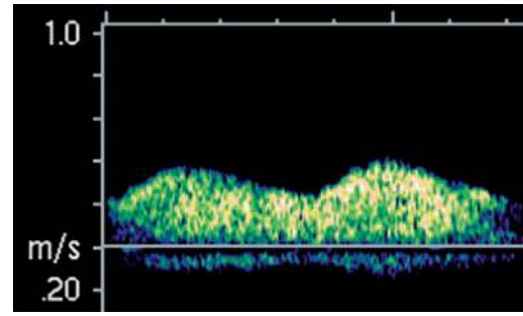

Fig. 49.4 Renal Doppler ultrasound suggestive for renal artery stenosis: *parvus et tardus* waveform pattern (image kindly provided by Great Ormond Street Hospital, London)

In general, the experience with renal Doppler ultrasound in children is limited apart from detecting renal artery stenosis in transplanted kidneys [75]. The sensitivity and specificity for Doppler ultrasound was reported in small paediatric cohorts at 63–88% and 73–99% [5, 72, 76–78]—significantly lower than in adult studies. In those small paediatric studies, Doppler ultrasound failed to detect stenoses located in small renal artery branches, segmental renal arteries as well as in main and accessory renal arteries.

CTA and MRA

Magnetic resonance angiography (MRA) or contrast-enhanced multidetector computer tomography angiography (CTA) can be used as next diagnostic steps in diagnosing renovascular disease in children. We do however advocate going directly to DSA in children with a strong suspicion of RAS. With the criteria given in Table 49.1, a majority of children will be shown to have arterial disease and MRA or CTA can never rule out RAS in these cases.

CTA and MRA are able to detect mid-aortic syndrome and sometimes stenosis of intestinal arteries and to assess non-vascular structures like occult renal or suprarenal neoplasms in addition to ultrasound [79].

The advantages of renal CTA versus MRA are a shorter examination time without the need for sedation or general anaesthesia in small children and an easy generation of high-quality 3D images. However, the disadvantage is the significant radiation exposure, even with low-dose protocols [80].

The spatial resolution of MRA has improved significantly with technological advancements and can evaluate renal parenchymal disease with high anatomical detail and improved diagnostic accuracy. However, there is a lack of data in children. To date, only small retrospective studies evaluated whether CTA/MRA can correctly predict renovascular disease, confirmed by DSA [5, 79]. MRA was less sensitive (80%) and specific (62%) than CTA (88% and 81%) and missed renovascular disease mainly in younger children [5]. Lee et al. described a sensitivity of CTA of 90% and MRA of 75% [81].

In summary, CTA and MRA are promising non-invasive imaging methods in diagnosing renovascular disease but currently cannot replace DSA. CTA seems to be more accurate than MRA in diagnosing renovascular hypertension in all age groups, but in particular in young children with small vessel size.

Renal Scintigraphy

Pre- and post-captopril renal scintigraphy with [99mTc] dimercaptosuccinic acid (DMSA) or 99m-Tc-mercaptoacetyltriglycine (MAG3) was initially thought to be a useful screening method for locating renal artery stenosis. However, paediatric studies could not reliably predict renovascular disease and showed low sensitivities of 47–73% [82–84]. Therefore, pre- and post-captopril renal scintigraphy cannot be recommended as a screening method.

DMSA scintigraphy can be informative by evaluating the relative function of each kidney and documenting focal ischemic deficits caused by renal artery stenosis and/or renal scarring. It is important to recognise that kidneys or renal segments with no initial DMSA uptake and suspected no function have the ability to recover after revascularization by angioplasty (Fig. 49.5), especially if the size of the kidney as measured on renal US is still good.

Invasive Imaging

Digital Subtraction Angiography (DSA)

DSA is the diagnostic gold standard in establishing the diagnosis of renovascular disease in children whereas all other non-invasive imaging methods have 10–40% false-negative and false-positive results. DSA provides the best spatial and temporal resolution producing excellent images of the renal arterial lumens and branches, especially if the sizes of the vessels are small.

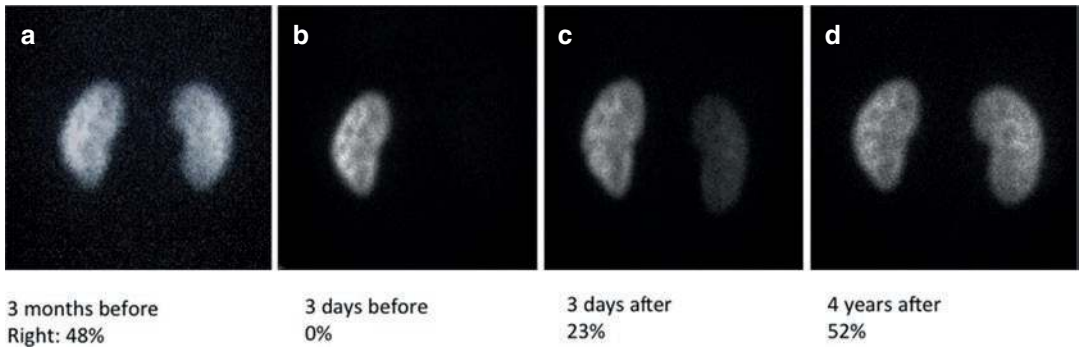

Fig. 49.5 DMSA scan with recovery of renal function after percutaneous transluminal angioplasty (PTA). (**a**) Right kidney with normal DMSA uptake 3 months before angioplasty (partial function 48%), (**b**) lacking uptake (0%) 3 days before angioplasty, (**c**) beginning recovery 3 days after angioplasty (23%) and (**d**) stable right partial function 4 years after invention (images kindly provided by Great Ormond Street Hospital, London)

Renal artery stenosis can be defined as a significant reduction of intraluminal diameter (e.g. >60%) and presence of collateral vessels.

DSA is an invasive diagnostic method with a mainly femoral artery approach, requiring general anaesthesia in children, and has a significant ionising radiation exposure. However, the main advantage of DSA is that potentially curative treatment with percutaneous transluminal angioplasty (PTA) can be performed during the same intervention. DSA and angioplasty are recommended to be performed only in clinical centres specialized in paediatric renovascular disease providing a multidisciplinary team including paediatric nephrologists, experienced interventional radiologists and vascular surgeons.

Renal Vein Renin Sampling

In case of bilateral renal artery disease and/or suspected location of stenosis in a segmental artery, renal vein sampling and measurement of plasma renin activity can be useful to identify the more relevant stenotic renal artery or to localize the segmental artery stenosis to a small area of one kidney. Usually, blood samples will be taken from the infrarenal vena cava inferior, the main renal veins and the larger intrarenal branches in order to perform renal vein renin studies during the diagnostic angiography.

Further Imaging

Cranial MR with Angiography

In case of neurological symptoms at initial presentation and suspected cerebrovascular involvement, a cranial MR evaluating cerebral parenchyma and vasculature is recommended.

Echocardiography

To evaluate cardiac function and left ventricular hypertrophy as target organ damage of severe hypertension, echocardiography should be performed in all children at initial presentation and on an annual basis.

Treatment

Treatment of renovascular hypertension is complex and requires input from a multidisciplinary team including interventional radiologists, vascular surgeons and nephrologists. In certain cases input from cardiologists, neurovascular specialists, anaesthetists and other specialists is needed. The goals of treatment are normalization of blood pressure and restoration or preservation of kidney function. Antihypertensive pharmacotherapy will nearly always be the first measure, but is almost never sufficient to control blood pressure. Angioplasty and sometimes open surgery will be needed in nearly all cases.

Pharmacological Treatment

The blood pressure in children with RVH is typically elevated very markedly. Antihypertensive medications usually improve blood pressure somewhat and many children receive combined therapies with multiple drugs [85]. It is however uncommon that blood pressure normalises by pharmacotherapy alone.

While there is no single most effective antihypertensive drug class in children with RVH, RAAS blockers are quite effective in selected individual cases. They do however bear a major chance of harming kidney function as they work by lowering the intra-glomerular pressure. These drugs are therefore contraindicated in children with renal artery stenosis. Selected cases where both angioplasty and surgery are technically unfeasible can benefit from renin-angiotensin blockade. This can often effectively reduce blood pressure but will also impair the function of the whole or part of a kidney (Fig. 49.6).

After successful angioplasty most children will acquire a much improved blood pressure and antihypertensive drugs can be weaned. It is however not uncommon that continued pharmacological treatment is required, albeit with much fewer drugs, to achieve total normalization of blood pressure.

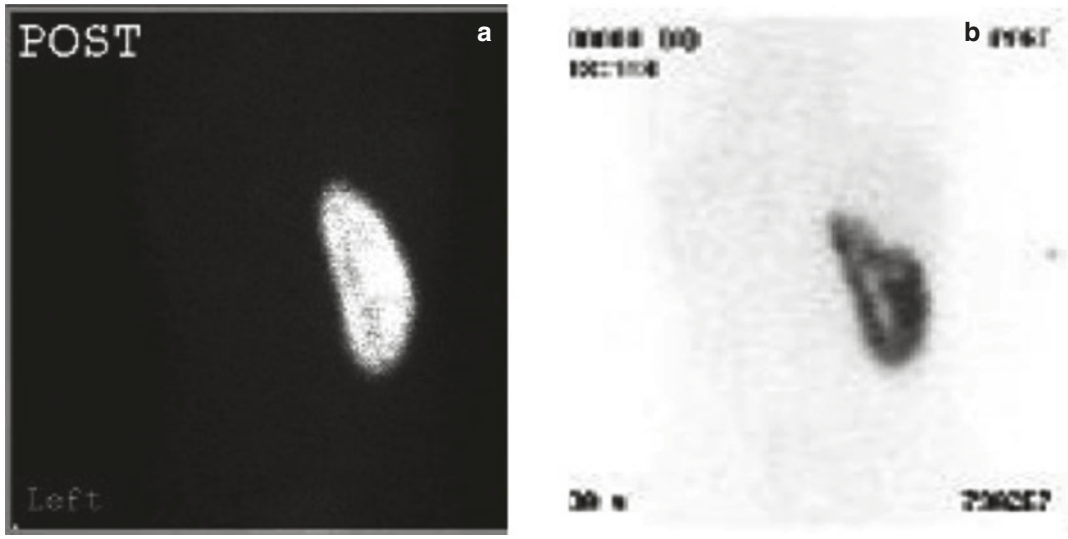

Fig. 49.6 Loss of upper pole after starting treatment with renin-angiotensin-blockade, shown in DMSA scan. (**a**) Normal left kidney in DMSA scan, (**b**) Missing upper pole of left kidney in DMSA scan (images kindly provided by Great Ormond Street Hospital, London)

Angioplasty

The vast majority of children will require interventional treatment. Percutaneous transluminal angioplasty (PTA) is the most commonly used procedure (Fig. 49.7). It is performed under general anaesthesia. The femoral artery is most commonly used for catheterization but in some cases an axillary or and radial approach is needed. The equipment used for adult coronary arteries are generally suitable. Mild to severe stenosis of the main renal arteries and sometimes also the aorta are most amenable to treatment [86]. Even completely occluded arteries can be re-canalized in many cases. Particularly in children with NF type 1 the stenotic tissue may be difficult to open up and a cutting balloon can be needed to reestablish a good-sized vascular lumen (Fig. 49.7).

Unfortunately, the artery can have elastic recoil and cause a residual stenosis after initially successful angioplasty [87]. In some of these cases a stent can be placed to keep the artery open [9, 88–90]. The lumen of the stent can however narrow in size with time. This can be due to intimal hyperplasia within the stent, stent thrombosis, or even stent fracture. Importantly, the fixed diameter of the stent may cause re-stenosis over time as children, and their arteries, grow in size. This is a particular issue in rapidly growing young infants. The use of a stent is therefore generally advised against in children.

A blood vessel that has had successful angioplasty can re-stenose with time and it is therefore not uncommon that a child needs to undergo repeated interventions to achieve optimal results [91].

Children with severe mid aortic syndrome can also benefit from angioplasty. We have seen cases diagnosed with an atretic aorta that have been possible to re-canalize and restore a reasonably wide aorta, normal blood pressure, and normal quality of life [92].

Some children with stenotic vascular lesions that are not amenable to angioplasty due to the small size of the blood vessel can be treated with ethanol ablation of a segment of a kidney [93, 94]. This is particularly useful in polar arteries supplying only a small part of the kidney.

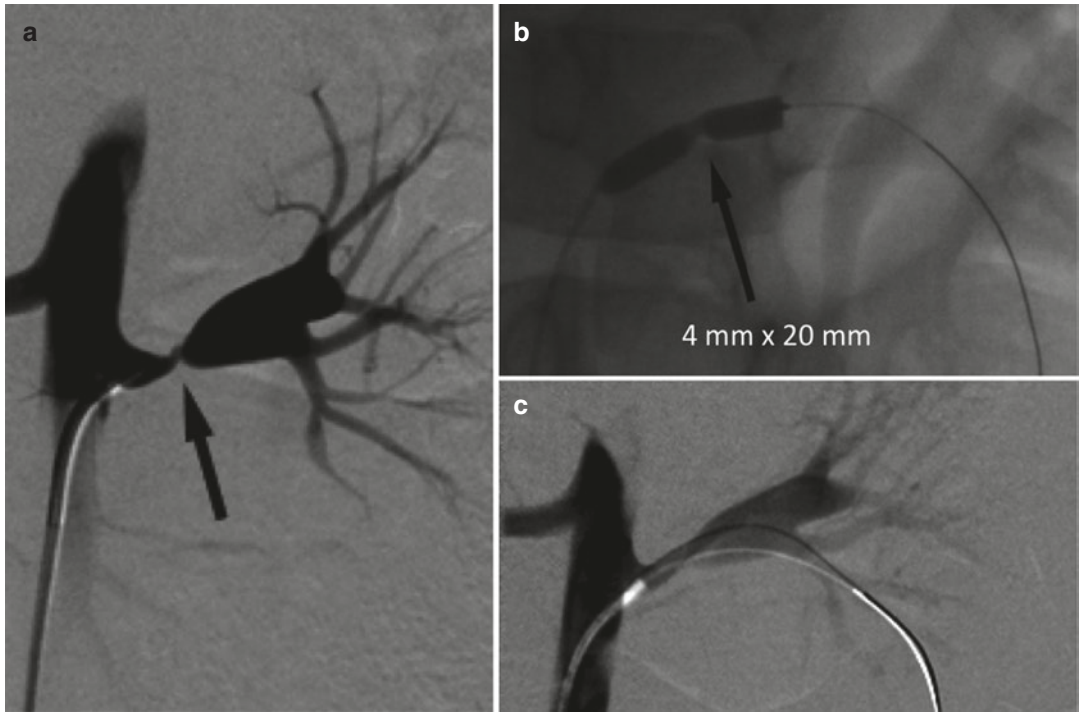

Fig. 49.7 Percutaneous transluminal angioplasty (PTA) of the left renal artery. (**a**) Left renal artery stenosis in angiography, (**b**) Positioning a cutting balloon catheter (4 mm × 20 mm) at the stenotic area, (**c**) improved vascular lumen of left renal artery directly after angioplasty procedure (images kindly provided by Great Ormond Street Hospital, London)

Complications to angioplasty include contrast-induced nephropathy, arterial spasm, thrombosis, arterial dissection and perforation. The risks for these complications vary from 0% to 43% in different publications [95, 96]. Haemodynamically insignificant dissections occur rather often and might be part of the remodelling process after angioplasty. Fatal complications are very uncommon for PTA but we and other centres recommend that vascular surgery should be readily available in case of arterial rupture or active extravasation of contrast [97].

Surgery

Surgery should be used in children where angioplasty has not achieved appropriate blood pressure control, and for aneurysmal disease that is not amenable to endovascular treatment. This assessment needs multi-professional consensus from interventional radiology, vascular surgery and nephrology. A variety of surgical revascularization procedures is available, including autologous or synthetic grafts [12, 13, 98, 99]. Autologous grafts can be the splenic or the gastro-duodenal artery that is pulled down to the kidney, or the use of a part of the saphenous vein or internal iliac artery can be used. Dacron is often used for synthetic grafts. Surgery on the renal arteries can be so complicated and time-consuming that it needs to be done outside of the child ("bench surgery", Fig. 49.8), with an ensuing auto-transplantation. In children with very complicated pathology, e.g., stenosis of both renal arteries and mid-aortic syndrome, a so-called trouser graft can be used. This extends

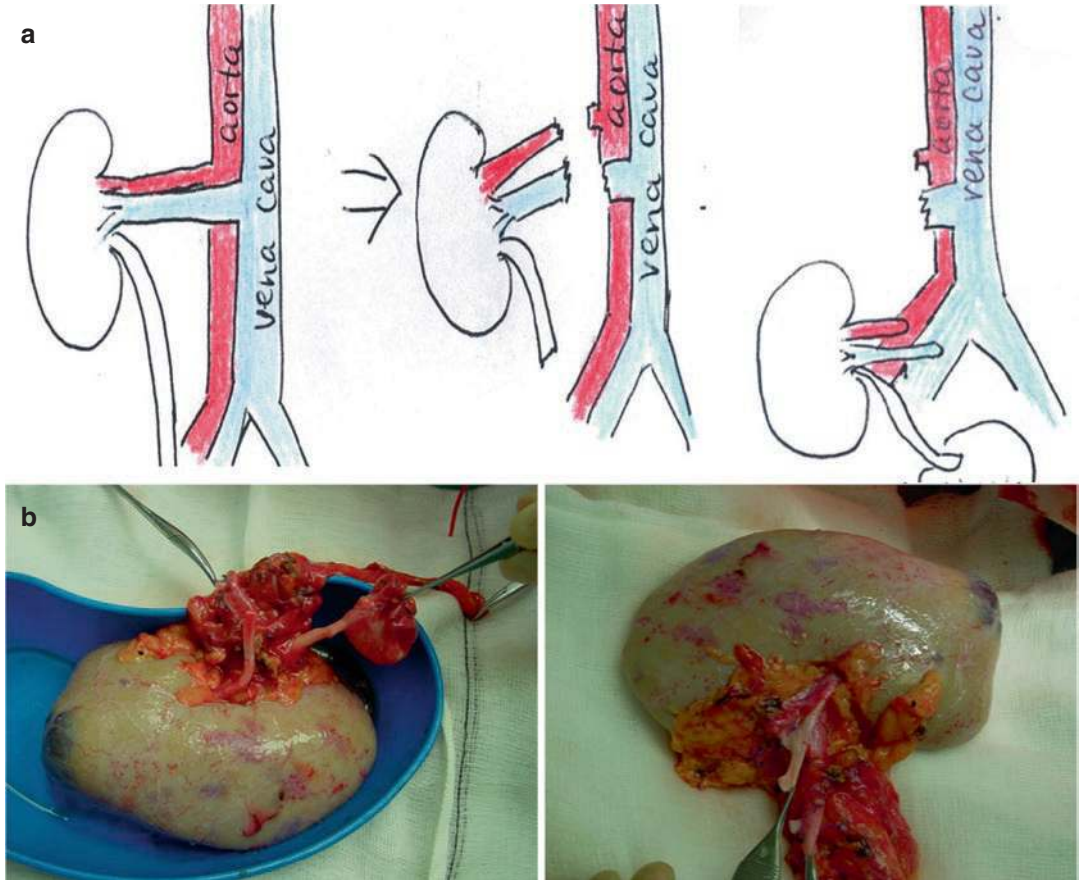

Fig. 49.8 Auto-transplantation: (**a**) Sketch and (**b**) bench surgery (images kindly provided by Great Ormond Street Hospital, London)

from the aorta above the MAS to the aorta below the stenotic lesion and to one or both renal arteries (Fig. 49.9).

In cases where no curative intervention is possible, nephrectomy can be an alternative option. This can be very successful and cure the blood pressure in children with unilateral disease and small non-functioning kidneys [100, 101]. A word of caution is, however, warranted; in some cases kidneys that show less than 10% function on a pre-treatment DMSA scan recover function after successful angioplasty or revascularization surgery, even up to 50% relative function (Fig. 49.4). These kidneys thus appear to survive on collateral circulation that does not give any relevant kidney function as measured with DMSA. We use the size of the affected kidney measured on ultrasound to decide when to try to recover function or to go directly to nephrectomy.

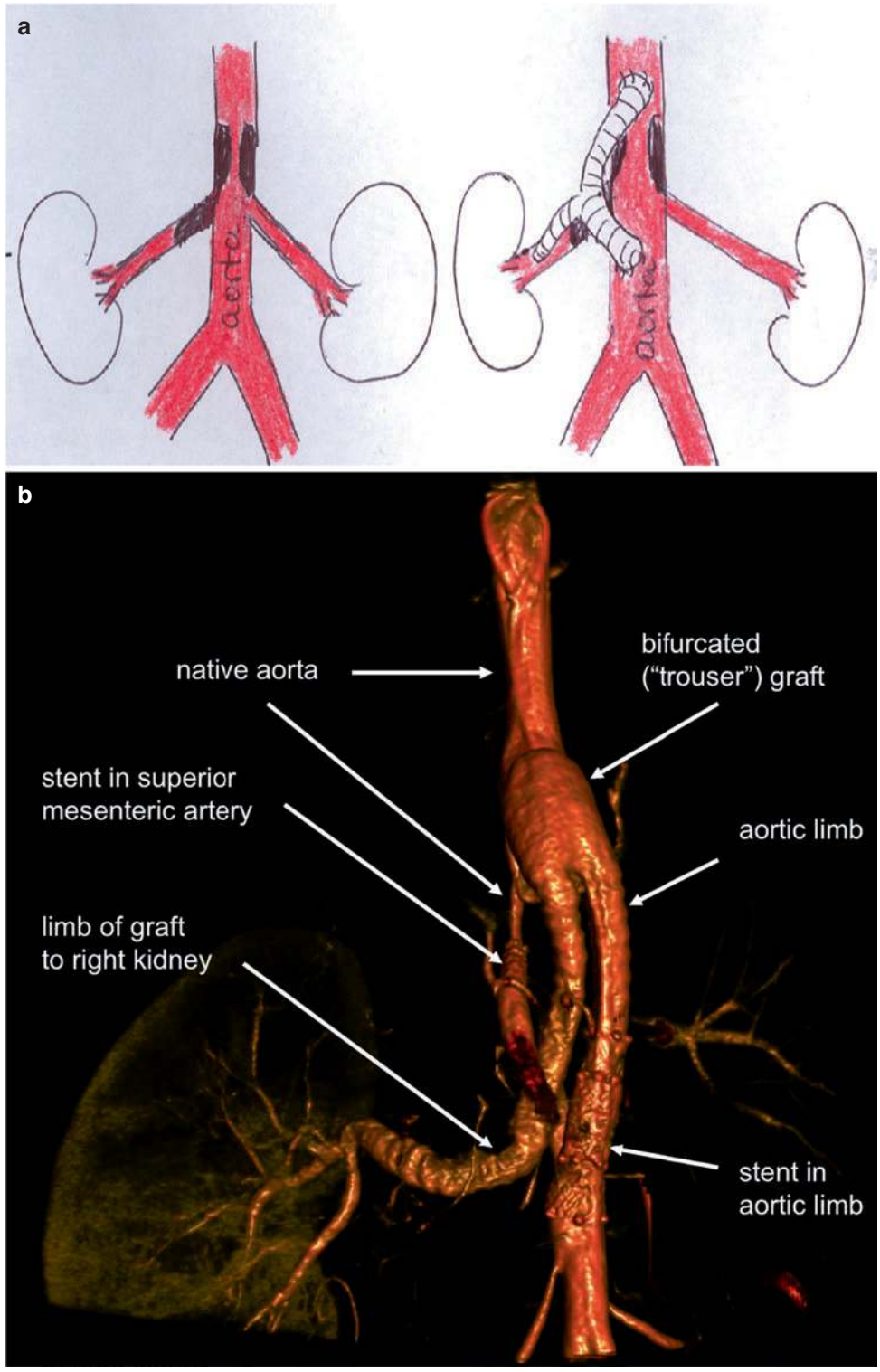

Fig. 49.9 Renal artery trouser graft in mid-aortic syndrome and right renal artery stenosis. (**a**) Sketch of renal artery and mid-aortic stenosis and implementing a renal artery trouser graft in order to plan vascular surgery. (**b**) Complex vascular situation with renal artery trouser graft (images kindly provided by Great Ormond Street Hospital, London)

Outcome

Technical Results

Angioplasty will, in most cases, widen the lumen of the artery. In some cases e.g. with total occlusion of the blood vessel or with a very long and slender renal artery this will however not be possible. In 25–28% of children re-intervention with angioplasty was required [9, 97, 102, 103]. In some children the artery recoils very quickly despite several successful attempts with angioplasty. Some of these children are amenable to surgery. In a recent cohort, 18% of children underwent surgery after initial angioplasty [81].

Blood Pressure

Improvement of blood pressure is the most commonly reported treatment outcome. With the current techniques, PTA normalizes or improves blood pressure in 53–63% of children [9, 97, 103–105]. It typically takes some days before blood pressure settles down and medication can be weaned. Quite often there is a persistent need for one blood pressure drug even after successful intervention.

It is important to analyse the reason when blood pressure improvement is not achieved. Re-stenosis, occurring immediately or with temporal delay, can in many cases explain a failure to improve the blood pressure. In children with widespread renovascular disease, successful treatment of individual stenotic arteries might not be sufficient and the remaining disease continues to drive high blood pressure [9]. Notably, vascular disease in the contralateral kidney and involvement of small intrarenal branches occurs in 50–70% of those children [9, 103] that are not amenable to either PTA or surgery.

Kidney Function

Most studies have not systematically reported the short- and long-term effects of treatment on kidney function. Recovery or stabilization of kidney function is however a very important therapeutic goal. Some kidneys that display no function on DMSA scan regain up to normal function (50% partial function on DMSA) after successful revascularisation. The value of serum creatinine in monitoring kidney function before and after intervention is limited since a major loss of function in one kidney can be "covered up" by a normally functioning contralateral kidney.

Summary

Renovascular stenosis is an unusual but very important cause of severe arterial hypertension in children. It is important to suspect and to diagnose. DSA is the gold standard investigation and angioplasty can be performed at the same time. Surgery is needed in an important minority of cases. Most but not all children will eventually benefit from treatment.

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Renal Hypertension: Etiology and Management

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Elke Wühl and Franz Schaefer

Introduction

Blood pressure (BP) is regulated by several setting mechanisms and, apart from the cardiovascular system, the central nervous system and the adrenal glands, the kidneys are key players in BP control.

In 1836 Bright first described the association of a small contracted kidney with left ventricular hypertrophy (LVH) and linked hypertension to kidney disease [1]. In children and adolescents with chronic kidney disease (CKD), arterial hypertension is often the earliest and most prevalent complication of CKD [2]. Large cohort studies in pediatric CKD in Europe [3], Canada [2] and the US [4–6] found controlled or uncontrolled hypertension in more than 40% of children with CKD stage 1 and in up to 90% of those with CKD stage 3 to 5 [2, 6]. Even among patients receiving antihypertensive treatment, BP was uncontrolled in up to 50% of patients [6]. Evaluated by ambulatory BP monitoring (ABPM), the overall prevalence of uncontrolled hypertension ranged between 27% and 48%. The fraction of children with elevated BP not receiving antihypertensive treatment was between 21% and 45% [3, 7–9].

Because untreated or uncontrolled hypertension is an independent risk factor for kidney damage and kidney disease progression as well as for cardiovascular morbidity and mortality, BP control is of utmost importance, especially in patients with prevalent CKD.

This chapter will focus on the pathophysiology of hypertension in pediatric kidney disease and on the indications for antihypertensive treatment, therapeutic options and BP targets in childhood CKD.

The Role of the Kidney in Hypertension

Mechanisms of Blood Pressure Regulation by the Kidneys

The *Pressure-Natriuresis-Diuresis Hypothesis* plays an important role in BP control and the pathophysiology of hypertension. By this mechanism the kidneys regulate arterial pressure by adjusting blood volume. When arterial BP or renal perfusion pressure changes, blood volume is adjusted accordingly by altering the excretion or retention of sodium and water to return arterial BP to normal values [10]. This is accomplished by the interplay of angiotensin II, atrial natriuretic peptide, vasopressin, nitric oxide (NO),

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kallikrein-kinin, prostaglandins, renal nerves, and other factors. Through this mechanism, the kidneys are even able to override other BP control mechanisms.

To protect the kidneys from BP peaks, transmission of elevated systemic pressure to the glomeruli and to periglomerular capillaries is prevented by preglomerular vasoconstriction [11]. This autoregulation of glomerular filtration rate (GFR) and renal blood flow (RBF) maintains RBF constant within a defined perfusion pressure ranging from approximately 80 to 200 mmHg.

Ideally, higher salt intake leads to an increase in natriuresis and diuresis but almost no change in arterial pressure. However, sensitivity to salt intake varies individually and subjects can be classified according to their arterial pressure response to changes in salt intake.

Subjects in whom arterial pressure is relatively insensitive to changes in sodium intake are so-called *salt-insensitive* individuals while those in whom arterial pressure is related to salt intake are called *salt-sensitive*. In salt-sensitive individuals the pressure-natriuresis relationship is shifted towards higher pressure levels needed to achieve increased sodium excretion; low salt intake results in normal BP but BP steadily increases with increasing salt intake [12].

Subjects with low nephron endowment, e.g., children born small for gestational age or preterm, are especially susceptible to salt sensitivity and increased BP level. Reduced nephron endowment may result in the histopathological finding of 'oligomeganephronia'. In a kidney biopsy study in adult patients with primary hypertension, the total number of glomeruli was reduced by 50%, while the size of the glomeruli was increased 2.3-fold [13].

In a neonate with low nephron number the kidney tries to adapt to the excretory overload by increasing the filtered sodium load per nephron and compensatory growth of the proximal tubule with a consequent increase in sodium reabsorption. A reduced flow at the macula densa induces an increase of glomerular pressure and filtration resulting in restoration of sodium delivery to the macula densa. Thus, extracellular fluid homeostasis is maintained by increased arterial pressure to excrete the sodium load [14]. However, the underlying pathophysiology is not yet fully understood. Pathophysiological mechanisms include genetic predisposition, kidney damage mediated by inflammation, the renin-angiotensin-aldosterone system, and neuronal alterations. After manifestation of salt sensitivity, an individual usually remains salt sensitive [15]. The prevalence of salt-sensitivity increases with age [16] and is associated with an increased risk for cardiovascular events over time [17, 18]. Supported by the observation that 50% of patients with essential hypertension are salt-sensitive, current hypertension guidelines recommend restriction of salt intake [19–21].

Also, dysfunctions of tubular ion transporters have a crucial role in the pathogenesis of hypertension. The Na^+/H^+-exchangers (NHEs), the Na^+-K^+-$2Cl^-$-cotransporter (NKCC), the Na^+-Cl^--cotransporter (NCC), the epithelial sodium cotransporter (ENaC), and the sodium-potassium-ATPase (Na^+/K^+-ATPase) play a major role in sodium homeostasis.

The *renin–angiotensin-aldosterone system* (RAAS) is one of the most powerful regulators of salt homeostasis and BP. The RAAS is activated by either the macula densa, baroreceptor activation, or the sympathetic nervous system. Activation of one of these sensors leads to stimulation of renin release by juxtaglomerular cells. Renin cleaves angiotensinogen to angiotensin I, and angiotensin I is rapidly converted to angiotensin II by the angiotensin-converting enzyme (ACE). Angiotensin II is the most effective component of the RAAS and plays a central role not only in the regulation of fluid volume but also in regulation of vascular resistance. Effects of angiotensin II are mainly mediated via the angiotensin (AT)-1 receptor, i.e., vasoconstriction, aldosterone and vasopressin release, salt and water retention through the kidney, and sympathetic activation, as well as important autocrine and paracrine effects on cell proliferation and migration, and on extracellular matrix formation. The AT-2 receptor-mediated effects of angiotensin II are mainly vasodilatory and anti-

proliferative and seem to antagonize the effect of angiotensin II at the AT-1 receptor [22]. Additionally, angiotensin II can directly stimulate the activity of ENaC in the collecting duct [23]. This subtle balance between salt homeostasis, BP and the RAAS changes with aging or with decreasing kidney function.

An important role in BP regulation and cardiac function has been ascribed to *renalase*, an amine oxidase mainly expressed by the kidneys [24]. Renalase expression and enzymatic activity are rapidly triggered by modest increases in BP and by brief surges in plasma catecholamines. The active enzyme degrades circulating catecholamines, causing a fall in BP.

Arginine-vasopressin (or *antidiuretic hormone* (ADH)), is a neuropeptide synthesized in the hypothalamus and released in response to increased plasma osmolality, decreased systemic BP, or reduced blood volume. Vasopressin regulates renal water excretion by increasing the osmotic water permeability of the renal collecting duct by activation of the vasopression-2 receptor. In addition, activation of the vasopressin-2 receptor induces NO production, attenuating vasopressin-1 receptor-mediated vasoconstrictor effects [25].

The *kallikrein–kinin system* (KKS) also contributes to the regulation of BP. Kinins, including bradykinin, are formed from kininogen by kininogenase and tissue kallikrein. Bradykinin is mainly degraded by ACE. In the tubular lumen bradykinin causes natriuresis, whereas interstitial bradykinin regulates medullary blood flow [26]. Bradykinin and kallikrein may also act in a paracrine manner on the preglomerular microvessels via release of nitric oxide and prostaglandins [27].

NO, involved in the renal regulation of BP, is produced in the renal medulla and mediates endothelium-dependent vasodilatation. It enhances arterial compliance, reduces vascular resistance and exerts an antiproliferative effect on vascular smooth muscle cells. NO antagonists (e.g. asymmetric dimethylarginine [ADMA]) induce endothelial dysfunction and lead to an increase of BP and decrease in RBF.

Endothelins (ETs) are vasoconstrictor peptides released by endothelial cells. In the kidney, ET-1 is expressed in the glomeruli and medullary collecting ducts. The renal hemodynamic effects are exerted by activation of ET-A and ET-B receptors, located in podocytes, glomeruli, afferent and efferent arterioles, the proximal tubule, the medullary thick ascending limb, and the collecting duct. ET-A is the dominant ET receptor on vascular smooth muscle cells and activation causes vasoconstriction, whereas ET-B receptor activation on endothelial cells results in vasodilation [28].

Atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), C-type natriuretic peptide (CNP), and urodilatin have been named "*natriuretic peptides*" due to their ability to increase sodium and water excretion, resulting in reduction of intravascular volume and BP [29]. ANP and BNP decrease the secretion of renin and aldosterone, and antagonize the effects of angiotensin II on vascular tone and renal tubular sodium reabsorption. Natriuretic peptides are degraded in the lung and kidney by neutral endopeptidase [30].

Hypertension and the Kidneys

Patients with hypertension are at high risk for progressive kidney damage. The extent of the resulting nephrosclerosis depends on the individual susceptibility, degree of hypertension, the etiology of hypertension and the underlying kidney disease.

The pathogenic factors determining the degree of hypertensive kidney damage can be divided into systemic BP burden, pressure transmission to the kidney vascular bed, and local tissue susceptibility to damage. As long as BP remains within the upper limit of autoregulation, no severe changes occur. However, if this limit is exceeded, acute severe injury may occur. Autoregulatory responses can be compromised by hypertension-induced vascular injury, resulting in amplified kidney damage [31, 32]. Relevant reduction of kidney mass in CKD patients may additionally impair autoregulation [33–35] with consequent enhanced susceptibility to hypertensive injury and accelerated glomerulosclerosis [36, 37].

Pathomechanisms of Hypertension in Chronic Kidney Disease

Sodium retention and consequent *fluid overload* has long been recognized as a critical cause of hypertension in CKD patients. In contrast to patients with essential hypertension, plasma volume is elevated in CKD and correlates with BP. Also, extracellular fluid expansion is consistently found in hypertensive end-stage kidney disease (ESKD) patients. However, the correlation between interdialytic weight gain and BP is weak, suggesting additional volume-independent mechanisms in BP control [38–43]. Furthermore, the high prevalence of arterial hypertension in early CKD, when plasma and extracellular fluid volumes tend to be normal, supports a role of fluid independent mechanisms [44]. This is particularly seen in children with renal hypo/dysplasia, who tend to lose considerable amounts of sodium and water and yet are commonly hypertensive. Additional evidence for volume-independent mechanisms of hypertension in CKD comes from patients undergoing bilateral nephrectomy. In dialyzed children nephrectomy lowers mean BP despite causing anuria [45]. The removal of the native kidneys markedly reduces BP and total peripheral vascular resistance, suggesting an excessive vasopressor function of failing kidneys. Interestingly, previously hypertensive, but not normotensive patients respond to salt and water loading by an increase of BP. Hence, the vascular tone must be affected by kidney-related as well as kidney-unrelated mechanisms.

Activation of the RAAS plays a pivotal role in renal hypertension. Although plasma renin activity is typically significantly elevated in patients with isolated kidney artery stenosis, many patients with CKD have 'inappropriately normal' renin levels considering their degree of hypertension and fluid overload [46, 47]. Enhanced renin secretion by poorly perfused areas such as cysts, scars or due to microangiopathic damage or tubulointerstitial inflammation [48, 49] leads to angiotensin II mediated vasoconstriction and aldosterone-mediated salt retention, increasing both total peripheral resistance and blood volume. In addition, the local angiotensin tone in the diseased kidney is affected by multiple mechanisms, independently of plasma renin activity.

Additionally, *sympathetic overactivity* plays an important role in the pathogenesis of hypertension in CKD. Sympathetic nerve activity is increased in CKD and in dialyzed patients [50, 51], and persists even after kidney transplantation as long as the native kidneys are in place. After bilateral nephrectomy, sympathetic nerve activity and BP normalizes [50]. ACE inhibitor treatment, but not calcium channel blockers, normalizes sympathetic activity, suggesting an effect of renal angiotensin tone on afferent neural signaling [51]. Overactivation of the sympathetic drive is also observed in renovascular and polycystic kidney disease-related hypertension [52].

In addition, renalase expression and blood levels are directly correlated with glomerular filtration rate and are markedly reduced in patients with ESKD. Renalase deficiency may thus contribute to the sympathetic overactivation, hypertension and cardiac disease associated with CKD.

The *vascular endothelium* exerts important endocrine and paracrine functions, including active control of the vascular tone. In CKD, endothelium-dependent vasodilation is impaired [53, 54] and NO production is decreased [55, 56] as a result of impaired biosynthesis and bioavailability of L-arginine, reduced NO synthase (NOS) expression and increased circulating endogenous NOS inhibitors [56]. ADMA, a potent NOS inhibitor, accumulates in CKD due to impaired renal excretion and enzymatic degradation. ADMA independently predicts overall mortality and cardiovascular events in patients with ESKD as well as progression of CKD [57, 58]; however, these findings do not appear to be related to clinical differences in BP [59].

ET-1 is the most potent vasoconstrictor known to date. In ESKD patients, ET-1 plasma levels are

increased and correlate with BP level [60]; hence, circulating and possibly renal ET-1 may contribute to hypertension in CKD. With decreasing kidney function, activity of the sympathetic nervous system and oxidative stress are increasingly relevant for the risk of hypertension.

Pressure autoregulation is thought to be impaired in CKD [35], resulting in unrestricted transmission of systemic BP to the glomeruli and subsequent glomerular damage and reduction of renal mass. According to the *Brenner hypothesis*, any critical reduction of functional renal mass leads to *hyperfiltration and intraglomerular hypertension* in the remaining nephrons [61]. The increased filtration pressure causes or aggravates preexisting proteinuria. The exposure of tubular and mesangial structures to macromolecular proteins elicits a marked and persistent tissue response. In addition, proteinuria and enhanced angiotensin II formation stimulate the synthesis and release of several pro-inflammatory cytokines and chemokines, resulting in a local inflammatory and fibrotic tissue response and atrophy of the nephron.

A large body of evidence from epidemiological studies and clinical trials indicates that hypertension is an important driver of *CKD progression*. Numerous interventional trials have demonstrated that lowering BP preserves kidney function in hypertensive patients at risk for progressive kidney disease [62–74]. In addition to hypertension, proteinuria is a major risk factor contributing to CKD progression. Although hypertension aggravates proteinuria and the two risk factors are strongly interrelated, they independently influence kidney survival. Two prospective pediatric trials have demonstrated that hypertension and proteinuria are major independent risk factors for progressive kidney failure in children with CKD [63, 75, 76]. Detailed information on role of hypertension in kidney disease progression is provided in Chap. 55.

Various modifying risk factors, such as low birth weight, high fructose diets or obesity may increase the risk for hypertension [77] in CKD patients.

One potential factor influencing the risk of hypertension is hyperuricemia [78], which is often related to obesity or metabolic syndrome, but also to reduced kidney function. Uric acid levels correlate with endothelial dysfunction and higher BP [79]. Hyperuricemia is involved in the generation of reactive oxygen species (ROS), low NO levels, activation of the RAAS, and endothelial dysfunction. The resulting pro-vasoconstrictive conditions cause ischemia and chemokine release, local inflammation and intrarenal generation of vasoconstrictors that further promote ischemia and block pressure-natriuresis. Sodium retention leads to an increase in serum osmolality, activation of the sympathetic nervous system, constriction of vascular smooth muscle cells, and an increase in systemic vascular resistance. Increasing BP ameliorates tubular ischemia by shifting the pressure-natriuresis curve towards higher pressure and the salt-resistant state [77].

Secondary *hyperparathyroidism*, a complication of CKD, may be another contributor to the high prevalence of hypertension. A retrospective study in adults with CKD demonstrated higher systolic and diastolic BP in patients with elevated parathyroid hormone levels [80]. Since BP correlated highly with serum calcium levels, a mechanistic role of increased cytosolic calcium in patients with severe hyperparathyroidism has been postulated.

BP may also be increased due to *adverse effects of drugs* used in the treatment of CKD. For example, erythropoiesis stimulating agents (ESAs) might increase BP by increasing hematocrit. Hypertension may be secondary to the direct vasoconstrictive actions of steroids, calcineurin inhibitors and other immunosuppressive or anti-inflammatory drugs prescribed in acute or chronic immune-mediated kidney disease or after kidney transplantation.

Factors involved in the pathogenesis of hypertension in CKD are summarized in Fig. 50.1.

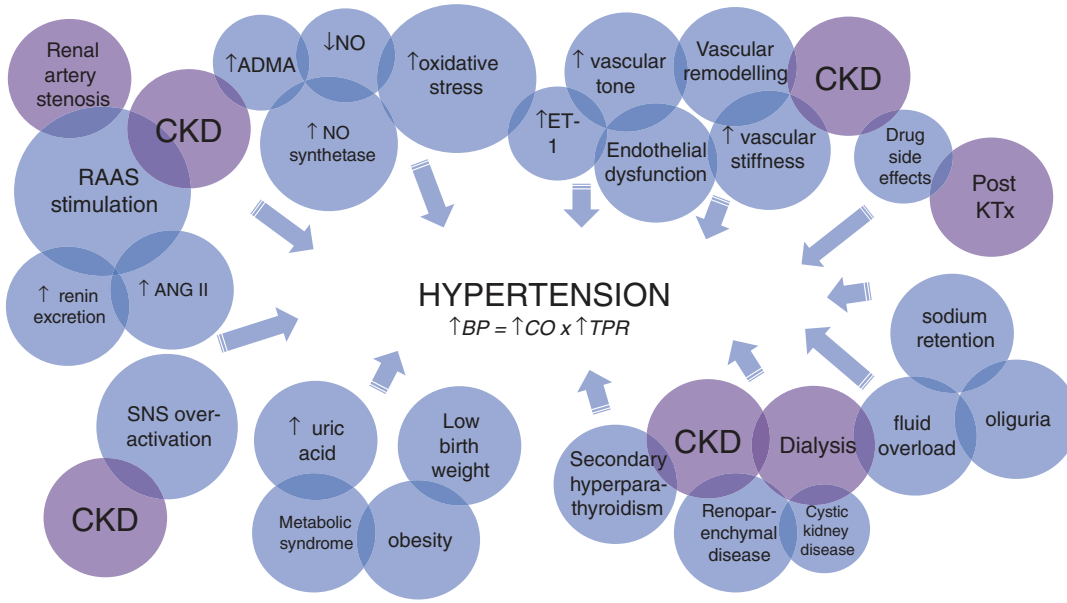

Fig. 50.1 Pathophysiology of hypertension in chronic kidney disease. *RAAS* renin-angiotensin-aldosterone system, *ANG II* angiotensin II, *SNS* sympathetic nervous system, *CKD* chronic kidney disease, *NO* nitric oxide, *ET-1* endothelin-1, *KTx* kidney transplant, *CO* cardiac output, *TPR* total peripheral resistance, *BP* blood pressure

Hypertension in Specific Renal Conditions

Renoparenchymal hypertension is common in various forms of *acute and chronic glomerulonephritis*. The most common underlying histopathological entities associated with hypertension, even in the absence of kidney failure, are poststreptococcal, focal-segmental, membranoproliferative and crescentic glomerulonephritis. Persistent hypertension is also common in patients with glomerulonephritis secondary to systemic vasculitis, such as systemic lupus erythematosus, and in patients with hemolytic uremic syndrome (HUS).

Hypertension in Acute Kidney Injury

In acute kidney diseases, changes in BP usually mirror changes in disease activity.

In *acute glomerular disease*, e.g., poststreptococcal glomerulonephritis or nephrotic syndrome, patients are often volume expanded due to sodium retention by acute renal impairment [81]. BP increases due to fluid overload and even in subjects with subclinical volume overload the incidence of hypertension may already be increased. While in these conditions the release of atrial natriuretic peptide is enhanced by sodium retention, the RAAS is often suppressed. In contrast, in patients with acute vasculitis of the kidneys ischemia-induced activation of the RAAS is the main underlying mechanism.

In hypertensive patients with *HUS*, a difference in the pathophysiological mechanisms involved in the origin of hypertension between patients with shiga-toxin associated HUS (STEC-HUS) and patients with atypical, complement-disorder associated HUS (aHUS) is hypothesized. In patients with STEC-HUS, fluid overload and salt retention are supposed to play a major role, but in patients with aHUS complement activation and endothelial dysfunction are probably key players [82, 83]. Of note, while malignant hypertension may indicate aHUS and thrombotic microangiopathy (TMA), in patients with malignant hypertension extremely high BP per se can induce a thrombotic microangiopathy.

Hypertension in Chronic Kidney Disease

Chronic renoparenchymal hypertension is not limited to *glomerular disease*, but is also observed in patients with *congenital anomalies of the kidneys and urinary tract (CAKUT)* or with tubulointerstitial disorders leading to kidney scarring. Recurrent pyelonephritis, reflux nephropathy, obstructive uropathies or polycystic kidney disease, can all lead to activation of the local RAAS, tubulointerstitial fibrosis and tubular atrophy.

The risk of hypertension is more closely associated with the type of underlying kidney disease than with the degree of kidney dysfunction. At any given level of CKD (stage 1–5), children with acquired glomerulopathies or polycystic kidney disease tend to have higher BP than those with renal hypoplasia and/or uropathies. In a survey of the ESCAPE trial group in pediatric CKD patients with CKD 2 to 4, the prevalence of hypertension was 88% in patients with acquired glomerulopathies, 38% in children with hypo-/dysplastic kidney disorders and 57% in other congenital or hereditary kidney diseases [8, 9]. Renoparenchymal disorders are responsible for approximately 75% of cases of secondary hypertension in childhood [84].

A condition that can be associated with malignant hypertension in newborns or in early infancy is autosomal recessive polycystic kidney disease (ARPKD). Children with severe ARPKD may present with episodes of hypertensive crisis associated with high mortality risk, including sudden heart failure, cerebral ischemic or hemorrhagic stroke.

Autosomal dominant polycystic kidney disease (ADPKD), while becoming clinically symptomatic only in adults, has a high prevalence of hypertension in childhood when kidney function is still normal [85].

As described above, the pathophysiological mechanisms in renal parenchymal hypertension encompass an increased activity of the RAAS, sodium and water retention, enhanced activity of the sympathetic nervous system, oxidative stress and endothelial dysfunction.

Renovascular hypertension accounts for about 10% of pediatric patients presenting with secondary hypertension. Renovascular hypertension results from vascular lesions that impair blood flow to one or both kidneys or to kidney segments [86, 87]. Post-stenotic decreased renal perfusion results in stimulation of the RAAS. The most common causes of renovascular hypertension in childhood are idiopathic stenosis of the kidney artery, fibromuscular dysplasia, mid-aortic syndrome, and genetic or syndromic disorders such as neurofibromatosis type 1 (NF1, von Recklinghausen), which can affect not only the renal, but also intrarenal arteries, Williams-Beuren syndrome, and Alagille syndrome.

Kidney artery stenosis should be ruled out in children with very high BP (stage 2 hypertension), hypertensive complications (e.g., hypertensive crisis with cerebral symptoms, heart failure, facial neve palsy), uncontrolled hypertension on more than two antihypertensive drugs, worsening of kidney function after initiation of ACE inhibitor (ACEi) or angiotensin receptor blocker (ARB) therapy, or increased plasma renin activity with hypokalemia.

The preferred therapeutic intervention in childhood renovascular hypertension is revascularization of the stenosis. Detailed information on the management of kidney artery stenosis can be found in Chap. 49.

Hypertension in Pediatric Dialysis Patients

The prevalence of *hypertension in pediatric peritoneal or hemodialysis patients* (CKD stage 5D) is very high. At time of dialysis initiation, up to 80% of patients are hypertensive; out of these 60% are untreated or have uncontrolled hypertension despite antihypertensive drug therapy. After 1 year on dialysis more than 50% of patients remain hypertensive [88–90]. Hypertension may be maintained by the underlying kidney disease, especially in patients with glomerulopathies or PKD, but also by inappropriately high fluid and sodium intake.

In a survey of more than 1300 pediatric dialysis patients in the European ERA/EDTA registry, the overall prevalence of hypertension was similar in hemodialysis (HD; 69.7%) and peritoneal dialysis (PD; 68.2%) patients. However, the percentage of uncontrolled hypertension was higher in HD compared to PD patients (45% vs. 35%) [91]. These epidemiologic data were derived from casual BP measurements, with a single BP recording per patient reported to the registries. For the interpretation of these data, consideration of the time of BP measurement is important because pre-dialysis measurements are usually higher compared with post-dialysis measurements, resulting in a higher probability to be classified as hypertensive when only pre-dialysis measurements are considered.

The dominant factor contributing to hypertension in dialysis patients is volume overload; other contributing factors include, as in non-dialysis CKD patients, activation of the RAAS and the sympathetic nervous system, endothelial dysfunction, increased arterial stiffness, hyperparathyroidism and exposure to BP elevating drugs.

Contrary to the physiologically expected suppression of the RAAS in a state of salt or fluid overload, plasma renin activity was found to be significantly higher in a study comparing hypertensive to normotensive dialysis patients. These findings suggest that in ESKD patients with adequately controlled sodium balance, the RAAS is an important factor involved in the pathogenesis of hypertension [92]. Also, the significant decline in BP observed after bilateral nephrectomy [93] points to volume-independent mechanisms of hypertension in dialysis patients. A further factor contributing to arterial hypertension in ESKD might be the markedly decreased plasma levels of *renalase*. Renalase deficiency and the resulting increase of circulating catecholamine levels may also contribute to the high prevalence of hypertension and cardiovascular disease in ESKD [24, 94].

Hypertension in Pediatric Renal Transplant Recipients

In children and adolescents after kidney transplantation, hypertension is almost as common as in dialysis patients and constitutes a serious comorbidity [91, 95, 96], not only with respect to cardiovascular morbidity and mortality, but also for kidney transplant survival [97]. Posttransplant hypertension might be caused by the native kidneys, vascular stenosis of the transplant kidney artery, acute or chronic graft dysfunction, and recurrent or de-novo glomerulonephritis in the transplant kidney. In addition, medication side effects of immunosuppressive drugs (steroids, calcineurin inhibitors), including excessive weight gain with steroid treatment, have an important role in posttransplant hypertension.

Hypertension Associated with Disorders of Renal Tubular Sodium Handling

In rare cases, hypertension may be caused by autosomal dominant or recessive single gene mutations (***monogenic hypertension***) [98–100]. Characteristically, all hereditary forms of hypertension lead to suppression of the RAAS with low levels of renin concentration or renin activity (‘***low renin hypertension***’) due to expansion of plasma volume. In a broader sense, the pathophysiology of these disorders comprises alterations of renal tubular electrolyte handling due to mutations in genes regulating glucocorticoid synthesis or action effecting the mineralocorticoid receptor, or in genes regulating the sodium excretion in the distal tubule.

Apparent mineralocorticoid excess (AME) is an autosomal recessive disorder in which inactivating mutations in the *HSD11B2* gene, encoding the kidney isozyme of 11-β-hydroxysteroid dehydrogenase 2, lead to increased concentrations of cortisol in the kidneys and to activation of the mineralocorticoid receptor due to cross-reaction of cortisol with the non-selective mineralocorticoid receptor. This induces aldosterone-like effects in the tubule, typically causing hypokalemia, metabolic alkalosis, hypernatremia and hypertension.

Glucocorticoid remediable aldosteronism (GRA; familial hyperaldosteronism type 1) is an autosomal dominant disorder due to altera-

tions in the adjacent 11β-hydroxylase and aldosterone synthase genes on chromosome 8q24.3. Unequal cross-over between these genes results in ACTH-stimulated ectopic secretion of aldosterone from the adrenal zona fasciculata. Increased aldosterone secretion stimulates the mineralocorticoid receptor, resulting in suppressed renin levels and hypertension due to upregulation of potassium excretion and sodium reabsorption in the renal tubule.

Hypertension in congenital adrenal hyperplasia (CAH) is caused by autosomal recessive inherited defects in 11β-hydroxylase (Type IV) or 17α-hydroxylase (Type V), leading to overproduction of 21-hydroxylated steroids and overactivation of the mineralocorticoid receptor. The uncontrolled mineralocorticoid activity results in hypertension and hypokalemia. Additionally, in CAH type IV the enzyme block increases the production of sex steroids, with androgenic actions causing virilization in girls and precocious puberty in boys. In CAH type V, the synthesis of sex hormones is compromised, resulting in primary amenorrhea and delayed sexual maturation in girls and in ambiguous genitalia in boys.

Liddle's syndrome is caused by a dominant gain-of-function mutation in the amiloride-sensitive epithelial sodium channel (ENaC) located in the collecting duct. The overactive ENaC causes increased sodium reabsorption and intravascular volume expansion, which leads to hypertension. Moreover, these patients present with hypokalemia, metabolic alkalosis, and occasionally hypercalciuria.

Gordon's syndrome is characterized by familial hyperkalemia with normal kidney function, reduced renal sodium excretion, hypercalciuria and hyperchloremic metabolic acidosis due to dominant mutations in the WNK serine-threonine kinases WNK1 and WNK4 or in *KLHL3* or *CUL3*, associated with loss of inhibitory regulation of the NCC in the thiazide-sensitive distal convoluted tubule.

For accurate assessment of the different forms of low renin hypertension, analysis of serum renin, aldosterone, serum electrolytes and blood gas analysis, urinary potassium excretion and the urinary steroid profile is required. In addition, further hormonal studies and genetic testing for the disease-causing mutations may be required [98–100].

The etiology of hypertension in pediatric CKD is summarized in Table 50.1. For the diagnostic approach to renal hypertension see Table 50.2. Additional information on the diag-

Table 50.1 Etiology of pediatric renal hypertension

Etiology	Underlying kidney disease/ condition
Renoparenchymal	• Acute glomerulonephritis (e.g. post streptococcal glomerulonephritis, Henoch-Schönlein nephritis) • Chronic glomerulonephritis (e.g., FSGS, IgA nephropathy) • Hemolytic uremic syndrome • Interstitial nephritis • Congenital hypo- or dysplasia of the kidneys • Congenital anomalies of the kidneys and urinary tract • Cystic kidney disease (ARPKD, ADPKD) • Recurrent pyelonephritis with scarring • Acute pyelonephritis • Urological interventions • Kidney tumors • Kidney trauma • Chronic kidney failure • Status post kidney transplantation
Renovascular	• Kidney artery stenosis (e.g., fibromuscular dysplasia with neurofibromatosis, Williams-Beuren syndrome) • Vasculitis (e.g., periarteritis nodosa, Takayasu arteritis) • Compression of kidney artery by tumor, bleeding, abscess
Other renal conditions	• Fluid overload in dialysis patients (misjudgment of fluid status) • Side effects of drug treatment in immunosuppressed patients (e.g., steroids, cyclosporine, tacrolimus) • Forms of monogenic ('low-renin') hypertension

FSGS focal-segmental glomerulosclerosis, *ADPKD* autosomal dominant polycystic kidney disease, *ARPKD* autosomal recessive polycystic kidney disease

Table 50.2 Baseline diagnostic measures in suspected renal hypertension

Medical history	
Family history	Hypertension, cardiovascular disease, hereditary kidney disease
Perinatal history	Birth weight, gestational age, oligohydramnios
Current findings	Urinary tract infections, renal or urinary diseases/malformation
Potential renal findings	Dysuria, polydipsia, polyuria, nycturia, hematuria, edema, weight loss, failure to thrive
Indicators for target organ damage	Headache, nose bleeding, vertigo, dizziness, blurred vision, facial palsy, seizures, stroke, dyspnea
Medication	Antihypertensives, steroids, CNI (ciclosporine A, tacrolimus), …
Physical examination	
General	Height, weight, body mass index
Hypertension-associated syndromes	Neurofibromatosis, Williams-Beuren syndrome, …
Cardiovascular exams	Measurements of pulse rate and blood pressure at all 4 extremities Murmur, flow noise at heart, abdomen, flanks, back, neck, head, signs of heart insufficiency
Abdomen	Palpable mass → Wilms tumor, autosomal-dominant or recessive polycystic kidney disease, obstructive uropathy, hepatosplenomegaly → autosomal-recessive polycystic kidney disease
Virilization, ambiguous genitalia	Forms of monogenic hypertension
Basic diagnostics	*Finding → indicating …*
Creatinine, urea (serum)	Creatinine, urea increased → kidney insufficiency
Electrolytes (serum)	Hypokalemia → kidney artery stenosis Hyperkaliemia → kidney insufficiency
Blood gas analysis	Metabolic acidosis → kidney insufficiency
Urinalysis: erythrocytes, leukocytes, glucose, protein, albumin[a]	Hematuria, proteinuria → glomerulonephritis Leukocyturia → pyelonephritis Glucosuria → tubulopathy, diabetes mellitus
Ultrasound of kidneys and urinary tract	Enlarged hyperechogenic kidneys → glomerulonephritis, pyelonephritis Kidney cysts → polycystic kidney disease, cystic dysplastic kidney disease, multicystic dysplastic kidney disease Small, hyperechogenic kidneys → kidney hypoplasia, kidney dysplasia Side differences → unilateral kidney hypo/dysplasia, vesicoureteral reflux with scarring, indicating kidney artery stenosis Tumor
Ophthalmological	Hypertensive retinopathy → end organ damage
Echocardiography	Left ventricular hypertrophy → end organ damage
Further diagnostics	*Finding → indicating…*
Renin, aldosterone levels Renin activity Renin/aldosterone ratio	Hyperreninism → kidney artery stenosis, renoparenchymal hypertension Low-renin hypertension → monogenic hypertension
Urinary steroid profile	→ Forms of monogenic hypertension
Genetic testing	→ Forms of monogenic hypertension
Doppler ultrasound	Flow in kidney arteries, side-different kidney size and resistance indices → kidney artery stenosis
CT or MRI angiography	→ Kidney artery stenosis
Angiography	→ Kidney artery stenosis
Renal scintigraphy	Kidney scars, kidney function
MRI abdomen	Tumor localization and size

[a] Quantitative excretion of protein and albumin in 24 h urine collection or protein/creatinine and albumin/creatinine ratio in morning spot urine samples

nosis of hypertension and BP monitoring in CKD patients is provided in Chap. 49.

Antihypertensive Treatment Strategies in Acute and Chronic Kidney Disease

The main goal of BP control in acute kidney injury is to attain a BP level in the normal range and to avoid acute hypertensive damage to the kidneys and the cardiovascular system.

In CKD-associated hypertension, the growing understanding of the epidemiology, pathophysiology and target organ damage has promoted the search for effective long-term therapeutic strategies (for further information see also Chaps. 49 and 51). These relate to both BP targets and preferred antihypertensive drug choices.

Blood Pressure Target

The therapeutic goal in all CKD children with hypertension is long-term and consistent BP control irrespective of the underlying condition. Hypertension-related cardiovascular end-organ damage such as left ventricular hypertrophy, hypertensive retinopathy, and progressive kidney function deterioration has to be avoided and the risk of long-term cardiovascular sequelae and kidney failure should be minimized. While in children with primary hypertension a BP goal below the 95th BP percentile or below the adult thresholds, whichever is lower, is recommended and this target might be also applicable for hypertension in acute kidney injury, there is ample evidence for beneficial effects of lower BP targets in CKD patients.

In adult CKD patients with diabetic or non-diabetic kidney diseases, meta-analyses of antihypertensive trials showed an almost linear relationship between achieved BP and the annual GFR loss [101]: The better the BP control, the better was kidney survival. As a result, the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC7) published in 2003 recommended a BP goal of <130/80 mmHg in patients with CKD or diabetes, as compared to <140/90 mmHg in primary hypertension [102]. However, at that point in time the renoprotective superiority of very *strict BP control* had not yet been unequivocally demonstrated in adult nephropathies [103–108]. Thus, the usefulness of lower BP targets in adults with CKD had been questioned. Meanwhile, the findings of the SPRINT trial, published in 2015 [109], gave new impetus to the discussion on BP targets. In this prospective randomized controlled trial including more than 9000 participants aged 50 and older with increased cardiovascular risk (26% in CKD stage 3–4), patients with intensified BP control (systolic BP <120 mmHg) showed 25% lower fatal and nonfatal cardiovascular event rates, 27% lower all-cause mortality, and a 43% reduction of relative risk to die from any cardiovascular cause with the intensive intervention compared to standard treatment (systolic BP <140 mmHg). However, there was no difference in primary cardiovascular outcomes in CKD patients [109] and some methodological aspects of the study are critically discussed [110].

In children with CKD, the Efficacy of Strict Blood Pressure Control and ACE Inhibition on Renal Failure Progression in Pediatric Patients (ESCAPE) Trial has provided evidence for a nephroprotective effect of intensified BP control [63]. CKD children randomized to a target 24-h mean arterial pressure below the 50th percentile for age were 35% less likely to lose 50% GFR or progress to ESKD within 5 years than children with a more conventional BP target between the 50th and 95th BP percentile. The risk to attain the study endpoint was increased by 15% for each mm Hg above the 50th percentile, while a BP below the 50th percentile did not significantly affect renal risk [63]. The protective effect of low normal BP on kidney function seen in the ESCAPE trial was independent of RAAS inhibition since all subjects received the same dose of the ACE inhibitor ramipril. While the benefit was greatest in children with glomerular disorders, it was also significant in children with renal hypoplasia or dysplasia. Survival analysis stratified by the achieved 24-h BP suggested that any BP

exceeding the 50th percentile was associated with a compromised kidney outcome [63]. Also in this study, proteinuria was found to be an important modifier of the renoprotective efficacy of intensified BP control. Patients with significant proteinuria had a greater benefit from intensified BP control than non-proteinuric patients.

Based on the findings of the ESCAPE trial [63], the European Society of Hypertension (ESH) pediatric guideline recommends that antihypertensive treatment should be initiated if BP exceeds the 90th percentile and aim for a mean 24-h ambulatory BP target below the 75th percentile in non-proteinuric and below the 50th percentile in proteinuric children and adolescents with CKD [111]. In adolescents aged 16 years and older a BP below 130/80 mmHg should be aimed for in non-proteinuric and below 125/75 mmHg in proteinuric CKD [111]. For young adults with kidney disease, the guideline of the European Societies for Cardiology and Hypertension (ESC/ESH) recommends a BP target of 130–139/70–79 mmHg [21].

The American Academy of Pediatrics Practice Guideline for Management of Hypertension in Children and Adolescents [112] recommends that all children or adolescents with CKD be treated to lower 24-h mean arterial pressure to <50th percentile by ABPM, irrespective of the degree of proteinuria, whereas children with primary hypertension be treated by non-pharmacological or pharmacological intervention to a reduction in systolic and diastolic BP to below the 90th percentile (or below 130/80 mmHg in adolescents ≥13 years old).

Choice of Antihypertensive Drugs

Treatment of Hypertension in Children with Acute Kidney Injury

In view of the different etiologies of acute kidney injury, treatment of hypertension should be individualized. In patients with acute glomerulopathies and edema, such as those presenting with hypertension due to poststreptococcal glomerulonephritis or nephrotic syndrome, where fluid overload and salt retention play a major role, the initial therapy is diuretics. Loop diuretics should be prescribed in patients with severely reduced GFR since thiazides are ineffective when GFR falls below 30. RAAS antagonists can be effective as second line treatment for persistently elevated BP [113], although acute glomerulonephritis typically is a state of low-renin hypertension.

In patients with acute vasculitis, ACE inhibitors are recommended as first line antihypertensive therapy since activation of the RAAS due to renal ischemia is the main pathophysiological mechanism. Since STEC-HUS leads to RAAS activation, ACE inhibitors and angiotensin receptor blockers are the treatment of choice in this condition. In children with hypertension due to atypical HUS mediated by complement activation, treatment of the underlying complement disorder is essential to normalize BP in addition to acute antihypertensive therapy using either RAAS antagonists or calcium channel blockers [83].

Treatment of Hypertension in Children with CKD

The major recommended antihypertensive drug classes (ACEi, ARB, calcium channel blockers (CCB), β-blockers) exert comparable BP lowering effects in CKD patients. A list of antihypertensive drugs used in children with CKD is given in Table 50.3.

In view of the crucial role of the RAAS in kidney failure progression, *RAAS antagonists* (ACEis and ARBs) might confer specific nephroprotective properties beyond their antihypertensive action. RAAS antagonists reduce the intraglomerular pressure, thereby lowering proteinuria and suppressing local inflammatory processes, with subsequent reduction of glomerular hypertrophy and sclerosis, as well as tubulointerstitial inflammation and fibrosis. Most randomized clinical trials have demonstrated superior renoprotective efficacy of RAAS antagonists (ACEis and ARBs) in adults with diabetic and non-diabetic CKD. Several meta-analyses have confirmed the nephroprotective benefit of RAAS antagonists, although the effect size is quite variable [114, 115]. One analysis suggested that the nephroprotection conferred by ACEi might be partially independent of their antihypertensive

Table 50.3 Pharmacological treatment of renal hypertension in children

Class	Drug	Mode of action	Side effects
ACE inhibitors	e. g., Captopril Enalapril Ramipril Fosinopril Benazepril Lisinopril	Inhibition of the conversion of angiotensin I to angiotensin II → vasodilatation, reduction of sympathetic tone, decreased aldosterone dependent salt and water retention, nephroprotection	Cough, hyperkalemia, increase of serum creatinine, hypotension, dizziness, fatigue, headache, laryngioedema, neutropenia *Caveat*: contraindicated in bilateral severe kidney artery stenosis *Caveat*: pre-renal acute kidney failure in dehydration episodes *Caveat*: pregnancy (teratogenic)
Angiotensin-receptor blockers	e. g., Candesartan Valsartan Losartan Irbesartan	Blockade of angiotensin-II-type-1-receptors Nephroprotection (see also ACE inhibitors)	Side effects similar to ACE inhibitors, no cough Rhabdomyolysis, thrombocytopenia
Calcium channel blockers	e. g., Nifedipine Nitrendipine Amlodipine	Reduced influx of calcium into the cells → relaxation of the arterial vascular muscle cells, decrease of peripheral vascular resistance	Headache, flush, tachycardia/palpitations, peripheral edema, fatigue, hypotension, gingival hyperplasia
Diuretics	e. g., Furosemide, Torsemide Hydrochlorothiazide	Increase of renal sodium excretion, decrease of peripheral resistance	Hypokalemia, hyponatremia, alkalosis, extracellular volume depletion, hyperuricemia, diuretics enhance ACE inhibitor effect *Cave* acute kidney failure in dehydration episodes
β-Blockers	e.g., Atenolol Metoprolol Propranolol Carvedilol	Reduction of cardiac output, renin-aldosterone release and of sympathetic activity	Tiredness, dizziness, depression, reduced physical capacity, bradycardia, hypotension, nausea, bronchospasm, *Cave* β-blocker in diabetes mellitus or pulmonary obstruction/asthma
Alpha-blocker	e.g., Doxazosin Phenoxybenzamine	Relaxation of vascular smooth muscle cells, direct vasodilatory effect	Edema, dizziness, fatigue, headache
Centrally acting adrenergic drugs (alpha2-agonists)	e.g., Clonidine	Reduction of sympathetic outflow, decrease in peripheral vascular resistance, decrease of heart rate	Dizziness, headache, fatigue, xerostomia
Vasodilators	e.g. Minoxidil Hydralazine	Activation of gated potassium channels, vasodilatation, lowering of total peripheral resistance	Edema, fluid retention, salt retention, headache, hypertrichosis, leukopenia, nausea, palpitations/tachycardia

Examples of antihypertensives with (some) clinical experience in pediatric hypertension
ACE angiotensin-converting enzyme

and antiproteinuric actions [114]. Thus, RAAS antagonists are considered the first-line pharmacological therapy in hypertensive CKD patients.

For pediatric CKD, the Chronic Kidney Disease in Children (CKiD) Study found an increased prevalence of uncontrolled hypertension in children not receiving ACEi or ARBs, supporting the use of RAAS antagonists as preferred antihypertensive agents in pediatric CKD [5]. Both ACEi and ARBs have been shown to reduce systolic and diastolic BP efficiently [63] in a dose-dependent manner [116]. The drugs

were very well tolerated and side effects requiring ACEi or ARB withdrawal due to acute increases of serum creatinine, hyperkalemia or hypotensive episodes were rare [63, 116].

As expected, in patients with underlying CKD the prevalence of reported adverse events is higher, and increases in serum potassium, creatinine and blood urea nitrogen are more commonly reported compared to non-CKD patients. Approximately 25–30% of CKD patients experience an eGFR decline of more than 25% [117]. However, it has been hypothesized that the observed increase in serum creatinine after start of RAAS blockade is due to hemodynamic changes in the glomerulus, which does not reflect kidney injury and should thus be reversible at withdrawal of RAAS blockade [118].

In some patients, the RAAS is incompletely suppressed by ACEi alone, and the possibility of partial secondary resistance due to compensatory upregulation of ACE-independent angiotensin II production ('aldosterone escape') has been suggested [119–121]. Interestingly, in those patients with breakthrough proteinuria followed in the ESCAPE trial [63], BP was still well controlled. Thus, the doses required to achieve the maximal antiproteinuric effect of ARBs may be much higher than the maximally active antihypertensive doses. Significant additional proteinuria lowering was achieved without increased side effects in adults with 64 mg and even 128 mg of candesartan, which has only a minor additional BP lowering effect beyond daily doses of 16–32 mg [122]. Similarly, *dual RAAS blockade* by combined use of an ACEi and an ARB does not exert significant additional antihypertensive effects compared to the maximum recommended dose in monotherapy, but still might improve proteinuria. However, the risk-benefit balance of combining ACEis and ARBs in younger subjects is still debated [123–125].

Other drug classes inhibiting the RAAS exert antihypertensive effects. Aliskiren is a direct *renin antagonist*, blocking the conversion from angiotensinogen to angiotensin I. Its BP lowering effect is comparable to that of ARBs and a combination of aliskiren and valsartan at maximum recommended doses provided significantly greater BP reduction than the respective monotherapies [126]. However, combination therapy of aliskiren with ACEi or ARBs significantly increased the risk of cardiovascular events in adults with diabetes or CKD and is therefore not recommended [127, 128].

Mineralocorticoid receptor antagonists (MRA) also lower BP; however, their use is limited by the risk of side effects, including hyperkalemia, gynecomastia, impotence, and amenorrhea. These side effects are more common for the steroidal MRAs spironolactone and eplerenone. Due to the high risk of hyperkalemia, the combination of ACEIs and steroidal MRAs in CKD patients has not been recommended [129]. The novel nonsteroidal MRAs finerenone and esaxerenone have a higher potency and selectivity for the mineralocorticoid receptor and thus seem to have fewer side effects.

Because the antihypertensive effect of MRAs is not superior to ACEis or ARBs, they are not used as first line agents in renal hypertension. However, recent studies support a future role, especially of the nonsteroidal MRAs, in the prevention of cardiovascular disease and CKD progression by reducing oxidative stress, inflammation, fibrosis, endothelial dysfunction and proteinuria [130, 131]. MRAs play an important role in the treatment of some forms of monogenic hypertension (see Table 50.4).

In a substantial number of pediatric CKD patients with hypertension, multidrug antihypertensive therapy is required [3, 63]. The choice of additional antihypertensive drugs in children with CKD is largely arbitrary.

Dihydropyridine *calcium channel blockers* have no antiproteinuric effect and may actually promote proteinuria and more rapid CKD progression [132]. However, their combination with ARBs or ACEis provides powerful BP lowering and even conferred a patient survival advantage as compared to the combination of ARBs or ACEis with thiazide diuretics [133, 134]. Non-dihydropyridine calcium channel blockers (diltiazem, verapamil) are antiproteinuric and therefore potentially renoprotective, but have a weaker effect on BP [132].

The use of *β-receptor blockers* appears rational in view of the sympathetic overactivation in

Table 50.4 Dosing of antihypertensive medication in children with CKD (*modified from* [156], *Springer International Publishing*)

Class	Drug	Usual pediatric dosing range[a]	Dosing modifications in CKD stage 3–5 D[a]
Angiotensin receptor blockers	Candesartan	*1–6 years*: 0.2 mg/kg/day up to 0.4 mg/kg/day *6–17 years*: *<50 kg*: 4–16 mg QD *>50 kg*: 8–32 mg QD	No known recommended adjustment but clearance reduced if GFR <30 mL/min; not removed by dialysis; give 50% of usual dose; consider dosing after HD session
	Losartan	0.75 mg/kg/day to 1.4 mg/kg/day; maximum 100 mg daily	Not recommended if GFR <30 mL/min; not removed by dialysis
	Olmesartan	*20–35 kg*: 10–20 mg QD *≥35 kg*: 20–40 mg QD	Clearance reduced if GFR <20 mL/min; do not exceed 20 mg daily in such patients; not removed by dialysis
	Valsartan	*<6 years:* 5–10 mg/day up to 80 mg daily *6–17 years*: 1.3 mg/kg/day up to 2.7 mg/kg/day; maximum 160 mg daily	Clearance reduced if GFR <30 mL/min; not removed by dialysis
Angiotensin converting enzyme inhibitors	Benazepril	0.2 mg/kg/day up to 0.6 mg/kg/day; maximum 40 mg daily	No pediatric data. 20–50% removed by dialysis; give 25–50% of usual dose; consider dosing after HD session
	Captopril	0.3–0.5 mg/kg/dose TID up to 0.6 mg/kg/day; maximum 450 mg daily	No pediatric data. 50% removed by dialysis. Give 25% of usual dose in HD patients; consider dosing after HD session. Give 50% QD of usual daily dose in PD
	Enalapril	0.08 mg/kg/day up to 0.6 mg/kg/day; maximum 40 mg daily	Not studied in children with GFR <30 mL/min. 50% removed by dialysis. Give 50% of usual dose; consider dosing after HD session
	Fosinopril	0.1 mg/kg/day (up to 10 mg/day) up to 0.6 mg/kg/day; maximum 40 mg/day	No known adjustments; not removed by dialysis
	Lisinopril	0.07 mg/kg/day (up to 5 mg/day) up to 0.6 mg/kg/day; maximum 40 mg daily	Not studied in children with GFR <30 mL/min. 50% removed by dialysis. Give 25% of usual dose; consider dosing after HD session
	Quinapril	5–10 mg/day up to 80 mg daily	No pediatric data. For adults with GFR 10–30 mL/min, do not exceed 2.5 mg/day; no data for GFR <10 mL/min
	Ramipril	1.6 mg/m^2 BSA/day QD up to 6 mg/m^2/day; maximum 20 mg daily	No pediatric data. In adults with GFR <40 mL/min, give 25% of usual dose; 20% removal by dialysis. Consider dosing after HD session
α- and β-blocker	Carvedilol	0.1 mg/kg/dose BID (up to 6.25 mg) up to 0.5 mg/kg/dose; maximum 25 mg BID	No adjustment needed; not removed by dialysis
	Labetalol	2–3 mg/kg/day BID up to 10–12 mg/kg/day; maximum 1200 mg daily	No adjustment needed; not removed by dialysis

(continued)

Table 50.4 (continued)

Class	Drug	Usual pediatric dosing range[a]	Dosing modifications in CKD stage 3–5 D[a]
β-Blocker	Atenolol	0.5–1 mg/kg/day up to 100 mg daily	If GFR 15–35 mL/min, do not exceed 50 mg daily (reduction to 50% of usual dose); if GFR <15 mL/min, do not exceed 25 mg daily (reduction to 25% of usual dose). 50% removed by dialysis; consider dosing after HD session
	Metoprolol	Immediate release: 1–2 mg/kg/day BID up to 6 mg/kg/day; maximum 200 mg daily Extended release: 1 mg/kg/day up to 2 mg/kg/day; maximum 200 mg daily	No adjustment needed; not removed by dialysis
	Propranolol	1 mg/kg/day TID-QID up to 8 mg/kg/day; maximum 640 mg daily	No adjustment recommended but can accumulate in kidney impairment; not removed by dialysis
Calcium channel blockers	Amlodipine	0.06 mg/kg/day up to 0.6 mg/kg/day; maximum 10 mg daily	No adjustment needed; not removed by dialysis
	Diltiazem	1.5–2 mg/kg/day up to 6 mg/kg/day; maximum 360 mg daily	No adjustment needed; not removed by dialysis
	Felodipine	2.5–10 mg/day; maximum 10 mg daily	No adjustment needed; not removed by dialysis
	Isradipine	0.05–0.15 mg/kg/dose TID/QID up to 0.8 mg/kg/day; maximum 20 mg daily	No adjustment needed; not removed by dialysis
	Extended-release nifedipine	0.25–0.5 mg/kg/day up to 3 mg/kg/day; maximum 120 mg daily	No adjustment needed; not removed by dialysis
Central α-agonist	Clonidine	5–20 μg/kg/day BID up to 15 μg/kg/day; maximum 0.9 mg daily	No known adjustments; 5% removed on HD
Peripheral α-blockers	Prazosin	0.05–0.1 mg/kg/day TID up to 0.5 mg/kg/day; maximum 20 mg daily	No known adjustments; not removed by dialysis
	Doxazosin	1 mg QD up to 4 mg daily; maximum adult dose is 16 mg daily	No known adjustments; not removed by dialysis
	Terazosin	1 mg QD up to 20 mg daily	No known adjustments; 10% removed on HD
Vasodilators	Hydralazine	0.25 mg/kg/dose TID up to 7.5 mg/kg/day; maximum 200 mg daily	No known adjustments; 25–40% removed by dialysis. Consider dosing after HD session
	Minoxidil	0.1–0.2 mg/kg/day QD-BID up to 1 mg/kg/day; maximum 50 mg daily	No known adjustments
Diuretics	Chlorthalidone	0.3 mg/kg/day up to 2 mg/kg/day; maximum 50 mg daily	Avoid in oligoanuria or with GFR <10 mL/min

Table 50.4 (continued)

Class	Drug	Usual pediatric dosing range[a]	Dosing modifications in CKD stage 3–5 D[a]
	Furosemide	0.5–2 mg/kg/dose QD-QID up to 6 mg/kg/day; maximum 600 mg daily	Avoid in oligoanuria; not removed by dialysis
	Hydroclorothiazide	0.5–1 mg/kg/day; maximum 25 mg daily	Not effective in GFR < 30

BID twice daily, *GFR* glomerular filtration rate, *HD* hemodialysis, *kg* kilogram, *μg* microgram, *mg* milligram, *PD* peritoneal dialysis, *QD* once daily, *QID* four times daily, *TID* three times daily

[a] Recommendations represent the authors' opinions although every effort has been made to confirm by consulting appropriate references. Manufacturers' prescribing information is frequently updated and should be consulted whenever possible

Table 50.5 Advantages of specific antihypertensive combination therapies

Combination	Advantages
ACEi or ARB + diuretic	Enhanced antihypertensive effect of RAAS blockade by diuretic-mediated salt loss
	Decreased risk of RAAS-mediated hyperkalemia by diuretic-mediated potassium excretion
	Reduction of diuretic-mediated increase in plasma renin activity by RAAS blockade
ACEi or ARB + CCB	Attenuated CCB-mediated activation of the sympathetic nervous system by RAAS blockade
	Reduced risk of (dose dependent) CCB-mediated peripheral edema by RAAS blockade
ACEi + ARB[a]	Enhanced RAAS blockade (caveat: increased risk of hyperkalemia, increase in serum creatinine)
	Additional blockade of ACEi-independent pathways in the RAAS → reduced risk of aldosterone rebound phenomenon

ACEi angiotensin converting enzyme inhibitor, *ARB* angiotensin receptor blocker, *CCB* calcium channel blocker, *RAAS* renin-angiotensin-aldosterone system

[a] Combination therapy of ACE + ARB not recommended

CKD. Metoprolol and atenolol were the first antihypertensive drugs used to demonstrate nephroprotective effects by good BP control [135]. Newer β-blockers, such as carvedilol, exert a significantly greater antiproteinuric effect than atenolol at comparable BP reduction [136, 137]. Dosing recommendations for antihypertensive drugs in CKD are given in Table 50.5.

Combination of different drug classes might not only exert additive effects on BP control, but also might counterbalance or increase side effects of antihypertensive agents. Therefore, some antihypertensive drug combinations seem to be more favorable than others (Table 50.6).

In patients with advanced kidney impairment, refractory, multi-drug resistant hypertension usually indicates fluid overload and is an indication to start dialysis therapy.

Because renal hypertension is characterized by the loss of the physiological nocturnal decrease in BP (nocturnal BP dipping) in the majority of patients, the ***timing of antihypertensive drug administration*** should receive additional attention. For example, bedtime dosing of antihypertensive medication may reconstitute the circadian BP rhythmicity by a more marked effect during the nighttime hours. In a recent study in adult CKD patients randomly assigned to take either all antihypertensive medications in the morning or to take at least one drug in the evening, bedtime dosing improved overall BP control, and reduced significantly the risk for cardiovascular events [138].

In addition to pharmacological treatment, ***non-pharmacological interventions*** are generally recommended in all children with renal hypertension [21, 111, 139, 140]. These life-style interventions should comprise a reduction of dietary salt intake, high consumption of fruits and vegetables ('DASH' diet), maintenance of a normal body weight, regular physical activity and refraining from smoking. However, it should

Table 50.6 Risk profile and pharmacological treatment[a] of patients with monogenic, 'low renin' hypertension

Form of monogenic hypertension	Age at onset	Cardiovascular symptoms/risks	Recommended therapy	
Apparent mineralocorticoid excess (AME)	Early childhood	Severe hypertension with extensive target organ damage; hypercalciuria, renal failure; low birthweight, failure to thrive	MRA If MRA not effective add dexamethasone K-supplementation Low sodium diet	Spironolactone 1 mg/kg/day in 1–2 doses (max dose 100 mg/day) Eplerenone (adult dosing) 50 mg/day QD or BID Dexamethasone up to 0.01 mg/kg/day
Glucocorticoid remediable aldosteronism (GRA)	Infancy/ childhood	Severe hypertension, intracranial aneurysms, cerebral hemorrhage, high associated mortality rate (>50% at age 30)	Physiologic doses of a glucocorticoid (suppression of ACTH secretion) If not effective in reducing BP, then add MRA	Hydrocortisone 10–20 mg/m^2/day Prednisolone 0.1 mg/kg/day Dexamethasone up to 0.01 mg/kg/day Spironolactone 1 mg/kg/day in 1–2 doses (max dose 100 mg/day) Eplerenone (adult dosing) 50 mg/day QD or BID
Congenital adrenal hyperplasia (CAH) (11βhydroxylase deficiency)	Infancy	Hypertension		Hydrocortisone 10–20 mg/m^2/day Prednisolone 0.1 mg/kg/day Dexamethasone up to 0.01 mg/kg/day
Liddle syndrome	Late childhood to adolescence	Hypertension	Direct ENaC inhibition Low sodium diet	Amiloride 0.4–0.625 mg/kg QD (max 20 mg/day) Triamterene 0.5–1 mg/kg/dose BID (max 3–5 mg/kg/day)
Gordon's syndrome	Adolescence to adulthood	Hypertension	Low dose thiazide Low sodium diet	Hydrochlorothiazide 0.5–1 mg/kg QD or BID

ACTH adrenocorticotropic hormone, *BID* twice daily, *BP* blood pressure, *ENaC* epithelial sodium channel, *mg* milligram, *MRA* mineralocorticoid receptor antagonist, *QD* once daily

[a] Recommendations represent the authors' opinions although every effort has been made to confirm by consulting appropriate references. Manufacturers' prescribing information is frequently updated and should be consulted whenever possible

be noted that in children with advanced CKD a high potassium diet might not be feasible. Also, low-salt diets may not be possible in children with renal salt wasting, as often occurs in kidney hypodysplasia.

Treatment of Hypertension in Dialysis Patients

In dialysis patients, where fluid and salt overload play a major role in the pathogenesis of hypertension, *adjustment of dry weight* and *restriction of dietary sodium intake* are the primary measures to correct an elevated BP.

Dry weight is defined as the lowest body weight at the end of a dialysis session at which a patient can remain normotensive without antihypertensive medication until the next dialysis treatment, or the lowest weight a patient can tolerate without exhibiting symptoms of hypotension [141]. However, the determination of dry weight is challenging and often has to be achieved by trial and error. In clinical practice, dry weight is assumed to have been achieved when patients develop signs of intravascular volume depletion, such as drop in BP, cramping, yawning, headache, or abdominal pain (i.e. symptoms often

associated with unpleasant and traumatizing experiences for patients). Simple but rather unprecise methods to assess dry weight include monitoring of pre- and post-dialysis weight and clinical assessment of edema, jugular vein distension and crackles on lung auscultation. Bioelectrical impedance analysis, or bioimpedance, is a method that determines the electrical resistance of the body to the flow of an electric current and is correlated with tissue water content. Bioimpedance can be applied to both HD and PD patients and can facilitate the assessment and achievement of dry weight in infants, children and adolescents on dialysis [142]. Further information on the assessment and attainment of dry weight can be found in the chapters on PD, HD and adequacy of dialysis.

To attain dry weight in HD patients, adjusting the duration of dialysis therapy and or the concentration of dialysate sodium are the main strategies to improve fluid removal. There is increasing evidence that matching dialysate sodium with the patient's pre-dialysis serum sodium concentration leads to reduction in thirst, interdialytic weight gain and hypertension [143–145].

In PD patients, optimization of water and sodium removal can be achieved by optimizing osmotic potential (dialysate dextrose concentration, dwell time) and fill volume.

Controlling dietary sodium intake facilitates achievement of dry weight [146], and is associated with decreased thirst, lower interdialytic weight gain, improved BP control, lower left ventricular mass index and decreased mortality in adults [147–149]. Fluid restriction should always be combined with reduction of sodium intake, because increased sodium intake inevitably increases thirst, leading to greater interdialytic weight gain [150]. However, restriction of sodium intake is difficult to achieve given the high sodium intake of most children: while the recommended sodium intake in hypertensive children with CKD is 1500–2300 mg daily (corresponding to 3.8–5.8 g of salt) [151], children with CKD stage 2–4 have been shown to ingest more than 3–5 g of sodium on average [152].

Antihypertensive drugs should only be prescribed if the adjustment of dry weight and sodium restriction do not result in normalization of BP. The preferable drug class in hypertensive pediatric dialysis patients is still unclear. Calcium channel blockers, beta blockers and RAAS antagonists are frequently used; however, RAAS blockade may have an increased risk for hyperkalemia and loss of residual diuresis while calcium channel blockers might complicate fluid removal during dialysis. For dose adjustments of antihypertensive drugs in dialysis patients, see Table 50.5.

Native kidney nephrectomy is typically considered the last resort in the treatment of hypertension in dialysis patients refractory to antihypertensive treatment after optimization of dry weight and salt balance [153].

Treatment of Hypertension in Transplant Patients

Hypertension in *transplant patients* usually requires pharmacological treatment similar to pre-transplant CKD. While CCBs are favorable with respect to counteracting calcineurin inhibitor-induced vasoconstriction, RAAS blockers are also safe and effective and exert antiproteinuric and potentially also renoprotective effects on kidney allograft function. In adult transplant patients, BP control was associated with improved graft survival [154]. However, whether strict BP control is superior to conventional BP control in pediatric kidney transplant patients remains to be shown.

Like in non-transplant CKD patients, non-pharmacological lifestyle measures, including weight reduction in overweight children, a healthy, low-salt, low-sugar, high-fibre diet (DASH-Diet) and physical activity, should be encouraged.

In patients with stable graft function, steroid withdrawal has been shown not only to improve BP, but also to restore nocturnal BP dipping [155]. Treatment options in hypertensive kidney disease are summarized in Fig. 50.2.

Treatment of Patients with Monogenic 'Low-Renin' Hypertension

Because BP elevation in these forms of hypertension is related to functional disturbances of renal tubular sodium handling and to fluid expansion, specific therapies targeting the underlying disease should be prescribed as initial treatment [98–100].

In patients with apparent mineralocorticoid excess (AME), hypertension ameliorates with MRA

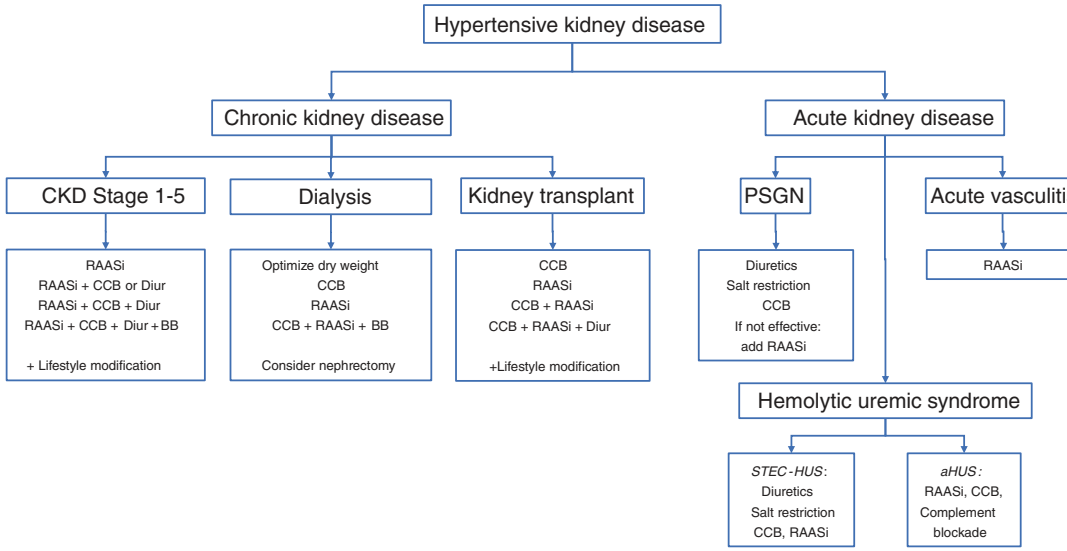

Fig. 50.2 Suggested treatment in hypertensive kidney disease and possible treatment escalation scheme. *CCB* calcium channel blocker, *RAASi* renin angiotensin aldosterone system inhibitor (i.e. ACE inhibitor or angiotensin type I inhibitor), *PSGN* poststreptococcal glomerulonephritis, *aHUS* atypical hemolytic uremic syndrome, *CKD* chronic kidney disease

treatment (spironolactone, eplerenone), along with potential potassium supplements and dietary sodium restriction. Persistently elevated cortisol levels may require glucocorticoid treatment to reduce ACTH-stimulated cortisol production and mineralocorticoid receptor activation.

In patients with *congenital adrenal hyperplasia (CAH type IV or type V)*, treatment consists of glucocorticoids to decrease ACTH secretion. Spironolactone, amiloride, and calcium channel blockers can additionally improve hypertension.

Hypertension in patients with *glucocorticoid remediable aldosteronism* (GRA) responds to treatment with glucocorticoids. If this is not effective to control BP, MRAs, and amiloride or triamterene can be added.

In patients with *Liddle's syndrome*, amiloride or triamterene, targeting the ENaC, are effective in lowering BP. MRAs are not effective in Liddle's syndrome because the ENaC activity is independent of mineralocorticoid regulation.

In Gordon's syndrome, hypertension should be treated with low-dose thiazide, which directly inhibits the underlying NCC overactivity.

Antihypertensive agents are usually of limited efficacy in monogenic forms of hypertension and are not the first-line therapy. For further information on recommended treatment of monogenic hypertension, see Table 50.4.

Monitoring of Patients with Renal Hypertension

Patients with CKD and renoparenchymal hypertension require long-term antihypertensive therapy and life-long monitoring of BP. In patients who recovered from acute kidney injury, BP should be monitored regularly since arterial hypertension and/or proteinuria may manifest years later in patients with subclinical residual kidney damage (e.g., after hemolytic uremic syndrome).

BP should be monitored at each medical appointment in all kidney patients. For further evaluation of BP control and to rule out masked or isolated nocturnal hypertension, conditions that are highly prevalent in CKD, *24-h ABPM* is recommended at least once a year [140]. In patients with insufficiently controlled hyperten-

sion at high risk for hypertensive end-organ damage, evaluation should be repeated at even shorter time intervals. Regular *home BP measurements* are recommended for monitoring of BP between outpatient appointments.

Echocardiographic examinations should be performed once yearly for timely diagnosis of left ventricular hypertrophy. If left ventricular hypertrophy is already present, echocardiographic follow-up should be performed every 3–6 months. Additional information on BP monitoring is given in Chap. 49.

Conclusions

Hypertension is a common condition in children with all stages of CKD and contributes to kidney disease progression by a variety of mechanisms, among which the RAAS plays a central role. Thus, RAAS antagonists are the drug class of first choice; other classes of antihypertensive agents should be added as needed until BP is controlled. In pediatric CKD patients, strict BP control is crucial to the preservation of kidney function and cardiovascular health. ABPM-guided treatment to achieve circadian BP control below the 50th to 75th BP percentile for age is the best currently known strategy to achieve maximal nephroprotection.

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Part XI

Acute Kidney Injury and Neonatal Nephrology

51 Acute Kidney Injury: Pathophysiology, Diagnosis and Prevention

Prasad Devarajan

Introduction

Acute kidney injury (AKI) has been traditionally defined as an abrupt loss of kidney function leading to a rapid decline in glomerular filtration rate (GFR), reduction in urine output, accumulation of waste products such as blood urea nitrogen (BUN) and creatinine, and dysregulation of extracellular volume and electrolyte homeostasis. The term AKI has largely replaced acute renal failure (ARF) since the latter designation over-emphasizes the discrete event of a failed kidney. We now recognize that AKI embodies both the continuum of renal dysfunction that characterizes the clinical spectrum, as well as the diverse molecular, biochemical, and structural injuries to the nephron that occur well before the decline in function. Hence, AKI includes both structural injury and functional impairment. AKI is an increasingly common problem afflicting all ages, occurring in 10–30% of non-critically ill hospitalized children and >30% of children in critical care units. AKI is the leading reason to seek in-patient nephrology consultation and associated with serious short-term and long-term consequences, and therapeutic options are unsatisfactory. The etiology of AKI varies widely according to age, geographical region, and clinical setting [1–7]. Functional AKI induced by dehydration is usually reversible with early fluid therapy. However, the prognosis for patients with structural AKI in the intensive care setting remains guarded. Clinicians now recognize that critically ill patients are dying "of" AKI, and not just simply "with" AKI. Fortunately, the cellular and molecular tools of modern science are providing novel insights into the pathogenetic mechanisms of AKI. Newly discovered pathways are yielding early non-invasive biomarkers for the prediction of AKI and its consequences, as well as innovative strategies for the pro-active treatment and prevention of AKI.

Definitions, Staging, Risk Stratification

Traditionally, AKI has been defined as a rapid decrease in GFR, manifested by an elevated serum creatinine (or a rise from baseline serum creatinine), and/or a reduction in urine output. Based on these criteria, more than 35 different definitions of AKI have plagued the literature, resulting in a wide range of quoted incidence rates, risk factors, and outcomes in the pediatric literature [8]. Essential advances in the field have resulted from consensus AKI definitions, initially in the form of the RIFLE criteria (Risk, Injury, Failure, Loss and End-Stage Kidney Disease) in 2004 with pediatric modifications (pRIFLE) in

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2007 [9]. The pRIFLE classification of AKI includes three graded levels of injury (*R*isk, *I*njury, *F*ailure) based upon the severity of reduction in estimated GFR or urine output, as well as two outcome measures (*L*oss of kidney function i.e., persistent failure for >4 weeks, and *E*nd-stage kidney disease i.e., persistent failure for >3 months). Another refinement was the addition of a 0.3 mg/dL serum creatinine rise in less than 48 h, as also embodied in the Acute Kidney Injury Network (AKIN) criteria [10], since studies have shown independent associations with poor outcomes at this creatinine threshold [8]. However, a systematic review of 12 pediatric studies that employed the RIFLE or pRIFLE classification showed continued wide variations in the relationship between RIFLE class and measures of morbidity and mortality [9]. More recently, the above definitions have been harmonized by the Kidney Disease Improving Global Outcomes (KDIGO) AKI Consensus Conference [11, 12] into a single definition of AKI:

- Increase in serum creatinine by ≥0.3 mg/dL [≥26.5 μmol/L] within 48 h, OR
- Increase in serum creatinine to ≥1.5 times baseline within the prior 7 days, OR
- Urine volume ≤0.5 mL/kg/h for 6 h

The KDIGO staging of AKI is shown in Table 51.1. Both the definition and staging include a 0.3 mg/dL serum creatinine increase criterion to specifically be applicable to pediatric AKI. The staging also allows for a child with eGFR <35 mL/min/1.73 m^2 to be included in Stage 3, in contrast with the adult criterion of ≥4 mg/dL serum creatinine (which would be unusual in infants and young children). The KDIGO AKI definition has now been extensively validated in both children and adults, and is therefore currently recommended to guide clinical care, and as a standardized inclusion and outcome measure in AKI studies. A neonatal modified KDIGO definition has recently been proposed with slight modifications to the KDIGO criteria, and is the recommended definition for clinical and epidemiological studies in neonates [1]. It is likely that validation and widespread clinical integration of the novel AKI biomarkers discussed later in this chapter will further improve our ability to define and stage AKI.

Table 51.1 The Kidney Disease Improving Global Outcomes (KDIGO) AKI criteria (Adapted from Ref. [11])

Stage	Serum creatinine	Urine output
1	1.5–1.9 times baseline, OR ≥0.3 mg/dL (≥26.5 μmol/L) increase	<0.5 mL/kg/h for 6–12 h
2	1.0–2.9 times baseline	<0.5 mL/kg/h for ≥12 h
3	3.0 times baseline, OR SCr ≥4.0 mg/dL (≥353.6 μmol/L), OR Initiation of renal replacement therapy, OR eGFR <35 mL/min per 1.73 m^2 (<18 years)	<0.3 mL/kg/h for ≥24 h, OR Anuria for ≥12 h

Some limitations with the KDIGO AKI definition and staging are well recognized, with respect to both the eGFR and the urine output criteria. First, the eGFR measurement is dependent on serum creatinine, which is imprecise for the clinical diagnosis of AKI (as detailed in section "Clinical Presentation"). Second, the definitions outlined above call for an increase in serum creatinine to ≥1.5 times baseline, and the baseline serum creatinine is often unknown. In previously healthy children, it is generally recommended to employ a presumed baseline of 120 mL/min/1.73 m^2 [13]. In children with available height measurements, the estimated baseline serum creatinine may be imputed by using currently proposed eGFR equations for children [14]. Alternatively, published normative values for serum creatinine based on age and gender can be used. Imputed age- and gender-based serum creatinine norms have been applied to determine baseline serum creatinine and the diagnosis of pediatric AKI in both the hospitalized [15] and the community settings [16]. Third, although a time-dependent reduction in urine output is an important component of AKI diagnosis, most of the published literature is based solely on serum creatinine changes since it is typically more commonly measured and more easily extractable from electronic health records (EHRs). Accurate urine output determination requires an indwell-

ing urinary catheter, which is usually restricted to the critical care setting. In addition, urine output is confounded by hydration status and the clinical use of fluids and diuretics. Finally, the majority of nephrotoxic AKI is non-oliguric. Despite these limitations, recent studies have illustrated that degree of oliguria is strongly associated with poor outcomes in pediatric AKI, and that exclusion of urine output in the definition results in underdiagnosis of AKI, especially in the critical care setting [17, 18].

A new criterion for pediatric AKI was reported, based on a change in reference value of serum creatinine [19]. This study defined pediatric AKI according to pediatric reference change value optimized for AKI in hospitalized children (pROCK) as creatinine increase beyond reference change value of serum creatinine, estimated as the greater of 20 μmol/L or 30% of the initial creatinine level. AKI incidence was lower using the pROCK criteria when compared with the pRIFLE and KDIGO definitions (5.3% versus 15.2% and 10.2%, respectively). In a subsequent study of critically ill children, the pROCK criteria outperformed KDIGO in predicting mortality [20]. However, at the present time, the use of pROCK criteria over the more extensively studied and validated KDIGO criteria is not widely recommended.

The duration of an AKI episode can be used to further refine the diagnosis. For example, transient or reversible AKI (lasting <48 h) portends an improved prognosis when compared to sustained AKI (typically of 2–7 days duration). A new definition of acute kidney disease (AKD) has been proposed for AKI that lasts beyond 7 days and up to 3 months (beyond which the condition is labelled as chronic kidney disease [CKD]). AKD represents a complex syndrome where recurrent bouts of acute injury interact with ongoing regeneration and repair processes. A recent consensus on the evolving definition, management strategies, and research priorities in AKD has been published [21]. AKD is now defined by abnormalities of kidney function and/or structure with implications for health and with a duration of ≤3 months. AKD may include AKI, but also includes abnormalities in kidney function that are not as severe as AKI or that develop over a period of >7 days. The cause(s) of and mechanisms underlying AKD should be actively sought and managed to prevent progression to CKD [21].

In the critical care setting, a simple bedside risk stratification system for AKI has been proposed, which can be incorporated into the real-time EHR for automated alerts. Termed the renal angina index (RAI), it is a composite of known clinical risk factors, including vasopressor use, invasive mechanical ventilation, percent fluid overload, estimated creatinine clearance, and the presence of bone marrow or solid organ transplantation. The RAI has been shown in prospective studies to improve prediction of severe AKI (KDIGO Stage 2 or 3) in critically ill children when compared to an increase in serum creatinine alone [22, 23]. Recent studies have demonstrated the ability of the RAI to predict AKI in children presenting to the emergency department [24] and in children with septic shock [25]. The RAI may be especially useful in identifying children who might benefit best from additional investigative monitoring, including novel biomarkers, and potential early intervention [26–28].

Epidemiology

The precise incidence and prevalence of pediatric AKI are not fully known. However, there is growing evidence to indicate that pediatric AKI is not only common but also rising in incidence globally. While the mounting AKI incidence rate may be attributed in part to increased awareness and better consensus definitions, it is also very likely a consequence of advancements in the non-renal care of otherwise critically ill children. The incidence varies based on the definition used, clinical setting, geographic location, available resources, and the underlying clinical risk factors, as outlined below.

Few data are available to define the incidence of pediatric AKI in the general community setting. In a diverse outpatient cohort of more than 1.5 million children who received care within

the Kaiser Permanente Northern California system from 2008 to 2016, the overall incidence of community-based KDIGO-defined pediatric AKI was about 1 per 1000 per year [29]. In contrast, the recent use of EHR systems has provided higher estimates of KDIGO-based AKI in non-critically ill hospitalized children in the 10–30% range. In a retrospective single center study from the United States that utilized EHR to identify AKI in a development and a validation cohort, the incidence of AKI in non-critically ill children was 722/2337 (31%) and 469/1474 (32%), respectively [30]. A prospective cohort study of hospitalized children from Wales used an electronic alert system to identify AKI in 77.3 cases per 1000 person-years [31]. A retrospective multicenter study from China identified AKI in the EHR of 20% among 101,836 hospitalized children [32]. A retrospective analysis from six hospitals in England revealed AKI in 11% of children using the National Health Services AKI e-alert algorithm [33]. In a single center retrospective EHR review in the United States, the incidence of AKI was 10.2% out of 8473 hospitalized children [34]. In the Kaiser Permanente cohort, the incidence of AKI in hospitalized children was 9% [29].

The incidence of AKI in critically ill children and neonates is even higher. In a recent prospective multinational study of 4683 children from 32 ICUs across North America, Europe, Asia, and Australia (AWARE), the overall incidence of AKI was 27% [35]. In a retrospective multicenter analysis of 2022 critically ill neonates from North America, Australia, and India (AWAKEN), the overall incidence of AKI was 30% [36]. This varied with gestational age, with the highest incidence (48%) noted in the 22–29-week gestational age group.

The incidence and etiology of pediatric AKI differs with geographic location and available resources. In resource-rich countries, the etiology has shifted in the past three decades to a hospital-acquired complication of other multisystem illnesses. The AKI is often multifactorial, frequently due to an underlying comorbid condition or its management, and often manifests in the context of multiorgan failure. Primary kidney diseases now account for only 7–10% of AKI in hospitalized children in high-resource settings. Thus, advances in critical care have increasingly rendered pediatric AKI a hospital-acquired disease, especially in developed countries. In contrast, in resource-limited countries, the most common causes of AKI remain in the community-acquired setting and include acute tubular necrosis (ATN) due to gastroenteritis or sepsis, and primary kidney diseases such as acute glomerulonephritis or hemolytic uremic syndrome [37]. In the Global Snapshot conducted by the ISN "*0by25*" AKI initiative, a prospective observational study of 341 children with AKI from 41 countries identified dehydration, infection, and primary kidney disease as the most common etiologies in low-income countries [38].

The primary risk factors for AKI include critical illness, comorbidities, and nephrotoxin use. The risk of AKI is greatest in critically ill children, in whom the incidence is about 30%. Major contributing factors include sepsis, multiorgan failure, hypotension, shock, congenital heart disease, nephrotoxins, and malignancy [35, 39–41]. Outcomes (including mortality and short- and long-term morbidity) in this population is directly related to AKI severity [42]. Critically ill neonates are also at high risk for AKI, in whom the incidence is also about 30% [36, 43]. Major contributing factors include very low birth weight, low gestational age, sepsis, congenital heart disease, and perinatal asphyxia [44, 45].

Nephrotoxin use is an important risk factor for pediatric AKI worldwide. Non-steroidal anti-inflammatory drugs (NSAIDs) are the most common culprits, accounting for up to 7% of AKI cases in hospitalized children. This risk is further increased in children with dehydration [46]. NSAIDs also cause sub-clinical AKI in children, as signaled by elevation of novel non-invasive biomarkers [47]. Other commonly implicated nephrotoxins include aminoglycosides, vancomycin, piperacillin-tazobactam, antiviral agents, angiotensin converting enzyme (ACE) inhibitors, and loop diuretics. Combinations amplify the risk [48, 49]. The overall risk of radiocontrast agents to cause AKI remains controversial but appears to be low in children with normal kidney function

and with the dominant current practice of using low osmolar contrast agents. In a retrospective study of hospitalized children with GFR ≥60 mL/min/1.73 m^2 who underwent contrast-enhanced CT scanning, the AKI rate was only 2.2% [50].

The global incidence of AKI is higher in pediatric populations with severe chronic illness or underlying comorbid conditions. For example, AKI incidence is high in children with congenital heart disease that requires surgical intervention [51–58], malignancies [59, 60], hematopoietic stem cell transplants [61, 62], liver transplants [63], nephrotic syndrome relapse [64], or sickle cell vaso-occlusive pain crisis [65].

The worldwide COVID-19 pandemic has now added an important new etiology and risk factor for AKI. While this is a rapidly evolving field, recent published data indicate that hospitalized children with COVID-19 infections are at high risk for developing AKI, with an incidence of 20–30% [66–69]. The incidence is even higher—up to 46%—in children with multisystem inflammatory syndrome [70]. The pathogenesis of AKI in COVID-19 infection appears to be multifactorial, including direct viral infection of podocytes and proximal tubular cells, cytokine storm, macrophage activation syndrome, endothelial dysfunction, hypercoagulability, rhabdomyolysis, and complement-mediated injury. AKI in children with COVID-19 infections is directly associated with increased morbidity and mortality, and residual renal impairment at discharge [68, 69].

Clinical Classification

Traditionally, AKI has been classified based on the anatomic location of the initial injury, as prerenal (functional response of structurally normal kidneys to hypoperfusion), intrinsic renal (involving structural damage to the renal parenchyma), or postrenal (congenital or acquired anatomic obstruction to the lower urinary tract). This classification remains convenient for understanding mechanisms as well as the approach to diagnosis and management. With the discovery and validation of novel AKI functional and structural biomarkers discussed below, the terminology is evolving to include functional, structural and even sub-clinical AKI to better depict the clinical situation and therapeutic response.

AKI may also be classified according to the clinical setting in which it occurs. Community-acquired AKI is more likely to be associated with a single insult, most commonly volume depletion, and is frequently reversible. The medical approach is often conservative, including discontinuing the insult, supportive fluid and electrolyte management, and awaiting spontaneous recovery of renal function. In contrast, hospital-acquired AKI, especially in the critical care setting, is frequently multifactorial, and often part of a more extensive multiorgan dysfunction. Management is more aggressive, with early initiation of renal replacement therapies to optimize the overall care of the patient. Recovery may be partial, and there are significant short- and long-term consequences.

AKI may also be classified according to the urine output, as being non-oliguric (urine output for greater than 6 h of >1 mL/kg/h in infants and >0.5 mL/kg/h in children), oliguric (urine output <1 mL/kg/h in infants, <0.5 mL/kg/h in children, or <400 mL/day in adults), anuric (no urine output), or polyuric (urine output >3 mL/kg/h). Measurement of urine output is especially useful in the critical care setting, since the degree of oliguria reflects the severity of the kidney injury, has important implications for fluid and electrolyte therapy, and provides prognostic information. However, even moderately severe forms of AKI due to nephrotoxins, and the majority of AKI seen in neonatal intensive care, are typically non-oliguric in nature. Furthermore, some children with AKI from ATN may present with polyuria due to a urinary concentrating defect, and polyuria is a characteristic of the recovery (diuretic) phase of AKI.

Functional AKI (Prerenal AKI, Volume-Responsive AKI)

Prerenal AKI is an appropriate functional response of structurally normal kidneys to hypoperfusion. It is the most common form of AKI encountered

globally, is often community-acquired, and accounts for 40–55% of all cases. The oliguria in this situation represents a renal mechanism for preserving intravascular volume and has colloquially been termed as "acute renal success". Prerenal AKI is often caused by true volume depletion (e.g., dehydration, bleeding, excessive intestinal or cutaneous losses), early sepsis, or other etiologies leading to effective kidney hypoperfusion (decreased cardiac output, decreased intravascular volume, decreased blood pressure, or "third-spacing"). When prerenal AKI is caused by dehydration, it is usually rapidly reversed by restoration of renal perfusion. However, it is critical to understand that prerenal AKI does not automatically imply fluid responsiveness. AKI due to sepsis or decreased blood pressure often requires the administration of vasoactive agents in addition to fluid resuscitation. In some situations leading to prerenal AKI, such as liver failure, heart failure and nephrotic syndrome, fluid restriction is a mainstay of treatment. The common causes of prerenal AKI are listed in Table 51.2.

Pathophysiology of Prerenal AKI

The kidneys normally receive 25% of the cardiac output, and any decrease in circulatory volume evokes a systemic response leading to the release of potent vasoactive agents (Fig. 51.1). These responses help maintain perfusion to other organs by normalizing circulatory volume and blood pressure, but at the potential expense of GFR. There is an intense baroreceptor-mediated activation of the sympathetic nervous system and renin-angio-

Table 51.2 Common causes of prerenal AKI

Mechanism	Etiology
Volume depletion	Dehydration, hemorrhage, diuretics, burns, shock, nephrotic syndrome, diabetes
Decreased cardiac output	Cardiac failure, arrythmias
Peripheral vasodilatation	Sepsis, anaphylaxis, anti-hypertensive agents
Renal vasoconstriction	Sepsis, non-steroidal anti-inflammatory drugs, angiotensin converting enzyme inhibitors, hepatorenal syndrome

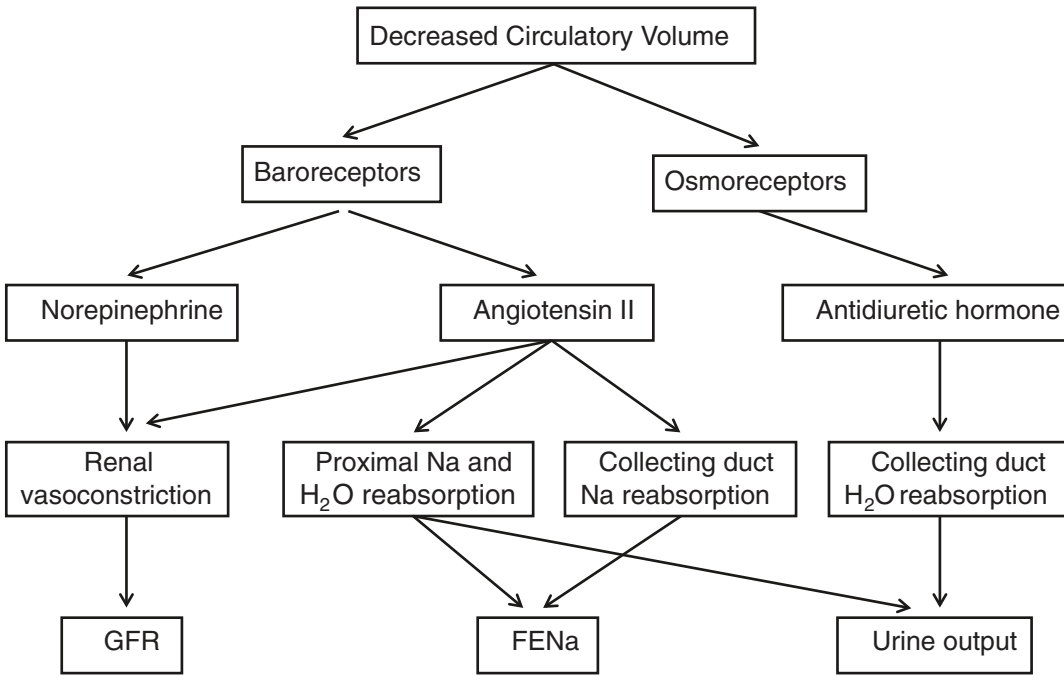

Fig. 51.1 Pathophysiology of prerenal AKI. These neurohormonal mechanisms are physiologically activated in the context of a reduction in effective circulatory volume, and result in an appropriate response by the kidney

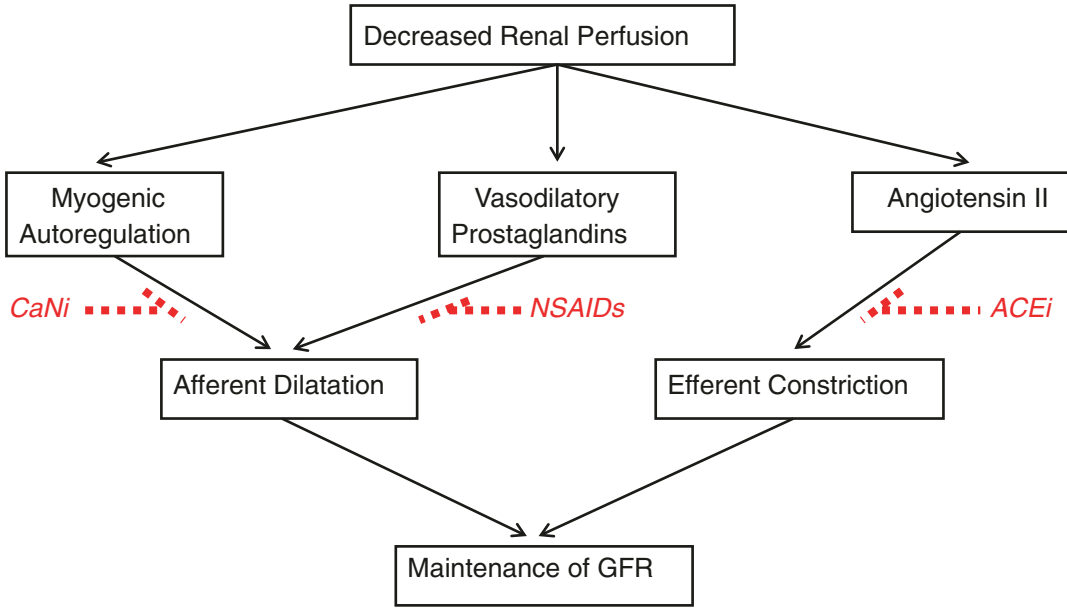

Fig. 51.2 Mechanisms that maintain GFR in prerenal AKI. Iatrogenic interference can precipitate a reduction in GFR. These include the use of calcineurin inhibitors (CaNi), non-steroidal anti-inflammatory drugs (NSAIDs), and angiotensin converting enzyme inhibitors (ACEi)

tensin axis, with resultant renal vasoconstriction mediated by angiotensin II and norepinephrine. Angiotensin II also promotes avid sodium and water reabsorption by the uninjured tubule cells, resulting in oliguria and a decreased fractional excretion of sodium that are the hallmarks of prerenal AKI. In addition, the release of anti-diuretic hormone in response to hypovolemia and a rise in extracellular osmolality results in enhanced water reabsorption by the intact collecting duct, which further contributes to the oliguria.

Concomitantly, at least three distinct intrarenal compensatory mechanisms are brought into play, which help maintain GFR in prerenal states (Fig. 51.2). Myogenic autoregulation refers to the rapid dilatation of the afferent arterioles in physiologic response to a reduction in lateral stretch following hypoperfusion. Calcineurin inhibitors such as tacrolimus and cyclosporine can interfere with the myogenic response and render the transplanted kidney more susceptible to prerenal azotemia. A more effective compensatory mechanism mediating afferent arteriolar dilatation involves the intrarenal production of vasodilatory prostaglandins [71]. Under normal physiologic conditions, the cyclooxygenase (constitutional COX-1 and inducible COX-2) enzyme systems catalyze the intrarenal production of prostaglandins that mediate afferent arteriolar dilatation. This system is dramatically upregulated by volume depletion. NSAIDs inhibit this response and can precipitate AKI, especially in the presence of decreased circulatory volume [72, 73]. For example, the use of postnatal indomethacin for patent ductus arteriosus closure in neonates results in AKI in as many as 40% of cases. When indomethacin is used to prevent preterm labor in pregnant women <32 weeks' gestation, the cumulative incidence of AKI within 15 days of life was 43.3% [74]. Another common pediatric scenario in which AKI might occur is the use of NSAIDs in febrile children with a dehydrating illness. In a recent meta-analysis, NSAIDs exposure was associated with an overall 1.6-fold rise in the odds of developing AKI in hospitalized pediatric patients [75]. A third mechanism for maintaining GFR involves the

differential effect of angiotensin II on the efferent arteriole. While angiotensin II tends to constrict both the afferent and efferent arteriole, this effect is more marked in the efferent arteriole, leading to increased hydrostatic pressure across the glomerulus and consequent preservation of GFR [76]. Interference with this compensation occurs following ACE inhibitor therapy. In clinical practice, the use of ACE inhibitors appears to increase the incidence of AKI in patients at risk, as has been documented for patients undergoing cardiac surgery, both pre-operatively [77, 78] and post-operatively [79].

It should be noted that compensatory mechanisms that maintain GFR in prerenal AKI are overwhelmed during states of prolonged reduction in renal perfusion pressure, and intrinsic AKI can ensue. Thus, prerenal and intrinsic AKI are along a continuum of renal hypoperfusion states and can co-exist in clinical situations. Furthermore, in some situations, prerenal AKI may be persistent and progressively detrimental, as exemplified by patients with heart failure and liver disease.

Structural AKI (Intrinsic AKI)

Intrinsic AKI is most frequently caused by prolonged ischemia, exogenous nephrotoxins (drugs), endogenous nephrotoxins (myoglobinuria and hemoglobinuria), or sepsis (Table 51.3). Primary kidney etiologies for AKI include vascular disease (e.g., typical and atypical hemolytic uremic syndrome, thrombotic thrombocytopenic purpura, and vasculitides), glomerular disease (e.g., acute glomerulonephritis), and tubulointerstitial disease (e.g., infections and allergic drug reactions). Intrinsic AKI can be associated pathologically with ATN. Consequently, it is common clinical practice to use the terms intrinsic AKI and ATN interchangeably. In the clinical setting, intrinsic AKI is frequently multifactorial, with concomitant ischemic, nephrotoxic, and septic components, and with overlapping pathogenetic mechanisms.

Table 51.3 Common causes of intrinsic AKI

Mechanism	Etiology
Acute tubular necrosis	Prolonged ischemia, nephrotoxins
Renal vascular diseases	Hemolytic uremic syndromes, vasculitis
Interstitial diseases	Interstitial nephritis, infections, infiltrations
Glomerulonephritis	Post-infectious, crescentic

Morphology of Intrinsic AKI

ATN is a misnomer since frank tubular cell necrosis is rarely found in human AKI. Even in patients with lethal sepsis and AKI, histopathological findings on post-mortem kidney biopsies taken immediately after death display only limited inflammation, coagulation and cell death. Biopsy findings in other forms of human AKI are also typically paradoxically mild when compared to the severe depression in GFR. There is effacement and loss of proximal tubule apical brush border, disruption of microvilli, patchy loss of tubule cells with exposure of denuded basement membranes, focal proximal tubular dilatation and distal tubular casts, and areas of cellular regeneration. Necrotic cell death is restricted to the outer medullary regions (S3 segment of the proximal tubule and medullary thick ascending limb (mTAL) of Henle's loop). On the other hand, patchy apoptotic cell death has been reported in both distal and proximal tubules, in both ischemic and nephrotoxic forms of human AKI. The relative contribution of damage to the distal tubule adjacent to the S3 segment of the proximal tubule remains controversial. An intense inflammatory response, with peritubular accumulation of leukocytes, is typical in experimental models of AKI but is less prominent in human AKI. In addition, peritubular capillaries in the outer medulla display a striking vascular congestion. The molecular, biochemical, and cellular mechanisms underlying these morphologic changes have uncovered several novel pathways in animal models. This is an extensively studied and rapidly advancing field, and only selected mechanisms that are currently providing promising therapeutic approaches [80] are outlined below.

Alterations in Hemodynamics and Microcirculation

Intrinsic AKI is characterized by persistent hemodynamic abnormalities (Fig. 51.3). Total renal blood flow is reduced to about 50% of normal due to persistent renal vasoconstriction in established AKI, a finding that resulted in the classical term of "vasomotor nephropathy". More importantly, there are regional alterations in renal blood flow, with marked congestion of the outer medullary region. Oxygen tensions are normally lowest in this region that ironically contains tubular segments with the highest energy requirements, namely the S3 segment of the proximal tubule and mTAL. The post-ischemic congestion worsens the relative hypoxia, leading to prolonged injury and necrotic cell death in these segments.

Mechanisms underlying these hemodynamic alterations relate primarily to renal microcirculatory and endothelial damage [81]. The renal microcirculation normally plays a crucial role in maintaining the kidney's functional and structural integrity. However, alterations in microcirculation and oxygenation due to endothelial damage can result in AKI regardless of systemic hemodynamic changes. At the sub-cellular level, AKI-induced mitochondrial damage is a prominent finding in endothelial injury [82]. This leads to a local imbalance of vasoactive substances, including enhanced release of the vasoconstrictor endothelin and reduced release of vasodilatory endothelium-derived nitric oxide (NO). There is substantial evidence from human AKI studies to indicate that endothelial dysfunction results in increased incidence, worsened severity, and prolonged duration of AKI. Plasma levels of endothelin, a potent vasoconstrictor, are increased (and NO levels decreased) in humans with septic AKI. However, while endothelin receptor antagonists ameliorate ischemic AKI in animals, human data are lacking. A human trial of an endothelin receptor antagonist for prevention of contrast-induced AKI resulted in a paradoxical exacerbation of nephrotoxicity. Several newer endothelin receptor blockers have recently been developed for potential use in hypertension and CKD and may hold promise in human AKI.

Similarly, carbon monoxide (CO) and carbon monoxide-releasing molecules have been shown to be protective in animal models of ischemic AKI, likely through vasodilatation and preservation of medullary blood flow as well as cytoprotective and immunomodulatory properties. However, much work is needed in translating these findings to humans, including determination of safe CO dosage, duration of treatment, and frequency of treatment for a given indication. NO, produced in renal endothelial and tubule cells from L-arginine by constitutive and induc-

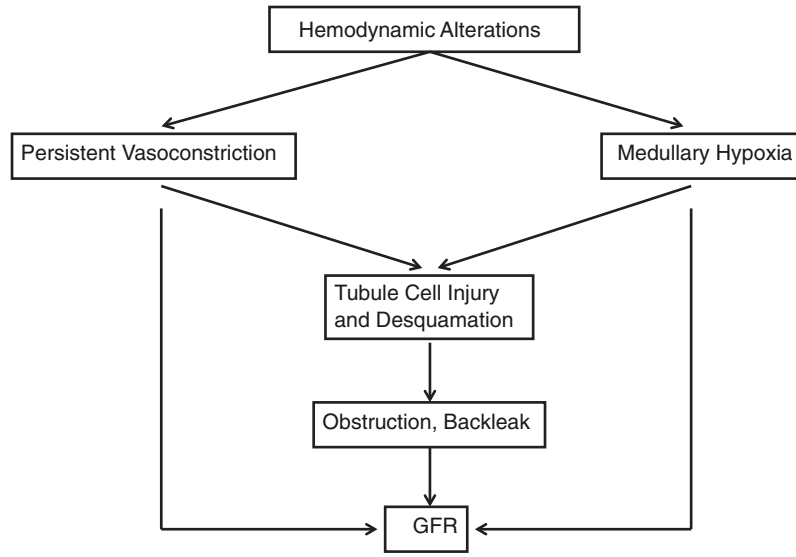

Fig. 51.3 Pathophysiology of intrinsic AKI—hemodynamic alterations and their consequences

ible NO synthetases (NOS), is also a known renal vasodilator. In a recent meta-analysis of five adult human studies, inhaled NO gas was associated with a reduced risk of AKI after cardiopulmonary bypass surgery with no adverse effects [83]. However, further studies are needed to determine the optimal dosage, timing and duration of NO administration in human AKI.

In the final analysis, it is now apparent that while hemodynamic abnormalities play an important role in the initial phases of AKI, they alone cannot fully account for the profound loss of renal function in established human AKI. Not surprisingly, several human trials of vasodilators have failed to convincingly demonstrate improvement in GFR in established AKI despite augmentation of overall renal blood flow. Persistent renal vasoconstriction may, in fact, represent an adaptive protective mechanism to minimize further cellular injury during the reperfusion period.

Alterations in Tubule Dynamics

Three well known derangements in tubular dynamics in intrinsic AKI include obstruction, back-leak, and activation of tubuloglomerular feedback. The consistent finding of proximal tubular dilatation and distal tubular casts in human AKI are indicative of obstruction to tubular fluid flow. The intraluminal casts stain strongly for Tamm-Horsfall protein, which is normally secreted by the thick ascending limb as a monomer. Conversion into a gel-like polymer is enhanced by the increased luminal sodium concentration typically encountered in the distal tubule in AKI because of impaired proximal tubule sodium reabsorption. This provides an ideal environment for cast formation along with desquamated tubule cells and brush border membranes. Cell death, most prominently noted in the highly susceptible outer medullary regions (S3 segment) of the proximal tubule, provides the sloughed cells and brush borders, with subsequent cellular cast formation and intratubular obstruction. However, it is unlikely that obstruction alone can account for the profound dysfunction in clinical AKI, since human studies using forced diuresis with furosemide or mannitol did not improve the renal recovery rate of patients with established AKI in most reported studies [84, 85]. Similarly, although movement of the glomerular filtrate back into the circulation has been shown to occur because of intratubular obstruction, this accounts for only a very minor component of the decrease in GFR in human AKI.

A role for activation of tubuloglomerular feedback has been proposed. By physiologic considerations, the increased delivery of sodium chloride to the macula densa due to abnormalities in the injured proximal tubule would be expected to induce afferent arteriolar constriction, mediated by adenosine via A1 adenosine receptor (A1AR) activation, and thereby decrease GFR. Thus, research efforts have focused on specific adenosine receptor antagonists as potential therapeutic agents, since tubuloglomerular feedback activation following ischemic injury may represent a beneficial phenomenon that limits wasteful delivery of ions and solutes to the damaged tubules, thereby reducing the demand for adenosine triphosphate (ATP)-dependent resorptive processes. The protective effects of adenosine inhibition on renal blood flow preservation may be mediated via more selective adenosine receptor activation, and the use of broad-spectrum adenosine receptor antagonists such as theophylline and caffeine is a subject of renewed clinical investigation [86–88].

Alterations in Tubule Cell Metabolism

The various cell types along the nephron display segment-specific susceptibilities to different types of injury, based on their metabolic requirements. The highly active proximal tubule cells need a steady oxygen supply to remain viable, whereas those in the thick ascending limb are relatively resistant to hypoxia. Epithelial cells in the straight segment (S3 segment) of the proximal tubule are most vulnerable to ischemic injury since they are highly dependent on oxidative phosphorylation for energy. The S1 and S2 segments are affected most by toxic nephropathy

because of their high rates of endocytosis. The mTAL is particularly vulnerable since it exists in a hypoxic precipice, with low oxygen tension but high oxygen consumption.

A profound reduction in intracellular ATP content is a hallmark of AKI that occurs very early after injury. Approximately 90% of renal intracellular ATP becomes depleted within 10 min of ischemic injury. With reperfusion, ATP levels recover in a bimodal fashion. There is an initial rapid but incomplete (up to 70% of normal) recovery phase, generated by re-phosphorylation of residual adenine nucleotides (ADP and AMP). This is followed by a second slower phase of ATP recovery, which requires resynthesis from purine nucleotide degradation products and salvage pathways. ATP depletion triggers several metabolic consequences in tubule cells. Events that provide insights into potential clinical interventions are detailed below, and their inter-relationship is illustrated in Fig. 51.4.

Alterations in tubule cell adenine nucleotide metabolism have been well documented. Oxygen deprivation leads to a rapid degradation of ATP to AMP and to hypoxanthine. These metabolites are freely diffusible, and their depletion precludes rapid re-synthesis of ATP during reperfusion. Provision of exogenous adenine nucleotides or thyroxine (which stimulates mitochondrial ATP regeneration) can ameliorate the cellular injury in animal models of ischemic AKI. However, a recent systematic review found a paucity of large, high-quality studies to inform analysis of thyroid hormone interventions for the treatment of humans with AKI. Current evidence suggests that thyroid hormone therapy may be associated with worse outcomes for patients with established AKI; therefore, its use for these patients should be avoided.

ATP depletion leads to impaired calcium sequestration within the endoplasmic reticulum, as well as diminished extrusion of cytosolic calcium into the extracellular space. The resultant increase in free intracellular calcium has been documented following AKI, but its role has remained controversial. Increased intracellular calcium could potentially lead to activation of proteases and phospholipases and cytoskeletal

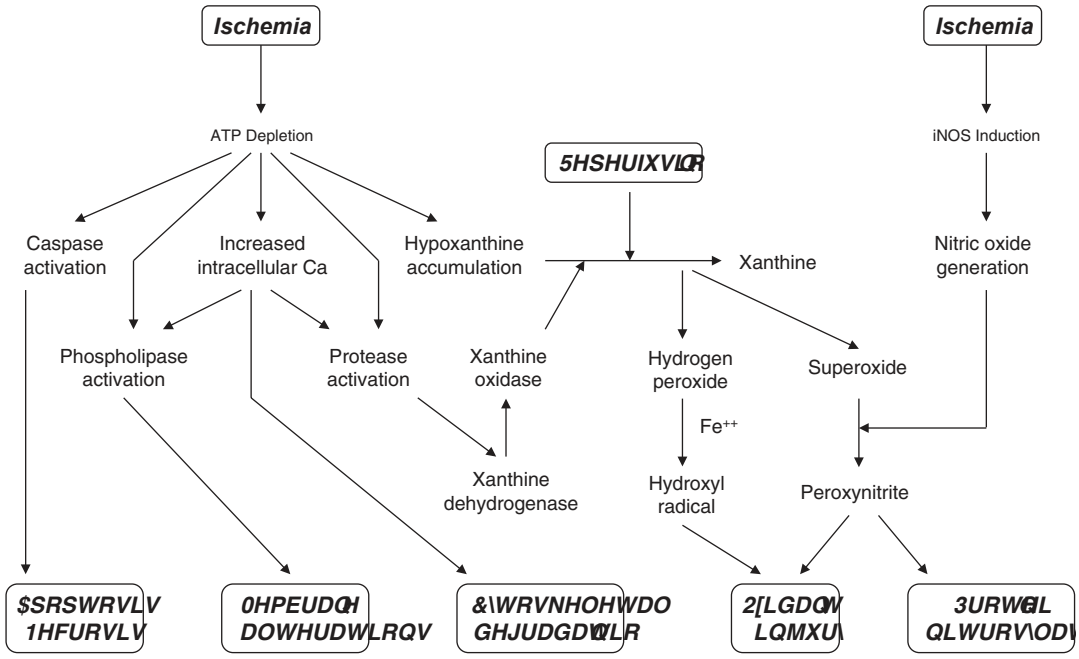

Fig. 51.4 Metabolic consequences of acute ischemia and reperfusion injury to kidney tubule cells. Inhibition of these pathways may provide novel therapeutic approaches to AKI

degradation. A previous meta-analysis suggested that calcium channel blockers may provide some protection from renal injury in the kidney transplant setting, but evidence for their efficacy in other forms of AKI is currently lacking. Any benefit from intracellular calcium blockade may be counterbalanced by the hypotension induced by these agents, with resultant worsening of AKI.

Abundant experimental, and to a lesser extent clinical data, now support a critical role for oxidative stress-related mechanisms in the early injury phase of AKI. Oxidative stress refers to metabolic disturbances, such as increased production of reactive oxygen species (ROS) that leads to the depletion of endogenous antioxidants with resultant cellular damage, dysfunction of proteins, and damage to DNA, lipids, and enzymes. Specifically, there is now substantial evidence for the role of ROS in the pathogenesis of intrinsic AKI. During reperfusion, the conversion of accumulated hypoxanthine to xanthine generates hydrogen peroxide and superoxide (Fig. 51.4). In the presence of iron, hydrogen peroxide forms the highly reactive hydroxyl radical. Concomitantly, ischemia induces NO synthase in tubule cells, and the NO generated interacts with superoxide to form peroxynitrate, which results in cell damage via oxidant injury as well as protein nitrosylation. ROS can cause renal tubule cell injury by oxidation of proteins, peroxidation of lipids, damage to DNA, and induction of apoptosis and autophagy. Studies have documented a dramatic increase in oxidative stress and autophagy, and reduction in antioxidant pathways, in experimental and human AKI. Several scavengers of ROS (such as superoxide dismutase, catalase, and *N*-acetylcysteine) protect against ischemic AKI in animal models, but human studies have been inconclusive or negative, except for prevention of contrast-induced AKI.

Free iron derived from red cells or other injured cells is one of the most potent factors in the generation of ROS, and the iron scavenger deferoxamine alleviates ischemia-reperfusion injury in animal models and in human clinical trials. However, the systemic toxicity of deferoxamine (hypotension and ocular toxicity) precludes its routine clinical use in human AKI. Several other molecules are under study for iron chelation [89]. Other potential approaches to minimize the nephrotoxic effects of iron and iron-containing proteins under investigation include administration of haptoglobin to facilitate sequestration of free hemoglobin, administration of hepcidin to prevent iron export from intracellular compartments into the circulation, and pharmacologic upregulation of heme oxygenase-1 (HO1) to accelerate the catabolism of toxic free heme.

Alterations in Tubule, Endothelial, and Glomerular Cell Structure

The cell biologic response of intrinsic kidney cells to ischemic or nephrotoxic AKI is multifaceted, and includes loss of cell polarity and brush borders, cell death, de-differentiation of viable cells, proliferation, and restitution of a normal epithelium, as illustrated in Fig. 51.5. While most prominently described in tubule epithelial cells, structural alterations in the endothelial and glomerular cell cytoskeleton are also of consequence. The major mechanisms underlying this morphologic sequence of events are summarized below.

Cellular ATP depletion results in an early, rapid disruption of the apical actin cytoskeleton and redistribution of actin from the apical domain and microvilli into the cytoplasm. This results in loss of brush border membranes, which contribute to cast formation and obstruction. Intracellular actin released from damaged tubule cells appears in the urine of patients with AKI. Similar changes in endothelial cells may potentiate vascular injury.

Disruption of the apical cytoskeleton also results in loss of tight (zonula occludens) junctions and zonula adherens junctions. Reduced expression, redistribution, and abnormal aggregation of several key proteins that constitute the tight and adherens junctions have been documented after ischemic injury in cell culture, animal models, and human studies. Loss of cadherin staining in the vascular endothelium also suggests that cadherin junctions are altered during injury.

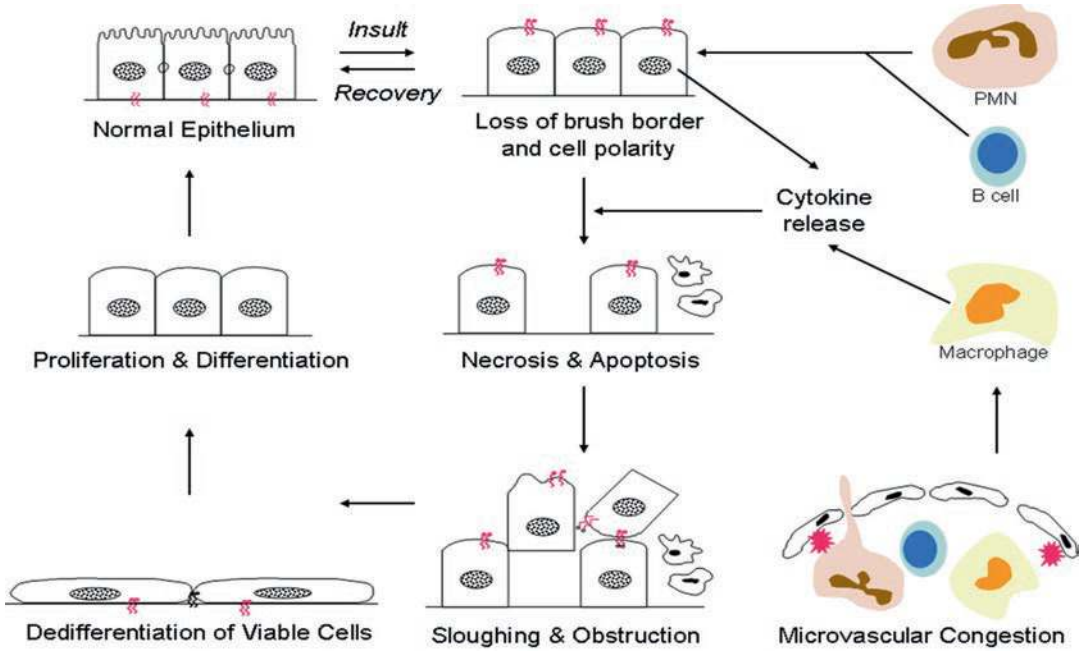

Fig. 51.5 Structural consequences of acute ischemia and reperfusion injury to kidney tubule cells. Novel therapeutic approaches in AKI have targeted prevention of cell death, inhibition of inflammation, and acceleration of the endogenous recovery process

The consequent loss of tight junction barrier function can potentially magnify the transtubular back-leak of glomerular filtrate induced by obstruction. Ischemic injury can also result in podocyte-specific molecular and cellular changes. In healthy podocytes, Neph1 (a component of the podocyte slit diaphragm) complexes with ZO-1 (an actin-related tight junction protein), to link tight junctions to the cortical actin skeleton, thereby providing a structural framework for the slit diaphragm. Slit diaphragms are an important component of the glomerular filtration barrier, and damage to slit diaphragms can lead to impaired filtration. Ischemia induces the dissociation of Neph1 from ZO-1, which results in podocyte effacement and loss of the Neph1-ZO-1 interaction. Although the interaction can be restored after reperfusion, the recovery of podocyte structure is often incomplete.

Other changes occur in the glomerulus during AKI. Podocyte foot processes coarsen during injury, and there is a decrease in heparin sulfate proteoglycan and sialic acid on the endothelial surface layer, which can lead to albuminuria and decrease GFR. Tumour necrosis factor-alpha (TNF-α) has been implicated as a mechanism mediating AKI via glomerular abnormalities. Mice deficient in TNFR1 have normal glomerular morphology and density with minimal cellular detachment, suggesting that TNF-α mediates damage to the glomerular endothelium and is a key determinant of AKI in sepsis.

Ischemic and nephrotoxic insults also result in the early disruption of at least two normally basolaterally polarized proteins, namely Na,K-ATPase and integrins. The Na,K-ATPase is normally tethered to the spectrin-based cytoskeleton at the basolateral domain via the adapter protein ankyrin, where it functions to pump intracellular sodium into the circulation. This provides the driving force for the normal reabsorption of salt and water by the proximal tubule via the apical membrane. A physiologic consequence of the loss of basolateral Na,K-ATPase is impaired proximal tubule sodium reabsorption and a consequent increase in fractional excretion of

sodium, which are diagnostic signatures of intrinsic AKI.

The β-1 integrins are normally polarized to the basal domain, where they mediate cell-substratum adhesions. Tubule cell injury leads to a redistribution of integrins to the apical membrane, with consequential detachment of viable cells from the basement membrane. This is followed by abnormal adhesion between these exfoliated cells within the tubular lumen, mediated by an interaction between apical integrin and the Arg-Gly-Asp (i.e., RGD) motif of integrin receptors. Administration of synthetic RGD compounds attenuates tubular obstruction and renal impairment in animal models of ischemic AKI, and the recent availability of orally active integrin antagonists as well as small-molecule peptidomimetics that inhibit RGD-binding integrins holds promise for clinical application in human AKI.

Alterations in Tubule Cell Death

Injured tubule epithelial cells may suffer one of several distinct cellular fates after AKI. Most cells remain viable, suggesting that they either escape injury or are only sub-lethally injured and undergo recovery. A subset of tubule cells displays patchy cell death resulting from at least five pathophysiologic mechanisms: necrosis, apoptosis, necroptosis, ferroptosis, and autophagy. Necrosis is an explosive, unregulated, chaotic process characterized by loss of membrane integrity, cytoplasmic swelling, and cellular fragmentation. Apoptosis is a quiet, regulated, orderly demise typified by cytoplasmic and nuclear shrinkage, DNA fragmentation, and breakdown of the cell into membrane-bound apoptotic bodies that are rapidly cleared by phagocytosis. Clearing dead cells and associated cellular debris is an integral part of tissue homeostasis. While diverse types of phagocytes remove various forms of dying cells during AKI, it remains unknown whether boosting removal of a specific form of dying cell would provide a benefit and which cell type should be targeted for phagocytosis-mediated therapy. Necrosis and apoptosis can coexist and are considered to present two ends of a spectrum. In AKI, the mode of cell death depends primarily on the severity of the insult and the resistance of the cell type. Necrosis occurs after more severe injury and in the more susceptible nephron segments and often is characterized by the activation of phospholipase A2, calpain, and eicosanoids. In contrast, apoptosis predominates after less severe injury, especially in the ischemia-resistant distal nephron segments. Apoptosis can be followed by "secondary necrosis," especially if the apoptotic cells are not removed rapidly.

Apoptosis is a major mechanism of early tubule cell death in contemporary clinical AKI, and considerable attention has been directed toward dissecting the molecular mechanisms involved. Several pathways, including the intrinsic (Bcl-2 family, cytochrome c, caspase 9) extrinsic (Fas, FADD, caspase 8), and regulatory (p53, NF-κB) factors, appear to be activated by ischemic and nephrotoxic AKI, as illustrated in Fig. 51.6. The extrinsic pathway functions by binding death ligands to cell-surface receptor, resulting in procaspase-8 activation, often through mediation with adaptor proteins such as Fas-associated protein with death domain (FADD) or TNF receptor 1-associated death domain. The role of the Fas-FADD pathway in animal models was suggested by demonstration of upregulation of these proteins in apoptotic tubule cells after ischemia and the functional protection afforded by siRNA duplexes targeting the Fas gene. However, convincing human data are lacking. On the other hand, growing evidence implicates an imbalance between the pro-apoptotic (Bax, Bid) and anti-apoptotic (Bcl-2, Bcl-xL) members of the Bcl-2 family in animals and humans so affected. Disequilibrium between Bac/Bcl-2 and Bad/Bid can lead to the formation of mitochondrial pores, which alters cell viability. The proapoptotic transcription factor p53 is activated by HIF-1α and induced at the mRNA and protein levels, and straddles both the intrinsic and extrinsic pathways. Inhibition of p53 by pifithrin-α suppresses ischemia-induced apoptosis by inhibiting transcriptional activation of Bax and mitochondrial translocation of p53. However, pifithrin-α is an unlikely candidate for therapeu-

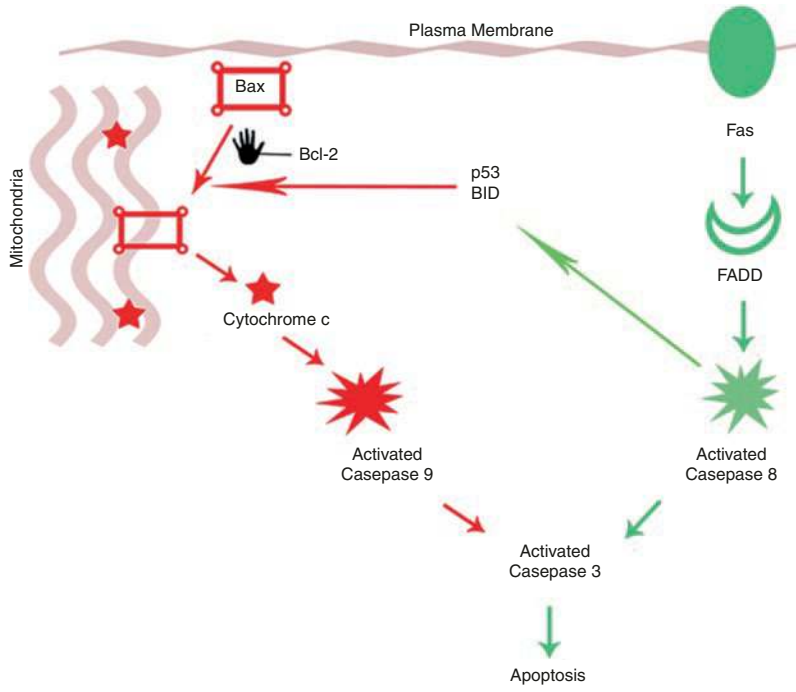

Fig. 51.6 Major tubule cell apoptotic pathways in human AKI. The extrinsic pathway (green) requires activation of Fas and TNF-R1, with subsequent signal transduction and activation of caspase 8. The intrinsic pathway (red) requires translocation of Bax to the mitochondria, thereby releasing cytochrome c and activation of caspase 9. Crosstalk between these pathways is provided largely by the regulatory molecule p53. Inhibition of the central regulatory molecule p53 and the terminal executor caspases hold promise in human AKI

tic consideration in humans because generalized inhibition of p53-dependent apoptosis is likely to promote survival of damaged or mutation-bearing cells in other organ systems. Studies have examined the efficacy of p53 si-RNA as a potential therapeutic agent, as administration of the treatment decreased serum creatinine and tubular necrosis in animal models. Clinical trials examining the effects of p53 si-RNA in human AKI are underway. A recent promising prospective, multicenter, double-blind, randomized, controlled phase 2 trial evaluated the efficacy and safety of a single 10 mg/kg dose of the p53 si-RNA teprasiran in reducing the incidence, severity, and duration of AKI after cardiac surgery in high-risk adult patients [90]. AKI incidence was 37% for teprasiran versus 50% for placebo-treated patients. AKI severity and duration were also improved with teprasiran: 2.5% of teprasiran versus 6.7% of placebo-treated patients had grade 3 AKI; 7% teprasiran versus 13% placebo-treated patients had AKI lasting for 5 days. No safety issues were identified with teprasiran treatment. Results of larger phase 3 studies, as well as investigations in other forms of AKI, are awaited.

Inhibition of other apoptotic pathways also hold promise for clinical application in human AKI. Caspase activation is by and large the final common "execution" step in apoptosis, and cell-permeant caspase inhibitors have provided particularly attractive targets for study. Currently available caspase inhibitors have largely been investigated only in animals, provide only partial protection, and are most effective when administered before the insult. A vast plethora of other agents, including mesenchymal stem cells, curcumin, erythropoietin (EPO), *N*-acetyl cysteine, thioredoxin, TNF-α antagonists, A1 adenosine receptor agonists, peroxisome proliferator-activated receptor ligands, NGAL, and poly(ADP-ribose) polymerase inhibitors (to name a few) have all provided encouraging struc-

tural and functional protection from experimental AKI, with inhibition of apoptosis and inflammation. Some of these agents are already widely available and have been used safely in other human conditions, and additional results with their use in human AKI should be forthcoming. Challenges for the future clinical use of apoptosis inhibition in AKI include determining the best timing of therapy, optimizing the specificity of inhibitor, minimizing the extrarenal side effects, and tubule-specific targeting of the apoptosis-modulatory maneuvers. The issue of timing is especially important, since inhibiting early cell death may be beneficial but interfering with late onset apoptosis is envisioned to interfere with the removal of dead and unwanted cells.

Necroptosis refers to a programmed cell death that is characterized by caspase-independent regulated necrosis resulting in plasma membrane rupture and subsequent release of damage-associated molecular patterns (DAMPs). Like apoptosis, necroptosis may be induced by multiple extracellular and intracellular stimuli, including tumor necrosis factor (TNF) family members, Fas ligands, interferon, and oxidative stress. However, the downstream mechanisms in necroptosis are distinct, independent of caspase activation, and the resultant cell death is characterized by several necrotic features, including a lack of chromatin condensation or DNA fragmentation. Several novel therapeutic agents that inhibit necroptosis pathways have already shown efficacy in experimental studies, and human translation appears to be promising.

Ferroptosis, a distinct form of iron-dependent cell death now well described in ischemia-reperfusion injuries in organs including the kidney, is characterized primarily by intracellular iron accumulation and lipid peroxidation. Morphologically, ferroptotic cells display mitochondrial changes, including reduced volume, increased membrane density, and decreased mitochondrial cristae and rupture of the outer mitochondrial membrane. The cell nucleus becomes devoid of chromatin condensation. Eventually, the cell membranes rupture and cell death occurs. Targeted regulation of ferroptosis and its signalling pathways are achieving promising results.

The study of autophagy as another mechanism of altered cell viability in AKI has been a topic of considerable recent attention. Autophagy is a physiologic process by which intracellular damaged macromolecules and organelles are degraded and recycled for the synthesis of new cellular components. The process of autophagy involves the formation of a double-membrane structure known as an autophagosome, which first sequesters the cellular constituents and subsequently delivers them to the lysosome for degradation. This results in the recycling of degraded products for the biosynthesis of new cellular components and for meeting the energy needs of the cell. The autophagy process uses lysosomal hydrolases to degrade large intracellular heterogeneous misfolded proteins, protein aggregates, damaged macromolecules, and even entire damaged organelles. Recent progress in identifying the interplay of autophagy, apoptosis, and regulated necrosis has revealed common pathways and molecules in this crosstalk during the pathogenesis of AKI. Autophagy and its associated pathways may pose potentially unique targets for therapeutic interventions in AKI.

The mechanisms whereby most tubule cells escape cell death and either emerge unscathed or recover completely after AKI remain under active investigation. HSPs have surfaced as potential mediators of this cytoprotection. Induction of HSPs is part of a highly conserved innate cellular response that is activated swiftly and robustly after ischemic AKI. The heat shock response is particularly robust in immature kidneys and may form the basis for the common observation that subsequent AKI is less likely to develop in premature infants than adults. Maneuvers that enhance the innate HSP response have potential benefit in human AKI, but clinical evidence is lacking.

Alterations in Tubule Cell Proliferation and Differentiation

Surviving renal tubule cells possess a remarkable ability to regenerate and proliferate after AKI. Morphologically, repair is heralded by the

appearance of de-differentiated epithelial cells that express vimentin, a marker for multipotent mesenchymal cells. These cells most likely represent surviving tubule cells that have de-differentiated. In the next phase, the cells upregulate genes encoding a variety of growth factors, such as insulin-like growth factor-1 (IGF-1), hepatocyte growth factor (HGF), and fibroblast growth factor (FGF), and undergo marked proliferation. In the final phase, cells express differentiation factors, such as neural cell adhesion molecule and osteopontin, and undergo re-differentiation until the normal fully polarized epithelium is restored. Thus, during recovery, renal tubule cells recapitulate phases and processes very similar to those during normal kidney development. Emerging data suggest that no pre-existing proximal tubule stem cell population exists, but rather all differentiated proximal tubule cells possess the capacity to proliferate during repair by de-differentiation and self-duplication.

In response to acute injury, animal studies have shown that the surviving, normally quiescent proximal tubule epithelial cells rapidly de-differentiate, proliferate, and enter the S-phase of the cell cycle. Understanding the molecular mechanisms of cell cycle re-entry may provide clues towards accelerating recovery from AKI. With mild injury, these endogenous repair mechanisms can result in the return to a normal structural and functional state. However, when the repair is more severe or dysregulated, the repair process can lead to fibrosis, which can facilitate progression to CKD. Recent experimental data have redefined the role of the surviving epithelial cells in fibrosis. After severe injury, the proximal tubule cell proliferative response is altered due to cell cycle arrest at the G2/M phase, resulting in generation of profibrotic factors including cytokines, growth factors and matrix proteins. Inhibition of the identified profibrotic factors may hold promise in preventing the AKI to CKD transition. For example, HGF is renoprotective and renotrophic in animal models of AKI, because of its proliferative, antiapoptotic, and anti-inflammatory actions. The use of HGF in humans, however, has been hampered at least in part by the widespread expression of its receptor, raising the possibility of extrarenal side effects. Recent studies have explored the effects of BB3/ANG3777, a small molecule with strong HGF-like activity, which, when first administered at 24 h after renal ischemia in rats, improved survival, augmented urine output, and improved kidney function. BB3/ANG3777 is currently being tested in renal transplant patients. In the case of IGF-1, enthusiasm for its renoprotective effects has been dampened by its exacerbation of inflammation and neutrophil infiltration in the post-ischemic kidney in animals. Human trials with recombinant IGF-1 have not demonstrated a beneficial effect. New growth factors have emerged as potential therapeutic targets that may accelerate renal recovery, including epidermal growth factor and α-melanocyte stimulating hormone (α-MSH), which likely acts via direct hemodynamic effects. α-MSH is an efficacious anti-inflammatory and antiapoptotic cytokine and protects from ischemic, nephrotoxic, and septic AKI in animal models. However, α-MSH therapy was not effective in human AKI trials.

Identification of the source of multipotent mesenchymal cells involved in the regeneration and repair process has been a matter of intense contemporary research. It is now established that renal tubule cells are capable of regeneration, instead of regeneration being driven by an extrarenal progenitor population. Current literature suggests that proximal tubule cells undergo transient de-differentiation after injury and then proliferate to repopulate the tubule. However, mesenchymal stem cells (MSCs) may have important autocrine, paracrine, and growth factor-like effects on kidney regeneration. MSCs are drawn to renal tubules that produce stromal cell-derived factor 1 and remain in the injured kidney for a short period of time. They secrete various growth factors that mediate kidney repair. Administered MSCs clearly enhance recovery from ischemic AKI in animals and recently were tested in clinical settings. Modified MSCs, which were modified to be immune privileged and genetically stable, were tested as a therapy for human AKI. In a cohort of adult patients with AKI after cardiac surgery, administration of allogeneic MSCs did not decrease the time to recovery of kidney function. Endothelial progeni-

tor cells (EPCs) are bone marrow-derived precursors that promote tubular regeneration in the kidney after ischemic injury. Microvesicles from EPCs can protect the kidney from ischemic injury, likely by delivering microRNA that reprograms resident renal cells to a regenerative program. Rats treated with EPC microvesicles had a reduced number of tubular lesions, improved kidney function, reduced apoptosis, and increased tubular proliferation. Six months after ischemic injury, rats showed less fibrosis and glomerulosclerosis, suggesting that EPC vesicles have long-term beneficial renoprotective effects in experimental animals.

Alterations in the Microvasculature

The role of endothelial alterations and endothelial dysfunction in the initiation and extension of AKI has received increasing attention. Morphologically, disruption of the actin cytoskeleton and junctional complexes, like those previously described in tubule epithelial cells, have been documented in endothelial cells in experimental AKI. Consequent endothelial cell swelling, blebbing, and death, with detachment of viable cells, have been observed, and circulating endothelial cells have been demonstrated in humans with septic shock. Sites of endothelial denudation are prone to prolonged vasoconstriction, and systemic or intrarenal administration of fully differentiated endothelial cells into post-ischemic rat kidneys results in functional protection. Furthermore, ischemic injury leads to a marked upregulation of angiostatin, a well-known antiangiogenic factor that induces apoptosis of endothelial cells. Collectively, these findings provide a rationale for the use of pro-angiogenic agents that can increase the pool of or mobilize endothelial progenitor cells. These agents include bone morphogenic protein (BMP), VEGF, statins, and EPO; their putative roles in AKI are currently under investigation. In particular, statins have been shown in pre-clinical studies to ameliorate AKI development via several microvascular mechanisms, including inhibition of vascular superoxide generation and restoration of endothelial derived NO synthase activity. The role of statins in clinical AKI remains controversial. In a recent large retrospective review of adult patients undergoing open cardiac surgery, preoperative statin exposure was a protective factor against all stages of postoperative cardiac surgery associated AKI as well as stage 3 AKI (OR, 0.671, 95% CI, 0.567–0.795), after adjusting for confounding factors [91]. These findings support the need for a larger prospective randomized controlled trial of AKI in adults and children, especially since several commonly available statins are approved for use in children over 8–10 years of age.

EPO is a well-known potent stimulator of erythroid progenitor cells and is commonly used to treat anemia in both children and adults with CKD. In animal models of ischemic AKI, EPO has protective effects via amelioration of microvascular injury as well as additional anti-apoptotic and anti-inflammatory mechanisms. However, several clinical trials in adults with AKI have yielded mixed results. In a meta-analysis of controlled trials in adult patients either at high risk for AKI or following kidney transplant, no reduction of incidence of AKI and no reduction in delayed graft function or improvement in 1-year graft survival after renal transplantation could be documented. Furthermore, in a recent study of extremely low gestational age neonates, EPO did not protect from any AKI or from severe AKI [92].

AKI also leads to increased endothelial expression of a variety of adhesion molecules that promote endothelium-leukocyte interactions. These include intracellular adhesion molecule-1 (ICAM-1), P-selectin, E-selectin, B7-1, vascular adhesion molecule-1 (VCAM-1), and thrombomodulin (TM). Endothelial cells are also involved with coagulation processes via interactions with protein C and TM. Derangements in the coagulation cascade, such as alterations in tissue-type plasminogen activator and plasminogen activator inhibitor-1 in the kidney, may account for the fibrin deposits characteristically found in the renal microvasculature after ischemic injury. The pathways and mechanisms that are involved in formation of interstitial fibrosis after AKI have

been examined. Impaired endothelial proliferation and mesenchymal transition processes contribute to vascular dysfunction after AKI. In contrast with tubule epithelial cells, cells of the renal vasculature lack an efficient regenerative capacity, which results in a persistent 30–50% reduction in vascular density after ischemic injury. Vascular dropout likely promotes hypoxia and impairs hemodynamic and sodium regulatory responses in the kidney after AKI and may augment the progression of CKD. Endothelial-to-mesenchymal transition may explain the loss of renal microvessels and the deposition of interstitial fibroblasts, which are seen during ischemic AKI recovery. Numerous genes and molecules have been identified as contributing to kidney fibrosis, including platelet-derived growth factor receptor-β (PDGFR-β), metalloproteinase inhibitor 3 (encoded by the TIMP-3 gene), a disintegrin and metalloproteinase with thrombospondin motifs 1 (encoded by ADAMTS1 gene), transforming growth factor β (TGF-β), and angiotensin II (Ang II).

Alterations in the Inflammatory Response

A growing body of evidence indicates that the inflammatory response plays a major role in ischemic AKI, including particularly in COVID-19 associated AKI [93]. The major components of this response include endothelial injury, leukocyte recruitment, and production of inflammatory mediators by tubule cells. Inflammatory cascades initiated by endothelial dysfunction can be augmented by the generation of several potent mediators by the injured proximal tubule (Fig. 51.5). These include proinflammatory cytokines (TNF-α, IL-6, and IL-1β) and the chemotactic cytokines (MCP-1, IL-8, RANTES). Human studies have shown that the plasma levels of the pro-inflammatory cytokines TNF-α, IL-6 and IL-8 are elevated in AKI and predict mortality. Toll-like receptors (TLRs) represent a major component of this pro-inflammatory response. TLRs are membrane-associated glycoproteins that primarily mediate the function of innate immunity upon induction with pathogen-associated molecular patterns, with resultant production of various pro-inflammatory cytokines and chemokines via intracellular activation of signalling cascades to eliminate the infective agents. TLRs also recognize endogenous host material released during cellular injury, rendering TLRs as crucial surveillance receptors to detect cellular injury. In the kidney, TLRs are expressed primarily in proximal and distal tubule cells. Renal tubular expression of TLR2 is enhanced after ischemic AKI, and TLR2 gene silencing by knockout and antisense treatment prevents ischemia-induced renal dysfunction, neutrophil influx, tubule apoptosis, and induction of MCP-1, TNF-α, IL-6, and IL-1β. Toll-like receptor 4 (TLR4) recently was suggested as another mediator of tubule cell injury. Activation of TLR4 supports the liberation of pro-inflammatory mediators, promotes leukocyte migration and infiltration, and triggers both innate and adaptive immune systems. Mice lacking TLR4 were observed to have reduced tubular damage and fewer proinflammatory cytokines after renal injury. Several compounds have been shown to offer renoprotection via inhibition of TLR4 in experimental animals. Many of these are safe for use in humans, rendering TLR4 inhibition as a novel therapeutic approach in clinical AKI.

Morphologically, several leukocyte subtypes have been shown to aggregate in peritubular capillaries, interstitial space, and even within the tubules after ischemic AKI, and their relative roles remain under investigation. These include neutrophils, macrophages, dendritic cells, B cells and T cells. Neutrophils are the earliest to accumulate in the postischemic kidney and often are found in the peritubular capillary network of the outer medulla, where they adhere to endothelial cells and can cause capillary plugging and congestion. Neutrophils also migrate into the interstitium and increase vascular permeability which aggravates tubular injury. Neutrophil depletion or blockade of neutrophil function provides partial functional protection in some but not all animal models. Furthermore, neutrophils are not a prominent feature of ischemic AKI in humans, casting doubt on the clinical significance of neutrophil infiltration.

Macrophages are the next to accumulate in animal models, classically thought to be largely in response to upregulation of MCP-1 in tubule cells and induction of its cognate receptor CCR2 on macrophages. There is recent evidence for the role of both resident macrophages as well as activated infiltrating macrophages after experimental AKI. Tubule cell injury results in increased expression of damage-associated molecular pattern (DAMP) molecules, Toll-like receptors (TLRs), and pathogen-associated molecular patterns (PAMPs). These signals rapidly recruit neutrophils, natural killer cells, activated macrophages, and resident macrophages to the site of injury. Macrophages recognize the initial damage signals through pattern recognition receptors (PRRs), a family of receptors that recognize DAMPs and PAMPs. This results in downstream stimulation of macrophage phagocytosis, phagolysosomes maturation, antigen presentation, and production of the proinflammatory cytokine, TNFα. Resident macrophages first act to engulf cellular debris as additional bone-marrow derived macrophages and monocytes are recruited. Resident macrophages further prolong inflammation by recruiting other leukocytes to the site of injury. The initial inflammatory macrophage events are subsequently followed by modulation and then inhibition of the inflammatory response. Macrophages have two distinct phenotypes during AKI; the first phenotype contributes to injury, whereas the second promotes kidney repair. Classically activated macrophages, which predominate in early ischemic injury, produce proinflammatory cytokines (such as IL-12). Alternatively activated macrophages, also known as M2 macrophages, are believed to modulate the inflammatory response and promote tissue repair and are prevalent during the recovery and repair phase of AKI.

Dendritic cells (DCs), like macrophages, have pro- and anti-inflammatory functions and work closely with other components of the immune system to respond to kidney injury. Dendritic cells help regulate immune effector cells, and present antigenic material to T cells. Macrophages and DCs share similar functions and have functional plasticity depending on the cues they receive from the microenvironment. There is a contiguous network of DCs, which are identified by presence of the chemokine receptor CX3CR1, in the kidney interstitium and mesangium. DCs are key initiators, potentiators, and effectors in the innate renal immune system. The role of DCs in renal injury is not fully resolved, because some experimental studies suggest that depleting DCs has protective effects while other findings suggest that deleting dendritic cells worsens injury.

CD4+/CD8+ T cells have been identified in animal as well as human models of ischemic AKI and often are observed to increase the production of proinflammatory molecules, such as TNF-α and IFN-γ. T cell depletion is protective in experimental AKI. Inconsistencies exist, however, and recent data suggest that the role of T cells in ischemic AKI may be complex, with the identification of both protective (TH2 phenotype) and deleterious (TH1 phenotype) subtypes of T cells. T helper lymphocytes enhance tissue injury by recruiting neutrophils and other inflammatory cells, while regulatory T cells conversely reduce renal injury and facilitate repair. Moreover, animals deficient in both T and B cells are not protected from ischemic AKI, and depletion of peripheral CD4+ T cells fails to bestow protection from ischemic AKI.

The potential role of B cells in ischemic AKI is intriguing. Compared with wild-type animals, B cell-deficient mice are protected partially from structural and functional ischemic renal injury, despite comparable neutrophil and T cell infiltrations. Wild type serum transfer, but not B cell transfer, into B cell-deficient mice was shown to restore susceptibility to ischemic AKI, implicating a soluble serum factor as a mechanism by which B cell deficiency confers renal protection.

Recent literature has focused on the proximal tubule's role in intra-renal cross talk. Crosstalk between the proximal tubule and the thick ascending limb (TAL) of the loop of Henle recently has been revealed, with TLR4 and Tamm-Horsfall Protein (THP) suggested as likely mediators of the process. THP, also known as uromodulin, is considered a protective molecule, and inhibits proximal tubule production of proinflammatory cytokines and chemokines. Crosstalk

between the TAL and proximal tubule suppresses tubular activation of innate immunity and reduces inflammatory injury. During the early phases of AKI, THP production is significantly diminished both at the RNA and protein level, in both experimental and human studies. However, THP is significantly upregulated within 48 h of ischemia in experimental models. The absence of THP in mice resulted in more severe inflammation, increased cast formation, reduced renal function, and diffuse tubular necrosis in the outer medulla. Neutrophil infiltration also was increased in THP-knockout mice, corroborating suggestions that THP functions as an anti-inflammatory and protective molecule during ischemic injury. There is now considerable evidence for a negative association between urinary THP and the development of human AKI [94]. Experimental administration of exogenous THP after AKI mitigates subsequent injury and hastens recovery.

Activation of the complement system in AKI, with resultant amplification of the inflammatory response in the kidney, has received widespread attention in recent years. Whereas ischemia-reperfusion injury in most organs activates the complement cascade along classic pathways, studies in animals and humans have implicated the alternative pathway in AKI. However, other reports have identified a role for the mannose-binding lectin pathway after animal and human ischemic AKI. Also controversial is the identification of the final active complement component. Although earlier studies pointed to the C6b-directed formation of a membrane attack complex, recent observations have identified a predominant role for C5a in ischemic AKI. C5a is a powerful chemoattractant that recruits inflammatory cells such as neutrophils, monocytes, and T cells. The kidney is one of the few organs in which the C5a receptor is normally expressed, in proximal tubule epithelial cells as well as in interstitial macrophages. C5a receptor expression in tubule epithelial cells is upregulated markedly after ischemia-reperfusion injury and sepsis. Inhibition of C5a generation using monoclonal antibodies was found to protect against renal dysfunction induced by ischemia, and in turn to inhibit neutrophil and macrophage influx in experimental models. Pre-treatment with orally active small molecule C5a receptor antagonists substantially reduced the histologic and functional impairment induced by ischemic AKI in animal models. Small molecule antagonists for C5a receptor represent promising agents for the treatment or prevention of ischemic AKI. In addition, the anti-C5 monoclonal antibody (eculizumab) ameliorates experimental ischemic AKI and is widely used in the AKI of atypical hemolytic uremic syndrome. Persistent systemic complement activation is also a hallmark of COVID-19 related AKI in humans, and eculizumab may be beneficial.

Alterations in Gene Expression

Attempts at unraveling the molecular basis of the myriad pathways activated by AKI have been facilitated by advances in functional genomics and transcriptome profiling technologies. Several investigators have used these techniques in human and animal models of AKI to obtain expression profiles of thousands of genes. When combined with bioinformatics tools, these studies have identified novel genes with altered expression, new signal transduction pathways that are activated, and even new drug targets and biomarkers in AKI [95–99]. A few clinically relevant examples are provided here.

One of the first induced molecules to be identified in the postischemic kidney using genomic approaches was kidney injury molecule 1 (KIM-1). KIM-1 protein subsequently was demonstrated to be upregulated in the postischemic animal and human kidney tubules, predominantly on the apical membranes of proximal tubule epithelial cells, where it may play a role in renal regeneration. An ectodomain is shed into the urine, making KIM-1 a promising non-invasive urinary biomarker of ischemic human AKI.

Another example is neutrophil gelatinase-associated lipocalin (NGAL), one of the most highly induced genes in the early postischemic kidney. NGAL protein is markedly upregulated in kidney tubules very early after ischemic AKI in animals and humans and is excreted rapidly in the

urine, where it represents a sensitive novel biomarker of early ischemic injury. In the postischemic kidney tubule, NGAL protein is highly expressed in tubule cells that are undergoing proliferation, suggesting its protective or regenerative role after AKI. Exogenous administration of NGAL in experimental models before, during, or even shortly after ischemic or nephrotoxic injury provides remarkable protection at the functional and structural levels, with induction of proliferation and striking inhibition of apoptosis in tubule epithelial cells. In this context, NGAL mitigates iron-mediated toxicity by providing a reservoir for excess iron and may provide a regulated source of intracellular iron to promote regeneration and repair. Exogenously administered NGAL also markedly upregulates HO1, a proven multifunctional protective agent in experimental AKI that works by limiting iron uptake, promoting intracellular iron release, enhancing production of antioxidants such as biliverdin and carbon monoxide, and inducing the cell cycle regulatory protein p21.

Gene expression studies have shown that extracellular signal-regulated kinases (ERK1 and ERK2) are activated 24 h after injury and likely alter cytoskeletal organization and focal complex assembly. ERK 1 and 2 are localized in damaged proximal tubule cells and are activated during renal reperfusion in response to ROS and Ras signaling. ERK1 and 2 have negative downstream effects by phosphorylating proteins that induce the dissolution and restructuring of focal adhesions. Synthetic ERK inhibitors, such as U0126 (already used in humans for chemotherapy), may be relevant in clinical settings as a method that preserves cytoskeletal structure during AKI.

Data mining of gene expression profiles from 150 microarray experiments performed in 21 different models of AKI (including mouse, rat, pig, and human models) identified novel upregulated genes that have now been well characterized—including *LCN2* (*encoding lipocalin 2 or NGAL*), *KIM-1* (kidney injury molecule-1), *CCL2* (*chemokine ligand 2 or MCP-1*), *HMOX1* (*heme oxygenase*), *TNF* (tumor necrosis factor), and *CLU* (Clusterin) [100]. More recent deep sequencing studies have identified significant differences in the responses between AKI subtypes. For example, there exists a remarkable diversity of changes in the kidney genomic response to ischemic and septic injuries [101]. In addition, a comparison of ischemic and volume depletion models of AKI, often considered to be a continuum and therefore predicted to have similar gene expression response, unexpectedly showed that less than 10% of expressed genes were differentially regulated in the two models despite identical elevations in the serum creatinine [102]. Volume depletion induced the metabolic pathways and anti-inflammatory molecules. By contrast, ischemic injury activated known and novel inflammatory, coagulation, and epithelial repair pathways, including *LCN2*, *KIM-1*, *CXCL1*, and *IL-6*, all of which were totally unchanged in the volume depletion model. For added complexity, different nephron segments responded with distinct signatures to different injuries. For example, volume depletion predominately affected the inner medulla, whereas ischemic changes were noted primarily in the outer medulla. Ischemic injury induces mRNA expression of *KIM-1* specifically in the proximal tubule and, in contrast, *LCN2* specifically in the distal nephron. Hence, different insults lead to diverse responses reflecting alterations in segment-specific pathophysiology.

Dramatic recent advances in single-cell RNA sequencing (scRNA-seq) and single-nucleus sequencing can now uncover the expression level of every gene in every cell type, enabling the rapid determination of serial gene expression changes in many thousands of cells, identification of previously unknown cell populations, and even novel heterogeneity within a given cell type. For example, scRNA-seq analysis of human kidney transplant biopsies has uncovered 16 distinct cell types and novel cell states within endothelial cells as well as proinflammatory parenchymal responses in the rejecting kidney. Single nuclear approaches have detected unique cell types and cell states within the human kidney, redefined cellular heterogeneity in the proximal tubule and thick ascending limb, and identified novel genomic signatures of fibrosis. Surprising recent data has begun to challenge the dogma that the fibrotic response of the kidney to injury is a late and final common pathway. In a murine bilateral ischemia-

reperfusion AKI survival model, early kidney sections at day 1 revealed surprising significant fibrosis adjacent to damaged S3 segments [103]. Single-cell profiling of AKI in mice has also revealed early activation of profibrotic transcriptional signatures [95]. Encouraging new experimental data suggest that this early fibrotic response can be prevented. In murine AKI due to ischemia-reperfusion, intraperitoneal administration of a peptide (pUR4) that binds fibronectin and inhibits fibronectin polymerization (an early event in the fibrotic cascade) soon after injury dramatically attenuated the early fibrotic response [104]. The pUR4 peptide was devoid of any adverse effects, rendering translational application to human AKI a realistic possibility. The NIH-funded Kidney Precision Medicine Project (KPMP) is analyzing human AKI kidney biopsies based on elevations in serum creatinine and a urinary biomarker. These single cell RNA-seq and other advanced deep sequencing studies are expected to yield a detailed molecular atlas of the human kidney, and potentially identify additional new pathways for future therapies.

One of the most remarkable and consistent finding from scRNA-seq studies is the rapid induction of an embryonic phenotype in injured tubule cells [95]. Specifically in injured proximal tubule cells, there is re-expression of genes normally present only in the developing kidney (e.g., *Sox4*, *Cd24a*). This switch to the embryonic state is likely critical for regeneration of tubule cells lost during AKI. The identified candidates that accelerate repair represent new future therapies.

Large-scale genome wide association studies (GWAS) can identify potentially pathogenetic genomic sequences that are statistically enriched in AKI cases compared to controls. A recent GWAS analysis of a discovery cohort of 1400 adults with critical illness (760 with AKI) followed by a separate replication cohort of 200 AKI cases [105] have yielded two single-nucleotide polymorphisms (SNPs) involving the transcription factor interferon regulatory factor 2 (*IRF2*), and an additional two SNPs close to the transcription factor T-box 1 (*TBX1*). The identification of SNPs near IRF2 suggests a potential role for the immune system in AKI, a concept with already strong biologic plausibility. TBX1 is expressed during kidney development, and this finding supports the intriguing concept that ontogeny recapitulates phylogeny after kidney injury, whereby genetic programs involved in nephrogenesis that become dormant after birth are once again reactivated and are essential for the recovery process after injury in post-natal life. Additional GWAS studies with even larger cohorts of control and AKI subjects are under way and may yield new AKI susceptibility genes of critical biological significance.

Alterations in Metabolomics

Recent metabolomic approaches have identified dramatic differences in the response of the kidney to injuries that were previously thought to be closely related. For example, experimental models of ischemia-reperfusion injury display the rapid appearance of alanine, leucine, and glucose in the urine, with a downregulation of urinary creatinine and nicotinamide [106]. In marked contrast, hypoxic injury rapidly induces the urinary excretion of benzoate and fructose, while citrate and isothionate are suppressed [107]. The differential appearance of these metabolites in the urine may hold important clues towards etiology-specific biomarkers and therapeutic targets in humans. Additional recent metabolomic studies in a mouse model of ischemic AKI have identified a deficiency in urinary and intra-renal nicotinamide adenine dinucleotide (NAD), an essential component of energy generation via glycolysis and the Kreb's cycle [108]. In a phase I study of oral NAM supplementation (which generates NAD via a salvage pathway) in adults undergoing cardiac surgery, the rise in serum creatinine was prevented compared to placebo [108]. Additional translational studies are under way.

Unique Aspects of Septic AKI

Although sepsis is one of the most common causes of AKI, the pathogenesis remains incompletely understood. Translational analysis of sep-

tic AKI has been limited by the fact that most animal models of this condition do not faithfully mimic the human condition. Kidney biopsies from humans with septic AKI have revealed only mild, nonspecific changes that do not correlate with the profound functional changes. Histological assessment of postmortem kidneys from non-survivors of septic AKI shows mild heterogeneous tubular injury with apical vacuolization, but with an absence of tubular necrosis and only minimal apoptosis. Studies have revealed the surprising finding that unlike in ischemic or nephrotoxic AKI, humans with early septic AKI demonstrate a paradoxical increase in renal blood flow despite a reduction in GFR. Thus, early septic AKI is a hyperemic injury that contrasts with the persistent vasoconstriction characteristic of ischemic and nephrotoxic AKI. In sepsis, at the glomerular level, there is evidence for afferent arteriolar vasoconstriction, efferent arteriolar vasodilatation, and shunting of blood flow via capillaries that bypass the glomerulus, resulting in decreased GFR and oliguria.

Recent evidence shows that microcirculatory dysfunction, inflammation, and metabolic reprogramming are the three fundamental mechanisms that may play a role in the development of septic AKI. Profound heterogeneous changes in microcirculatory flow have been demonstrated, including a decrease in the capillary density, a decrease in the proportion of capillaries with continuous flow and an increase in the proportion of capillaries with intermittent and stop flow. Multiple mechanisms may lead to microcirculatory alterations, including endothelial injury, autonomic nervous system response, shedding of the glycocalyx, and activation of the coagulation cascades.

During sepsis-associated AKI, a reprioritization of energy occurs that seeks to meet metabolic vital needs to ensure survival at the expense of cell function. Functions that consume ATP are downregulated, including protein synthesis and ion transport, especially in the proximal tubular epithelial cells. In addition, experimental studies have suggested that tubular epithelial cells may reprogram their metabolism by switching to aerobic glycolysis and oxidative phosphorylation to fulfill energy requirements during sepsis. Furthermore, mitochondria enter a series of quality control processes such as mitophagy and biogenesis to preserve the mitochondrial pool to confer protection and fulfill the necessary energetic requirements. Finally, an early response to septic AKI is cell cycle arrest in tubule cells, which may represent another defense mechanism to preserve energy. Approaches to enhance these survival mechanisms hold promise in human AKI.

AKI and Distant Organ Dysfunction

Substantial experimental and clinical evidence illustrates that AKI leads to dysfunction of other organs, including lung, heart, brain, liver, and intestine via aberrant organ-organ communication. This is clinically important, since the prognosis for patients who have AKI and another organ in a state of dysfunction is especially poor, with a morality rate of 60–80%. Interruption of normal immunological balance and generation of inflammatory mediators are important in AKI-induced distant organ crosstalk. Additional mechanisms include increased endothelial injury, cellular apoptosis, and oxidative stress.

Acute lung injury is a common complication of AKI, and manifests clinically as pulmonary edema and respiratory failure needing mechanical ventilation [109]. Fluid overload from decreased urine output and impaired cardiac function is the major cause of pulmonary edema. In addition, the integrity of alveolar-capillary barrier is impaired by systemic inflammation, oxidative stress, and uremia, causing fluid accumulation in the lung. Increased inflammatory cytokines in the plasma, such as IL-6 and IL-8, have been associated with prolonged ventilator weaning times and increased mortality in AKI patients with acute lung injury.

The term cardiorenal syndrome (CRS) refers to a complex pathophysiological disorder of the heart and kidneys whereby acute or chronic dysfunction in one organ may induce acute or chronic dysfunction in the other organ. In CRS Type 3, also called acute reno-cardiac syndrome, an abrupt worsening of renal function leads to acute cardiac disorder. Cardiovascular failure occurs in

about 60% of critically ill adults and is the second most common cause of death (after sepsis). Volume overload decreases myocardial contractility and induces maladaptive myocardial remodeling. Uremic toxin accumulation leads to cardiovascular toxicity and can increase risk for myocardial ischemia by compromising coronary vasoreactivity. Metabolic acidosis also diminishes myocardial contractility. Electrolyte imbalances can trigger arrhythmias.

Liver dysfunction can be observed in critically ill AKI patients and hepatic failure increases the in-hospital mortality in AKI patients. Several clinical studies have found development of hepatic dysfunction in AKI patients leading to alterations in protein synthesis and metabolism of lipid, protein, and drugs. During AKI, there are changes not only in renal drug metabolism, but also in non-renal metabolism, which can have considerable influence on clinical outcomes due to under- or over-dosing and related toxicity problems. The mechanisms by which AKI impacts liver drug metabolism may be related to uremic toxins, inflammatory cytokines, activated leucocytes, and other neuro-humoral factors. Drug dosing needs to be especially carefully monitored in AKI patients with hepatic dysfunction.

Clinicopathologic Correlations and Therapeutic Implications

The clinical course of AKI can be divided into four phases: initiation, extension, maintenance, and recovery. Clinical recognition of these phases can be facilitated using novel biomarker panels, as described later in this chapter (Fig. 51.7). Advances in our understanding of AKI pathogenesis now allow for postulation of temporal correlations between the clinical and cell biologic alterations. The *initiation phase* is the period during which initial exposure to the ischemic insult occurs, kidney function begins to fall, and parenchymal injury is evolving but not fully entrenched. Intracellular ATP depletion is profound, sublethal injury to the tubule epithelial and endothelial cells predominates, generation of reactive oxygen molecules is initiated, and activation of inflammatory mechanisms commences. Intrarenal protective mechanisms such as induction of HSPs in tubule cells also are brought to play during the initiation phase. If the injury is alleviated at this stage, complete restitution and recovery is very likely. Interventional approaches during this phase might include vasodilators, ATP donors, antioxidants, and iron chelators (Fig. 51.7).

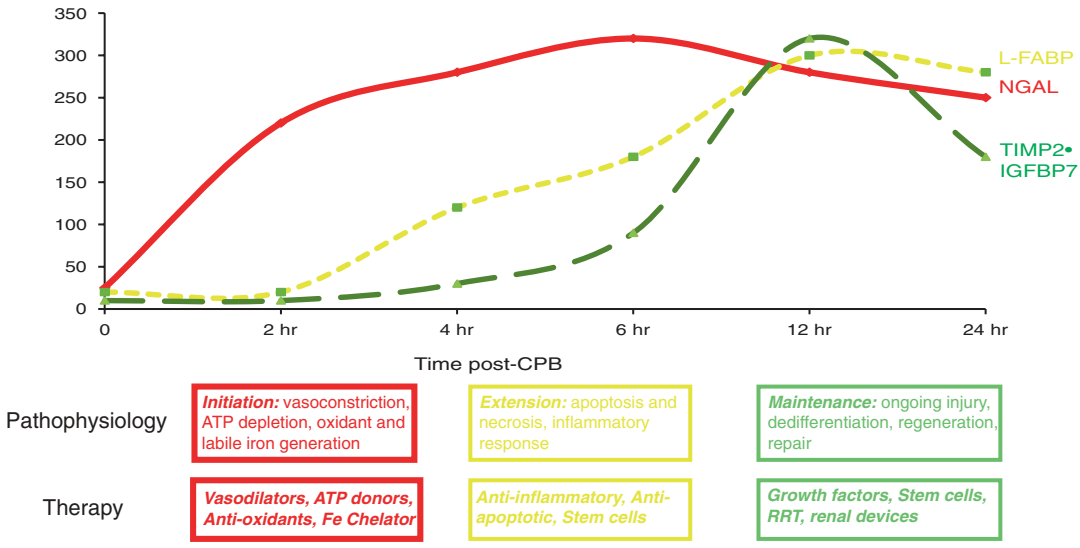

Fig. 51.7 Color coded correlations between time after the initiating insult (in this case, hours after initiation of cardiopulmonary bypass), appearance of non-invasive urinary biomarkers, phase of AKI and the underlying mechanisms, and suggested therapeutic approaches

Prolongation of the initial insult (including reperfusion after ischemia) ushers in the *extension phase*. Blood flow returns to the cortex, and tubules undergo reperfusion-dependent cell death but also commence the regeneration process. By contrast, medullary blood flow remains severely reduced, resulting in more widespread tubule cell death, desquamation, and luminal obstruction. Injured endothelial and epithelial cells amplify the raging inflammatory cascades, and the endothelial denudation potentiates the intense vasoconstriction. The GFR continues to decline. This phase probably represents the optimal window of opportunity for early diagnosis and active therapeutic intervention. Anti-inflammatory agents, anti-apoptotic measures, and stem cells are likely to be particularly efficacious during this phase.

During the *maintenance phase*, parenchymal injury is established, and the GFR is maintained at its nadir even though renal blood flow begins to normalize. Cell injury and regeneration occur simultaneously, and the duration and severity of this phase may be determined by the balance between cell survival and death. Repair of epithelial and endothelial cells appears to be critical to recovery. Measures to accelerate the endogenous regeneration processes may be effective during this phase. These include growth factors, stem cells, and kidney support therapies.

The *recovery phase* is characterized functionally by an improvement in GFR and structurally by reestablishment of tubule integrity, with fully differentiated and polarized epithelial cells. The origin of cells that replenish the damaged epithelial cells has been examined. Fate mapping studies have shown that bone marrow-derived cells do not make a significant contribution. Rather, it is the surviving tubule epithelial cells themselves that dedifferentiate and proliferate to repair the damaged tubule. The normally quiescent surviving proximal tubule cells proliferate by entering the cell cycle and activating cyclin-dependent kinases such as Cdk2 and Cdk4/6, resulting in adaptive repair. Re-expression of kidney developmental genes in injured tubule cells is likely critical for this process. However, the repair process may be incomplete or maladaptive, and AKI can progress to CKD.

Mechanisms Underlying Long-Term Sequelae of AKI

Several studies using large databases in both adults and children have now established the strong correlation between AKI and long-term sequelae, including CKD, hypertension, and end-stage kidney disease (ESKD). The cellular and molecular mechanisms that result in CKD after AKI have been intensely studied in animal models . The three primary morphologic findings include failed tubule recovery/maladaptive tubule repair, capillary rarefaction, and interstitial fibrosis.

The fate of tubule cells that do not regenerate and recover after AKI is variable. In severe AKI, tubule repair may not occur at all, leading to atrophic and fibrotic areas with disconnection of intact glomeruli from surviving tubules and subsequent decline in kidney function. Other surviving cells that undergo dedifferentiation become growth arrested in the G2 phase of the cell cycle and cannot engage proliferative pathways. Such abnormally dedifferentiated "failed-recovery" cells occur in small clusters along entire tubule segments. These senescent tubule epithelial cells acquire the senescence-associated secretory phenotype and actively secrete fibrogenic factors and pro-inflammatory cytokines, thereby creating microenvironments that promote fibrosis and inflammation. Mechanisms underlying this maladaptive repair are emerging.

Capillary rarefaction has been demonstrated after AKI in both animal models and in humans. Persistent hypoxia and resultant endothelial injury results in capillary disintegration. Failure of endothelial cells to regenerate leads to reduced capillary density. In addition, injury to pericytes, which are resident fibroblasts that normally support capillary structure and integrity, results in their detachment from capillaries and worsens capillary disintegration. The consequent persistent local tissue hypoxia drives secretion of profibrotic cytokines, inhibition of vascular repair, and endothelial-mesenchymal transition, all of which promote interstitial fibrosis. Loss of renal vasculature leads to renal VEGF deficiency, and administration of exogenous VEGF or VEGF-

derived chimeric molecules preserves capillary density and ameliorates CKD progression in animal models. As a strategy to combat tissue hypoxia, pharmacologic activation of hypoxia-inducible factor (HIF) prevented the development of fibrosis. Pharmacologic activation of Nrf2, a potent antioxidant, has also been effective in ameliorating the sequelae of AKI in animals. All three agents are available for human use and should be tested as ameliorators of the AKI to CKD transition.

The primary cellular drivers of interstitial fibrosis include activated fibroblasts, myofibroblasts derived from activated pericytes and/or endothelial-mesenchymal transition, and persistent influence of inflammatory macrophages and lymphocytes. Fibrosis typically occurs around damaged or atrophic tubules, and is not progressive *per se*. Indeed, the surrounding tubulointerstitium remains normal, and with time, fibrotic tissue shrinks as activated fibroblasts regress. However, fibrosis is progressive in the setting of repeated AKI episodes or if AKI occurs in the setting of previous CKD.

Postrenal AKI

Postrenal AKI is a result of obstruction to the outflow tract on both sides and is uncommon beyond the neonatal period. Postrenal AKI is usually reversed by relief of the obstruction but is accompanied by a very significant post-obstructive diuresis. The common causes of postrenal AKI are listed in Table 51.4. The pathophysiology and management of the obstructive nephropathy syndrome that can result from congenital anomalies are detailed in Chap. 45.

Clinical Presentation

AKI is largely asymptomatic, and its detection must begin with having a high index of suspicion and an awareness of the risk factors. AKI most commonly presents with a progressive accumulation of fluid and/or nitrogenous wastes, in a predisposed patient who has been exposed to one or more of the etiologic factors outlined in Tables 51.2, 51.3 and 51.4. Classic clinical presentations include edema, hypertension, hematuria, and oliguria. Less frequently, one encounters an increase in BUN and creatinine which is not readily explained. In all cases, the evaluation requires a complete history, physical examination, laboratory evaluation, renal imaging, and rarely a kidney biopsy. A diligent search for all drugs and medications ingested is especially important, even when another obvious cause for AKI is evident. The initial approach to a patient with known or suspected AKI should be directed towards (a) identifying the underlying cause, (b) distinguishing between pre-renal and intrinsic AKI, (c) discriminating between AKI and CKD, (d) determining the severity of AKI, and (e) considering a diagnostic fluid challenge.

Identifying the Underlying Cause

The initial history should be directed towards uncovering an obvious risk factor for AKI, such as those listed in Tables 51.2, 51.3 and 51.4. Additional relevant aspects of the AKI history and physical examination are outlined in Tables 51.5 and 51.6, respectively. These, in combination with a careful urinalysis with microscopy, will yield the etiology of AKI in most cases. A short duration of vomiting, diarrhea, or decreased oral intake associated with decreased urine output and typical physical findings of dehydration suggests prerenal volume-responsive functional AKI. Bloody diarrhea with oliguria is consistent with the hemolytic uremic syndrome. A history of pharyngitis or impetigo a few weeks prior to the onset of gross hematuria or edema suggests post-streptococcal glomerulonephritis. The presence of edema should

Table 51.4 Common causes of postrenal AKI

Mechanism	Etiology
Congenital anomalies of the kidney and urinary tract (CAKUT)	Urethral valves, ureteropelvic junction obstruction
Acquired causes	Calculi, clots, neurogenic bladder, drugs that cause urinary retention

Table 51.5 History taking in patients with suspected AKI

• Fluid loss
– Diarrhea, vomiting
– Burns
– Surgery, shock
• Nephrotoxic agents
– Non-steroidal anti-inflammatory drugs
– Aminoglycosides
– Contrast agents
• Glomerular disease
– Streptococcal infection (Post-streptococcal glomerulonephritis)
– Bloody diarrhea (Hemolytic-uremic syndrome)
– Fever, joint complaints, rash (Systemic lupus erythematosus)
• Obstruction
– Complete anuria
– Poor urinary stream

Table 51.6 Physical signs in AKI

• Signs of intravascular volume depletion
• Signs of AKI (edema, hypertension)
• Signs of underlying renal disease
– Butterfly rash, joint swelling (Systemic lupus erythematosus)
– Purpuric rash (Henoch-Schonlein Purpura)
– Fever, macular rash (Interstitial nephritis)
– Palpably enlarged kidneys (Polycystic/multicystic kidney disease, Renal vein thrombosis)
• Signs of obstruction
– Poor urinary stream
– Palpably enlarged bladder
– Therapeutic catheterization

also prompt a search for nephrotic syndrome, cardiac failure, or liver failure, which would suggest prerenal AKI that should not be treated with fluid resuscitation. Fever, joint complaints, and a malar rash are indicative of systemic lupus erythematosus. In hospitalized patients, nephrotoxic medications or periods of hypotension are commonly associated with intrinsic AKI and should be diligently searched for.

The urinalysis is an important noninvasive test in the diagnostic evaluation. Typically, the urine in conditions that result in prerenal AKI is highly concentrated and contains little protein or blood. In contrast, proteinuria and hematuria are prominent in etiologies leading to intrinsic AKI. A heme positive urine by dipstick in the absence of RBCs in the sediment on microscopy suggests hemolysis or rhabdomyolysis. Characteristic findings on microscopic examination of the urine sediment can suggest certain diagnoses. Muddy brown granular casts and epithelial cell casts are highly suggestive of intrinsic AKI or ATN. The finding of red cell casts is diagnostic of glomerulonephritis. The concurrent findings of red cell casts, dysmorphic red cells, heavy proteinuria, or lipiduria are referred to as a "nephritic" urinary sediment. This is commonly associated with AKI due to glomerulonephritides. Pyuria with white cell and granular or waxy casts are suggestive of tubular or interstitial disease or urinary tract infection. White cells and white cell casts may also be seen in acute glomerulonephritis. The presence of renal epithelial cells, renal epithelial cell casts and granular casts are characteristic of ATN. In prospective studies of adults with AKI, when a renal epithelial cell, renal epithelial cell cast or granular cast was present, the sensitivity for discriminating no AKI versus AKI was low, but the specificity was very high at 91–95%. In another prospective study of adults with AKI, a urinary sediment scoring system created based on the number of renal tubular epithelial cells and granular casts was significantly associated with severity of AKI and with increased risk of worsening AKI. Urine microscopy is very inexpensive, readily available, noninvasive, and specific for AKI diagnosis and severity, and should therefore be a routine part of evaluating any patient who is suspected to have AKI [110].

Distinguishing Between Functional and Structural AKI

This determination is potentially important because (a) the treatment and prognosis are different, and (b) prompt identification and management of prerenal AKI can prevent the progression to intrinsic AKI. As noted above, the urinalysis with microscopy will often provide an initial differentiation between prerenal and intrinsic AKI. The further distinction is based on the principle that prerenal AKI is associated with maxi-

mal reabsorption of solutes and water by the intact proximal tubule, whereas the proximal tubule cell damage typical of intrinsic AKI results in impaired reabsorptive capacity. Urinary indices based on this principle are shown in Table 51.7. The fractional excretion of sodium (FENa) is a convenient bedside screening test for making this distinction. It is calculated from measured concentrations of sodium (Na) and creatinine (Cr) in the urine (U) and plasma (P), as follows:

$$\mathrm{FENa} = \left(\left[\mathrm{U}/\mathrm{P}\right]\mathrm{Na}\right)/\left(\left[\mathrm{U}/\mathrm{P}\right]\mathrm{Cr}\right)\times 100$$

A FENa below 1% suggests prerenal AKI, in which the reabsorption of almost all the filtered sodium represents an appropriate response to decreased perfusion. A FENa above 2% suggests intrinsic AKI with proximal tubule injury. A FENa between 1% and 2% is non-diagnostic. However, limitations to the utility of FENa should be noted. In neonates, the FENa is generally higher because of their decreased ability to reabsorb sodium resulting from immaturity of proximal tubule function. The FENa can be high following fluid resuscitation, or administration of diuretics.

The fractional excretion of urea (FEUrea) has been proposed as a more accurate determinant of prerenal AKI, especially in patients receiving diuretics and in patients with sepsis. FEUrea is typically <35% in patients with prerenal AKI, and >50% in those with established intrinsic AKI. In the setting of diuretic use, there can be an uncoupling of renal sodium handling from tubular function. FEUrea may represent an alternative diagnostic approach because urea transport is not directly linked to sodium transporters. However, recent studies have revealed that in patients with heart failure receiving intravenous loop diuretic therapy, FEUrea commonly and significantly increased above the pre-diuretic baseline [111].

Table 51.7 Urinary indices in AKI

Measurement	Prerenal AKI	Intrinsic AKI
Urine specific gravity	>1020	<1012
Urine/plasma creatinine	>40	<20
Urine Na (mEq/L)	<20	>40
FENa	<1%	>2%

Multicenter studies in the complex critically ill population have revealed limited utility of both FENa and FEUrea in distinguishing prerenal from intrinsic AKI. These studies raise fundamental questions about the pathophysiologic validity of the prerenal AKI paradigm and suggest that AKI in the critical care setting is a continuum of injury that should likely not be divided into functional (prerenal or transient) or structural (ATN or persistent) sub-types.

By the same principle of increased proximal tubule solute reabsorption, the BUN/creatinine ratio (both expressed as mg/dL) in the serum is often markedly elevated (>20) in prerenal AKI. This is because the intact proximal tubule can avidly reabsorb urea but is impermeable to creatinine. However, increases in BUN without AKI can be encountered in patients receiving steroids or total parenteral nutrition, in catabolic states, and those with gastrointestinal bleeding. In addition, a BUN/creatinine ratio >20 is a poor indicator of prerenal AKI in critically ill patients and should not be used in that setting. This is because of at least two confounding factors. One, critical illness is associated with increased protein catabolism and increased urea generation rate, and a higher severity of illness would be expected to result in a higher BUN and a greater risk for intrinsic AKI. Second, critical illness could result in decreased muscle mass, and a paradoxical reduction in serum creatinine. Indeed, animal studies have demonstrated decreased production of creatinine in sepsis, the most common cause of severe AKI. Thus, in critical illness, both the high BUN and lower serum creatinine are explicable by factors other than a prerenal state.

Distinguishing Between AKI and CKD

A kidney and bladder ultrasound are a sensitive, non-invasive modality that can differentiate not only between AKI and CKD but can also rule out a postrenal etiology. Typically, the kidneys in AKI are normal or enlarged, with increased echogenicity, whereas those in CKD are frequently small and shrunken. Other distinguishing features are shown in Table 51.8.

Determining the Severity of AKI

Estimating the baseline pre-illness serum creatinine can determine the severity of AKI and allow for classification based on the KDIGO criteria. This is best achieved when a previous serum creatinine level is available. If not, the baseline serum creatinine in children can be estimated using the Schwartz formula and a presumed baseline eGFR of 120 mL/min/1.73 m^2:

$$\text{Estimated creatinine clearance in mL/min} = 0.413^{*}\,\text{L/Pcr}$$

Where L = height (cm) and Pcr = plasma creatinine in mg/dL. The constant of 0.413 provides a good approximation of GFR in children of all ages and both genders [14]. However, it should be emphasized that this constant was derived in patients with CKD Stage 2 to 5 and has not been validated for use in AKI.

Another important index of AKI severity is the degree of fluid overload, which is especially useful in the assessment of the critically ill patient. Several recent pediatric AKI studies have demonstrated that increasing degrees of fluid overload are independently associated with mortality and adverse outcomes. Extent of fluid overload during a hospitalization period can be estimated by the following formula:

$$\%\text{Fluid overload} = \left[\text{Total fluid in}(\text{L}) - \text{Total fluid out}(\text{L})\right] / \text{Admission weight in kg} \times 100$$

The importance of assessing fluid overload was first demonstrated by the Prospective Pediatric Continuous Renal Replacement Therapy (ppCRRT) Registry Group, via analysis of its 340-patient cohort using a tripartite classification for percent fluid overload at CRRT initiation. Patients who developed >20% fluid overload at CRRT initiation had significantly higher mortality (66%) than those who had 10–20% fluid overload (43%) and those with <10% fluid overload (29%). The association between degree of fluid overload and mortality remained after adjusting for intergroup differences and severity of illness. When fluid overload was dichotomized to >20% and <20%, patients with >20% fluid overload had an adjusted mortality OR of 8.5 (95% CI, 2.8–25.7). In addition to impacting mortality, fluid overload can directly worsen kidney function and AKI due to increased renal venous pressure, interstitial edema, and abdominal compartment syndrome. Fluid overload also leads to hemodilution and a falsely reduced serum creatinine concentration, thereby delaying or masking the diagnosis and classification of AKI. Fluid overload at a threshold of 10–20% is independently associated with adverse outcomes in several critically ill pediatric populations [112].

Table 51.8 AKI versus CKD

Acute kidney injury	Chronic kidney disease
Progressive rise in BUN and Cr	Stable elevated BUN and Cr
History of AKI etiology	History of chronic hypertension
Normal growth	Stunted growth
Normal bones	Renal osteodystrophy
No broad urinary casts	Broad waxy urinary casts
Anemia usually mild	Anemia usually severe
Normal or enlarged kidneys	Small shrunken kidneys

Routine laboratory evaluation for the presence of metabolic complications can also assist in establishing AKI severity. AKI is associated with several life-threatening complications, which require diligent monitoring by the clinician. Fortunately, these complications are uncommon in patients who receive dialytic therapies. Common complications are listed in Table 51.9. Hyponatremia is usually dilutional (secondary to fluid retention and administration of hypotonic fluids). Less common causes

Table 51.9 Major complications of AKI

Metabolic	Cardiovascular	Gastrointestinal	Neurologic	Hematologic	Infectious
Hyperkalemia	Pulmonary edema	Nausea, vomiting, anorexia	Altered mental status	Anemia	Pneumonia
Metabolic Acidosis	Arrythmias	Malnutrition	Irritability	Bleeding	Sepsis
Hyponatremia	Pericarditis	Gastritis	Seizures		Infected IV sites
Hypocalcemia	Myocardial infarction	GI Bleeding	Somnolence		
Hyper-phosphatemia	Hypertension	GI ulcers	Coma		

of hyponatremia include sodium depletion (hyponatremic dehydration) and hyperglycemia (serum sodium concentration decreases by 1.6 mEq/L for every 100 mg/dL increase in serum glucose above 100 mg/dL). Hypernatremia in AKI is usually a result of excessive sodium administration (inappropriate fluid therapy or overzealous sodium bicarbonate administration). Hyperkalemia is due to the reduction in GFR, reduction in tubular secretion of potassium, increased catabolism, and metabolic acidosis (each 0.1 unit reduction in arterial pH raises serum potassium by 0.3 mEq/L). Hyperkalemia is most pronounced in patients with excessive endogenous production (rhabdomyolysis, hemolysis, and tumor lysis syndrome). A high anion gap metabolic acidosis is secondary to the impaired renal excretion of acid and the impaired reabsorption and regeneration of bicarbonate. Acidosis is most severe in shock, sepsis, or impaired respiratory compensation. Hypocalcemia is due to increased serum phosphate and impaired renal conversion of vitamin D to the active form. Hypocalcemia is most pronounced in patients with rhabdomyolysis. Metabolic acidosis increases the fraction of ionized calcium (the active form). Therefore, overzealous bicarbonate therapy can decrease the concentration of ionized calcium and precipitate symptoms of hypocalcemia, including tetany, seizures and cardiac arrhythmias. Hyperphosphatemia in AKI is primarily due to impaired renal excretion and can aggravate the hypocalcemia. During recovery from AKI, the vigorous diuretic phase may be accompanied by significant volume depletion, hypernatremia, hypokalemia and hypophosphatemia.

Considering a Diagnostic Fluid Challenge

A common clinical scenario is one where patients present with an increase in BUN and serum creatinine, with a history and physical exam findings consistent with a prerenal etiology, but the duration of the prerenal insult is unknown. Another common diagnostic dilemma occurs when a subject presents with an increase in serum creatinine, but the cause is unclear. In both these cases, a fluid challenge may be diagnostic as well as therapeutic. Typically, fluid challenges in children consist of normal saline in the dose of 10–20 mL/kg repeated once or twice until urine output improves. A reduction in BUN and serum creatinine would suggest a prerenal etiology, whereas an absence of improvement in these parameters (and/or the development of fluid overload) would confirm the diagnosis of intrinsic AKI. Fluid challenges should be avoided in children with pre-renal AKI due to volume-unresponsive states such as liver failure, heart failure and nephrotic syndrome.

Considering a Kidney Biopsy

A renal biopsy is rarely indicated in AKI but should be considered when noninvasive evaluation fails to establish a diagnosis. In pediatric AKI, it is most indicated in patients with suspected acute glomerulonephritis (to identify crescentic forms or specific vasculitides), with suspected lupus nephritis (to classify the disease

and establish the activity and chronicity), or with kidney transplant dysfunction.

Problems with Serum Creatinine Measurements in AKI

A progressive increase in serum creatinine (typically 1–2 mg/dL/day) is the hallmark of intrinsic AKI and has served as a biomarker of AKI for several decades. However, serum creatinine concentration is a flawed AKI biomarker for several reasons [113]. Serum creatinine does not differentiate the nature, type and timing of the renal insult. Changes in serum creatinine concentrations often lag changes in GFR until a steady state has been reached, which can take several days. Dialysis readily clears serum creatinine, rendering this marker less useful in the assessment for improving renal function once dialysis has begun. Even normal serum creatinine can vary widely with age, gender, diet, muscle mass, nutritional status, medications, and hydration status. In the acute setting, it is estimated that more than 50% of kidney function must be lost before the serum creatinine even begins to rise because of the concept of renal reserve. However, animal studies have shown that while AKI can be prevented and/or treated by several maneuvers, these measures must be instituted very early after the insult, well before the serum creatinine rises. The lack of early biomarkers of AKI in humans has hitherto impaired our ability to launch potentially effective therapies in a timely manner.

Serum Cystatin C as a Functional Biomarker

The use of cystatin C as an endogenous marker of kidney function in children is well established [114–119]. Cystatin C is a ubiquitous cysteine protease inhibitor protein that is produced by all nucleated cells at a constant rate, freely filtered by the glomerulus, catabolized by the proximal tubule, and does not undergo significant tubular secretion. These qualities make cystatin C a more ideal functional marker of GFR than serum creatinine. Its measurement has been standardized on a clinical laboratory platform. Serum cystatin C is not affected by gender, diet, hydration, muscle mass, and age (cystatin C levels are nearly identical in adults and children over 12 months of age). Cystatin C outperforms serum creatinine for estimation of GFR in adults and children in the steady state, but its ability to rapidly detect acute changes in GFR is still unclear. Some recent studies have demonstrated that serum cystatin C is an early predictive biomarker of AKI in children [119–121] and adults [122]. However, serum cystatin C measurements are not uniformly available, and the assay is more expensive than serum creatinine. Also, serum cystatin C levels are influenced by steroids and other immunosuppressive therapies, thyroid dysfunction, diabetes, acute inflammation, and high cell turnover. Serum cystatin C remains a functional marker, and not an early marker of structural kidney damage. Additional prospective studies are required to improve our understanding about cystatin C diagnostic cut-off values for prediction of AKI. There remain confounding variables such as method of measurement (standardized clinical laboratory measurements by nephelometry versus turbidimetry, international standardization of reagents) and different reporting equations. The creation of a calibrated reagent (IFCC) has recently allowed for standardization of cystatin C measurements across clinical laboratories and the development of universal GFR estimation equations. New CKD-EPI equations for cystatin C combined with creatinine have now become the preferred method for estimating GFR in adults. For use in children, the Full Age Spectrum (FAS) equations were developed using the assumption that the average GFR of children, adolescents, and young adults is 107.3 mL/min/1.73 m^2. The FAS cystatin C based equations performed as well as or better than the CKD-EPI equations and is currently the preferred mode of reporting eGFR in children and young adults [123].

Novel Early Biomarkers of Kidney Damage in AKI

In the clinical continuum of AKI, we are currently primarily operating in the "established AKI" stage, when the GFR is already reduced

and biomarkers of functional injury become apparent, as shown in Fig. 51.8. The genomic and proteomic tools of modern science have identified novel markers for the early stress response of the kidney, which serendipitously appear in the urine or plasma during the "subclinical AKI" phase, well before a change in serum creatinine is detected [124–126]. Thus, they detect structural kidney injury before any functional impairment may be apparent. Many are being developed and validated as early non-invasive structural damage biomarkers for the prediction of AKI and its clinical outcomes in humans. This is a rapidly evolving and expanding field, and the current status of only the most promising examples is summarized in Table 51.10.

The most widely studied and validated early biomarker of AKI in children is neutrophil gelatinase-associated lipocalin (NGAL) [124]. In a prospective study of 71 children undergoing cardiopulmonary bypass, levels of NGAL in the urine and plasma were significantly elevated within 2 h of bypass in those who subsequently developed AKI (defined as a 50% increase in serum creatinine) 1–3 days after surgery [51]. A subsequent prospective study of 374 infants and children undergoing cardiopulmonary bypass confirmed these findings, and additionally established cut-off thresholds as well as a strong association between early NGAL measurements and adverse clinical outcomes, including length of hospital stay and the duration and severity of AKI [54]. A prospective multicenter study of 311 children undergoing cardiac surgery has confirmed the early rise of plasma and urine NGAL concentrations (within 6 h after surgery) in subjects who developed an increase in serum creatinine 2 days later [127]. Early NGAL concentrations were also shown to be associated with longer hospital and ICU stays, and with longer duration of mechanical ventilation. Studies in the heterogeneous pediatric intensive care and emergency department settings have also demonstrated the ability of early NGAL measurements to predict subsequent AKI and its severity [128–130]. Furthermore, urine NGAL levels effectively discriminate between pre-renal AKI and intrinsic AKI [131, 132]. In many reported studies, the addition of NGAL significantly improved the risk prediction for AKI over clinical models alone. Since the widespread

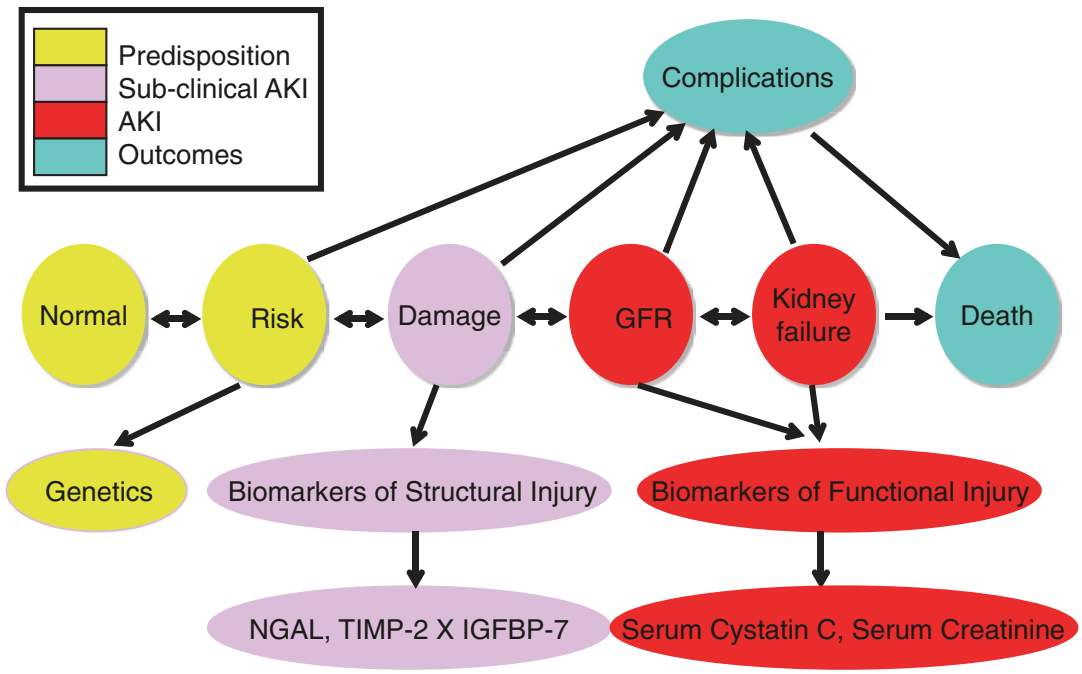

Fig. 51.8 Clinical continuum of AKI, showing the color coded correlations between phases of AKI, and the currently available laboratory methods for their detection

Table 51.10 Novel urinary biomarkers for the prediction of AKI and its outcomes

Biomarker	Source	Function	Cardiac surgery	Kidney transplant	ICU/ED
NGAL	Distal tubule and collecting duct	Regulates iron trafficking, promotes tubule cell survival	2 h post CPB 2 days pre-AKI Predicts AKI severity, dialysis, and death	6 h post-transplant 2–3 days pre DGF Predicts long-term graft loss	On admission 1–2 days pre-AKI Predicts AKI severity, dialysis, and death
IL-18	Proximal tubule	Promotes tubule cell apoptosis and necrosis	6 h post CPB 2 days pre-AKI Predicts AKI severity, dialysis, and death	6 h post-transplant 2–3 days pre DGF Predicts long-term graft loss	On admission 1–2 days pre-AKI Predicts AKI severity, dialysis, and death
L-FABP	Proximal tubule	Antioxidant, suppresses tubule-interstitial damage	6 h post CPB 2 days pre-AKI Not tested for outcomes	Fresh donor urine pre-transplant; predicts DGF	On admission 1–2 days pre-AKI Predicts AKI severity, dialysis, and death
KIM-1	Proximal tubule	Promotes epithelial regeneration, regulates apoptosis	12 h post CPB 1 day pre-AKI Not tested for outcomes	Fresh donor urine pre-transplant; Predicts DGF	On admission 1–2 days pre-AKI Predicts AKI severity, dialysis, and death
TIMP-2 X IGFBP-7	Proximal tubule	Biomarkers of G1 cell cycle arrest	12 h post CPB 1 day pre-AKI Not tested for outcomes	Not tested	12 h after admission; Predicts severe AKI

Times shown (in hours or days) are the earliest time points when the biomarker becomes significantly increased from baseline

AKI acute kidney injury, typically defined as AKIN Stage I or greater, *CPB* cardiopulmonary bypass, *DGF* delayed graft function, *ICU* intensive care unit, *ED* emergency department, *IL-18* interleukin-18, *KIM-1* kidney injury molecule 1, *L-FABP* liver-type fatty acid binding protein, *NGAL* neutrophil gelatinase-associated lipocalin

availability of commercial NGAL assays, there has been an explosion of studies validating the utility of NGAL as an early biomarker. Several multicenter pooled analyses of existing NGAL studies in children and adults have now confirmed the utility of this marker for the early diagnosis of AKI and its clinical sequelae in several clinical scenarios [133–143]. Collectively, these studies have identified proposed cut-offs for NGAL diagnostic thresholds. When urine or plasma NGAL is measured using standardized clinical laboratory platforms, a value of <50 ng/mL effectively rules out structural AKI (irrespective of the serum creatinine concentration). Measured NGAL values of >150 ng/mL are highly predictive of AKI, and values of >500 ng/mL strongly predict severe AKI [143]. These cut-offs still need to be rigorously validated using prospective, multicenter studies.

Studies have examined a combination of urinary biomarkers in children at risk for AKI, including following cardiac surgery [55, 144]. Urinary NGAL was increased in AKI patients within 2 h of bypass initiation, urine interleukin-18 (IL-18) and liver-type fatty acid binding protein (L-FABP) were increased within 6 h, and both urine kidney injury molecule-1 (KIM-1) and the cell cycle biomarkers (product of TIMP-2 and IGFBP-7) increased at the 12-h time point. All markers correlated with AKI severity and clinical outcomes and improved the risk prediction for AKI over clinical models. Thus, they represent temporally sequential markers, and a panel of such biomarkers may therefore help establish the timing of injury and plan appropriate therapies. This concept is illustrated in Fig. 51.7. Standardized clinical laboratory platforms for the measurement of urine and plasma NGAL as well as the cell cycle biomarkers are now available in most countries.

The concept of outcomes in "biomarker-positive, creatinine-negative" patients has been explored. Multicenter studies have enrolled cardiac surgical, critically ill or emergency department patients who were grouped according to their NGAL and serum creatinine status [133]. Studies found that measurement of the levels of NGAL complemented the information obtained by measurement of serum creatinine levels in establishing the diagnosis of AKI and predicting prognosis. A substantial proportion of patients (about 20%) had elevated NGAL levels even in the absence of loss of renal excretory function. This previously undetectable condition (which we now term "subclinical AKI") was associated with a two- to threefold increased risk of death or the need for renal replacement therapy compared to patients without elevations of serum creatinine or tubule damage markers. Notably, even in patients with significant loss of renal function, measurement of tubule damage biomarker levels still added prognostic information, as patients with increased levels of NGAL and serum creatinine levels displayed by far the worst prognosis. Overall, the data support that measurement of tubule damage markers such as NGAL results in a substantial added value to serum creatinine measurements.

Given the above considerations, NGAL and other tubule injury markers may complement a standardized diagnostic approach to AKI, and help clinicians improve their ability to make an early AKI diagnosis. Besides NGAL, other promising biomarkers of tubule damage currently include KIM-1, IL-18, L-FABP, and the cell cycle biomarkers (product of TIMP-2 and IGFBP-7), with additional candidates being continually discovered and verified in this area of intense contemporary research. However, markers of renal function will remain important even after tubule damage markers are fully established. Glomerular filtration markers such as serum creatinine or serum cystatin C are still valuable for the diagnosis and quantification of excretory function loss (e.g., for drug dosing) and prognosis (e.g., for development of CKD). Urine output will still represent a useful criterion for adjustments to fluid balance, and for the commencement or ending of renal replacement therapies. Structural AKI biomarkers may add substantively to our ability to detect AKI early, and to refine our ability to reliably classify AKI, as shown in Table 51.11.

Table 51.11 Biomarkers to refine AKI classification

Functional marker	Structural marker	Classification
–	–	Normal
+	–	Prerenal AKI
–	+	Subclinical AKI
+	+	Intrinsic AKI

Functional markers include serum creatinine, cystatin C, and other markers of GFR. Structural markers include neutrophil gelatinase-associated lipocalin, and others described in the text

Prevention of AKI

Proven measures for prevention of AKI include vigorous fluid administration in patients at high risk for developing AKI, adequate fluid repletion in those with hypovolemia, avoidance of hypotension in critically ill children by providing inotropic support as needed, and close monitoring of renal function and drug levels in children receiving nephrotoxic medications.

Hydration

Vigorous fluid administration (typically with isotonic crystalloid solutions) has been successfully employed to prevent AKI in patients at high risk, including hemoglobinuria, myoglobinuria, early tumor lysis syndrome, renal transplantation, other major surgical procedures, and use of nephrotoxic agents such as radiocontrast, cisplatin, and amphotericin. The efficacy of preoperative hydration strategies was demonstrated by a meta-analysis of 20 randomized controlled trials that investigated the reno-protective effects of perioperative hemodynamic optimization among 4220 adult surgical patients who were undergoing elective or emergent procedures [145]. Postoperative AKI was significantly reduced by perioperative hemodynamic optimization when

compared with the control group who did not receive similar goal directed therapy (OR 0.64, 95% CI 0.50–0.83). A more recent meta-analysis with an overall sample of 9308 patients indicated that goal-directed therapy by means of fluids and inotropes improves renal perfusion and oxygenation in high-risk patients undergoing major abdominal and orthopedic surgery [146].

Fluid Resuscitation

A child with a clinical history and physical examination findings consistent with hypovolemia and impending or established prerenal AKI requires immediate vigorous intravenous fluid therapy with normal saline (10–20 mL/kg over 30 min, repeated twice if necessary, until urine output is re-established). Isotonic crystalloids are most used for correcting extracellular volume depletion. Compared to crystalloids, colloids theoretically may result in a greater plasma expansion. However, the difference in required volumes for fluid resuscitation was minimal between crystalloids and colloids. Moreover, colloids carry the risks of hyper-oncotic reduction in glomerular filtration and osmotic tubular damage. It is recommended that serum chloride levels are monitored, since hyperchloremia can cause renal vasoconstriction. If urine output does not improve after restoration of intravascular volume, more invasive monitoring may be required to guide further therapy. In children not responsive to volume repletion alone, preservation of blood pressure and renal perfusion with appropriate inotropic agents is essential to prevent AKI. Patients with established intrinsic AKI require volume restriction to prevent worsening fluid overload. Such patients are best treated by maintaining current volume status by providing for insensible water losses and replacing any ongoing fluid losses.

Nephrotoxin Management

Nephrotoxins are an important risk factor and etiology for pediatric AKI. Monitoring kidney function and drug levels when possible are important for rational adjustment of drug dosing based on known alterations in pharmacokinetics and pharmacodynamics during AKI. It is crucial that clinicians caring for patients requiring potentially nephrotoxic drugs use appropriate drug dosing based on the knowledge of altered clearance rates in early AKI and be vigilant in monitoring for drug efficacy and toxicity. The importance of monitoring serum creatinine levels as a measure of kidney function in children receiving nephrotoxic drugs has been brought into focus in a retrospective single center study of 1660 non-critically ill, hospitalized children [147]. Children who developed AKI as defined by the serum creatinine-based pRIFLE criteria had significantly greater odds of exposure to one or more nephrotoxic medications than patients without AKI (OR 1.7; 95% CI 1.04–2.9). Both increasing dose and duration of nephrotoxin use were associated with increased development of AKI. A recent study demonstrated decreasing AKI duration when a systematic daily serum creatinine monitoring policy was put into practice for children who received multiple nephrotoxic medications. When a systematic daily serum creatinine monitoring program for all non-critically ill children receiving three or more nephrotoxic medications was put in place using an automated EHR-driven protocol in a single center, a 42% reduction in AKI days was observed [148]. This program has now been spread to multiple children's hospitals that could implement a systematic EHR-guided protocol, with excellent published results—follow-up studies have shown a nephrotoxin medication exposure rate decreased by 38% and a reduction in AKI incidence by 64% [149].

Additional quality improvement strategies in the prevention of AKI have been suggested [150]. Briefly, primary prevention at the community level includes raising awareness regarding AKI risk factors among health care professionals and patients by education and establishment of tools that measure these risk profiles. It is suggested that populations and patients at high risk for developing AKI should have a Kidney Health Assessment at least every 12 months to define

and modify their AKI risk profile. Primary prevention of AKI in hospitalized patients may be achieved by first screening for AKI risk factors, followed by at least an assessment of serum creatinine, urine dipstick analysis, and urine output. Once AKI has developed, secondary prevention may be directed towards early identification of AKI severity and complications (monitor serum creatinine, urine output, serum electrolytes), minimizing nephrotoxin exposure, ensuring optimal hemodynamic and nutritional status, and treating the underlying cause.

Unproven Pharmacologic Agents for Prevention of AKI

Unproven agents for prevention of AKI include mannitol, loop diuretics, low-dose dopamine, fenoldopam, atrial natriuretic peptide, N-acetylcysteine, and methylxanthines. Their potential use in AKI prevention is briefly discussed here.

Mannitol

Experimental studies suggest that mannitol might be protective by causing a diuresis (which minimizes intratubular cast formation), and by acting as a free radical scavenger (thereby minimizing cell injury). In the clinical setting, the efficacy of mannitol for prevention of AKI in high-risk patients is inconclusive. Indeed, its use may be detrimental, and can result in volume expansion, hyperosmolality, and pulmonary edema. Its use for prevention of AKI is not recommended.

Loop Diuretics

Loop diuretics such as furosemide induce a forced diuresis by reducing active NaCl transport in the thick ascending limb of the loop of Henle. The ensuing decrease in energy requirement may protect the tubule cells in the setting of a decrease in energy delivery. However, the available evidence from clinical studies in adults does not support the routine use of diuretics as prophylaxis for AKI. In some settings, the use of diuretics was harmful. In critically ill children, furosemide was found to be the most common nephrotoxin used and was associated with a twofold greater adjusted risk for developing AKI [151]. Therefore, the use of loop diuretics for prevention of AKI is not recommended. However, in certain select situations, such contrast induced AKI in susceptible populations, AKI prevention with loop diuretics can be associated with favorable outcomes if euvolemia is carefully maintained.

While controlled studies have demonstrated that the administration of diuretics to patients in the early stages of AKI does not significantly alter the natural history of the disease, furosemide can potentially convert AKI from oliguric to a non-oliguric form and therefore simplify fluid, electrolyte, and nutritional management. The concept here is to use diuretics in well-hydrated, diuretic-responsive patients (primarily furosemide, with sometimes the addition of thiazide diuretics) to maintain urine output, which would prevent fluid overload and allow for nutritional support, both of which would prevent worsening of AKI. In addition, a prospective assessment of a furosemide challenge, or furosemide "stress" test, was able to predict which patients would have worsening AKI based on a lack of response to furosemide within 2 h. In the setting of early AKI, low urine output following the furosemide stress test predicted progressive AKI, need for dialysis, and inpatient mortality [152]. Using a furosemide stress test in patients with increased AKI biomarker levels such as NGAL improves risk stratification and prediction of AKI progression [153].

Dopamine

The use of low "renal-dose" of the inotropic agent dopamine (0.5–3 μg/kg/min) is common in the critical care setting due to its renal vasodilatory and natriuretic effects. However, prospective randomized studies of adult patients at risk for AKI have not shown a beneficial reno-protective effect of "low-dose" dopamine. There are risks associated with even "low-dose" dopamine, including tachycardia, arrhythmias, and myocar-

dial, intestinal and even renal ischemia. Therefore, the routine use of dopamine for prevention of AKI is not recommended.

Fenoldopam

Fenoldopam is a potent, short-acting, selective dopamine A-1 receptor agonist that increases renal blood flow, increases natriuresis, and decreases systemic vascular resistance. Experience with fenoldopam in the pediatric age group is limited. A small, prospective, single center, randomized, double-blind, controlled trial of children undergoing cardiopulmonary bypass revealed a significant reduction in the urinary AKI biomarker NGAL at the end of surgery and 12 h after ICU admission in the group receiving fenoldopam [154]. Confirmation of the benefits of fenoldopam is required in a large, multicenter, randomized, controlled trial prior to routinely recommending this agent for the prevention of AKI.

Natriuretic Peptides

Atrial natriuretic peptide (ANP) and b-type natriuretic peptide (BNP) block tubular reabsorption of sodium and vasodilate the afferent arteriole. The reno-protective effects of these agents have been evaluated primarily in trials of adults undergoing cardiac surgery and with congestive heart failure. While initial data seemed promising, evidence from large, randomized trials have failed to show a conclusive clinic benefit from these agents. Pediatric data for the reno-protective effects of natriuretic peptides is limited. Pending further randomized controlled trial data, the routine use of natriuretic peptides for prophylaxis against AKI is not recommended.

N-Acetylcysteine

N-acetylcysteine (NAC) is a free radical scavenger antioxidant agent that counteracts the deleterious effects of ROS in the generation of tubule cell injury, and also has vasodilatory properties. Several recent meta-analyses have examined the efficacy of N-acetylcysteine in the prevention of AKI following cardiac and other major surgery as well as the prevention of contrast-induced nephropathy in adults. A recent meta-analysis evaluating the preventive effect of NAC on contrast-associated AKI in adults undergoing primary percutaneous coronary intervention suggested that NAC reduces the risk of AKI and all-cause in-hospital mortality [155]. NAC was also shown to prevent postoperative AKI in adults with pre-existing CKD undergoing cardiac surgery [156]. Given that the overall direction of the data is toward benefit and the agent is well tolerated and relatively inexpensive, the use of NAC in high-risk patients is generally recommended. While NAC is commonly used in children for treatment of acetaminophen toxicity and other forms of acute liver failure, data for its reno-protective effects in the pediatric population is limited. The routine use of NAC for AKI prophylaxis in children is not generally recommended, except for judicious use in children with CKD who are at high risk for contrast induced nephropathy. One approach is to use NAC in combination with IV hydration to prevent contrast induced nephropathy in children with CKD stage 3 or greater and a history of contrast-induced AKI in the past, or in children who are already on two nephrotoxins and contrast would be the third nephrotoxin to be used. The efficacy of this approach has not been systematically studied.

Methylxanthines

Methylxanthines are adenosine antagonists that act via A1 and A2A receptors in the kidneys. Previous human clinical trials using caffeine citrate, theophylline or aminophylline have suggested that methylxanthines may prevent AKI or improve renal function in special populations of high-risk neonates and infants, including those with perinatal hypoxia/ischemia or prematurity and undergoing cardiac surgery. In a recent multicenter in preterm neonates, caffeine administration in preterm neonates was associated with

reduced incidence and severity of AKI. In this retrospective analysis, for every 4.3 neonates exposed to caffeine, one case of AKI was prevented [86]. In two recent systematic reviews of prophylactic theophylline or aminophylline use for prevention of AKI in highly susceptible neonates with birth asphyxia, the pooled estimate showed a 60% reduction in the incidence of AKI and a significant improvement in fluid balance, with no increase in risk of complications [87, 88]. It should be noted that theophylline has wide variation in metabolism and a narrow therapeutic window, and it is therefore essential to monitor theophylline levels to avoid toxicity.

Prognosis and Outcome

In general, pediatric AKI has serious short- and long-term consequences. The outcome depends upon the etiology, age of the child, and comorbidities. The short-term outcomes of AKI include mortality and morbidity. Regarding mortality, retrospective analyses of large databases during the past decade in the United States showed that the overall in-hospital mortality rate of hospitalized children with AKI is approximately 15%. Mortality rates have ranged from 9.5% in non-critically ill children to 30% in children requiring intensive care. Even higher in-hospital mortality rates of 35–45% have been reported from other countries. The highest mortality rates are encountered in infants, those who have multiorgan failure, and those requiring renal replacement therapies. Data regarding long-term mortality after pediatric AKI is limited, although small studies have revealed a mortality rate of about 20% during a 2–5 year follow-up period. More recent multinational studies have revealed perhaps an encouraging improvement regarding in-hospital mortality. Both the AWARE study in critically ill children [35] and the AWAKEN study in neonates [36] reported an in-hospital mortality rate of approximately 10%. However, in the Global Snapshot study, children from low-income countries continue to experience a high mortality rate of 20% compared with only 1.2% in those from high-income countries [38].

Regarding short-term morbidity, it is quite clear that AKI is associated with adverse effects, including increased need for and longer duration of ventilatory support, as well as increased length of hospital stay [157]. The AWARE study demonstrated a stepwise increase in mechanical ventilation use as well as ICU lengths of stay (LOS), depending on AKI severity [35]. Critically ill children with stage 1, stage 2, and stage 3 AKI required mechanical ventilation 38.2%, 40.5%, and 50.2% of the time, respectively (vs. no AKI at 29.5%). In the AWARE study, patients with AKI (increase of 1.31 days) and severe AKI (increase of 3 days) had longer ICU LOS after adjusting for severity of illness [35]. These observations confirm previous findings of children who experienced AKI that had longer hospital LOS (36.6 days vs. 20.5 days) than those without AKI [9]. This association is also seen in neonates across all gestational ages. Two single center reports found that AKI increased hospital LOS among neonates by 3.4 days and 11.7 days, respectively. The association between AKI and LOS is evident even in non-critically ill children, including in the settings of nephrotoxin administration and the nephrotic syndrome [64].

Information regarding the long-term outcome of children after an episode of AKI is beginning to accumulate. In a multicenter pooled analysis of 3476 children with hemolytic uremic syndrome followed for a mean of 4.4 years, the combined average death and ESKD rate was 12%, and the combined average renal sequelae rate (CKD, proteinuria, hypertension) was 25%. Thus, long-term follow-up appears to be warranted after an acute episode of hemolytic uremic syndrome. Long-term follow-up of premature infants with neonatal AKI has shown a 45% rate of renal insufficiency. Prominent risk factors for progression include an increased random urine protein/creatinine ratio and a serum creatinine >0.6 mg/dL at 1 year of age. More recent long-term follow-up studies demonstrate that 40–60% of children surviving an AKI episode have a sign of CKD, including proteinuria, hyperfiltration, low eGFR or hypertension In the Translational Research Investigating Biomarker Endpoints in AKI (TRIBE-AKI) prospective study in children

after cardiac surgery, hypertension (17%), proteinuria (8%), and a eGFR <90 mL/min/1.73 (13%) were common 5 years later; however, these sequelae were not more common among the children who experienced perioperative AKI [158]. Similarly, the Assessment, Serial Evaluation, and Subsequent Sequelae in Acute Kidney Injury (ASSESS-AKI) prospective study followed children for 4 years after cardiopulmonary bypass [159]. The cohort prevalence of CKD was high (20%); hypertension prevalence was also high (30%). AKI was not significantly associated with the development of CKD or hypertension. However, a subsequently published retrospective study did find that cardiac surgery-associated AKI was associated with a greater risk for CKD stage 2 or greater [160]. The 5-year cumulative incidence of CKD for patients with cardiac surgery-associated AKI was 12%, in comparison with 3% in those without AKI (adjusted HR 3.8). In a longitudinal study of heterogenous critically ill children, AKI was associated with twofold higher odds for CKD or hypertension at 6 years of follow-up [161].

Beyond CKD, AKI episodes are also associated with ESKD. In a retrospective analysis of 1688 surviving children who required dialysis for an acute AKI episode, outcomes after a median of 9.6 years included death (7%), CKD (13%), ESKD (2.6%), and hypertension (12%) [162].

Collectively, these data strongly suggest that long-term follow-up is clearly warranted for children who survive an episode of AKI. Widely available interventions for hypertension and proteinuria hold promise for prevention of CKD progression after AKI, a concept that is especially pertinent to the pediatric population.

Concluding Remarks

AKI represents a very significant and potentially devastating problem in pediatric medicine, with dire immediate and long-term consequences. The incidence appears to be rising globally. Outstanding advances in basic research have illuminated the pathogenesis of AKI and have paved the way for several successful therapeutic approaches in animal models. However, translational research efforts in humans have yielded disappointing results. One reason for this is the lack of early markers for AKI, and hence an unacceptable delay in initiating promising therapies. Fortunately, several potential candidates are currently being developed and tested as sequential non-invasive early biomarkers for the prediction of AKI and its severity. It is likely that not any one biomarker but a collection of strategically selected proteins may provide the "AKI Panel" for the early non-invasive diagnosis of AKI and its consequences. Such a tool of biologically plausible sequential biomarkers would be indispensable for risk stratification, timely institution of potentially effective therapies, monitoring the response to therapies, and for prediction of adverse clinical outcomes. A judicious combination of clinical judgment, established functional markers, novel structural markers based on knowledge of underlying pathophysiology, and technical advances in therapies that counter the complex mechanisms holds the greatest promise for true progress in human intrinsic AKI.

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Management of Pediatric Acute Kidney Injury

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Introduction

Acute kidney injury (AKI) affects an increasing proportion of critically ill patients who now survive medical and surgical complications that were once often fatal. Despite increased efforts to recognize and prevent AKI, progression to kidney failure continues to occur with alarming frequency. The treatment of AKI in critically ill and injured children requires understanding of medical management of disease with ready availability of renal replacement therapy (RRT) as well as adaptability for use in pediatric patients of all ages and sizes. In this chapter, we review medical management of AKI as well as traditional and emerging RRT modalities.

Medical Management of AKI

Medical management of AKI includes optimizing renal perfusion, preventing or reducing fluid overload, correcting electrolyte abnormalities and acid-base disturbances, supporting patient nutrition, and closely monitoring administration of nephrotoxic medications all while considering the patient's need for renal replacement if medical management proves ineffective [1–3]. The prevention of AKI is the foremost means of management. The clinician must pay close attention to subtle changes in serum creatinine and corresponding urine output as creatinine increase is a late marker of AKI [4]. Chapter 46 discusses this topic in detail.

Renal Perfusion

Intravenous (IV) fluid is used to treat hypovolemia in an attempt to maintain end organ perfusion, but overly aggressive resuscitation may lead to fluid overload. Both crystalloid and colloid (typically albumin) are utilized in fluid resuscitation; however, two major studies in adults have failed to demonstrate a clear benefit on AKI outcomes or survival difference for colloid versus crystalloid infusions [5, 6]. However, colloid may be advantageous over crystalloid in patients requiring large amounts of fluid resuscitation in the setting of sepsis or burn injury [7].

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Vasopressors in conjunction with IV fluid resuscitation for vasomotor shock may improve kidney perfusion and are recommended in patients who have or are at risk of AKI [7]. Agents recommended are norepinephrine, vasopressin, and dopamine. Vasopressin and norepinephrine use has increased due to favorable side effect profiles versus the arrhythmic abnormalities noted with dopamine [8, 9].

The use of renal vasodilators to increase renal perfusion does not improve outcomes. Specifically, dopamine has been employed at low dosages in an effort to improve renal perfusion by promoting vasodilatation. Adult studies of "renal dose" dopamine show no benefit and may even suggest harm [10–13]. More recently the selective dopamine agonist fenoldopam has been utilized to augment renal blood flow. Adult literature from single-center studies suggests a decline in both mortality and the need for RRT [14]. Furthermore, meta-analysis of 16 trials of fenoldopam in adults concluded that fenoldopam decreased the incidence of AKI, the need for RRT, intensive care unit (ICU) stay and death from any cause [15]. To date, one available randomized controlled trial has suggested that use of high-dose fenoldopam in pediatric patients on cardiac bypass significantly reduced the use of diuretics and vasodilators during bypass [16]. Current Kidney Disease Improving Global Outcomes (KDIGO) recommendations are against the use of fenoldopam to prevent or treat AKI given the high risk for hypotension associated with its use in ICU patients in comparison to the relatively sparse data available regarding its efficacy [7]. Emerging data from randomized, blinded trials suggest that fenoldopam may be beneficial in complex cardiac surgeries to improve the quality of perfusion during cardiopulmonary bypass and prevent AKI in both adults and children [16, 17]. The role for fenoldopam in AKI requires further clarification.

Volume Status

Awareness of volume status is critical in a patient with AKI since patients may have hypovolemia, euvolemia, or hypervolemia (fluid overload). Volume status should be continually reassessed and correlated with patient intake and output as well as daily weights. Heart rate, blood pressure, capillary refill, and skin turgor are all key components of assessing volume status.

Hypovolemia should be addressed with isotonic fluid to restore intravascular volume, and if necessary, inotropic support. AKI secondary to prerenal azotemia is likely in a severely volume depleted child. Aggressive fluid resuscitation with 10–20 mL/kg normal saline boluses to reestablish intravascular volume is recommended, and if there is no urine output once volume status is restored, a significant dose of furosemide (2 mg/kg IV) may be given. It is important to recognize that administration of furosemide and subsequent diuresis may simply result in conversion of the AKI from oliguric to non-oliguric and will not alter the course of the renal injury itself [18]. If a furosemide trial is used, a single high dose should be administered with observation for response. Furosemide should not be continued if there is ongoing oliguria [19].

Fluid overload has been associated with increased morbidity and mortality in pediatric critical care patients; however, the pathophysiology leading to poor outcome is not fully delineated [20, 21]. Examples of AKI with hypervolemia include following aggressive fluid resuscitation in septic patients and in patients with left ventricular cardiac dysfunction. The use of excessive normal saline for fluid resuscitation is associated with hyperchloremia, which has been demonstrated to diminish renal sodium excretion [22, 23] and impair renal blood flow [24], thus heightening the risk for AKI. Congestive heart failure due to poor left ventricular function can result in poor renal perfusion secondary to deficits in forward flow, which further promotes edema formation through activation of the renin-angiotensin-aldosterone axis and subsequent sodium retention [25]. Diuretic use in the adult ICU patient with AKI is associated with heightened risk of both death and non-recovery of renal function [19]. A trial of high-dose furosemide may be used to relieve hypervolemia given evidence that persistent, positive fluid balance is

associated with increasing mortality in adult patients who develop AKI [26]. It is not advisable, however, to rely on diuretic therapy and fluid and nutritional restriction to avoid RRT given that such use of diuretics for either AKI "prophylaxis" or therapy does not improve outcomes [27, 28]. Patients with fluid overload not responsive to diuretics require consideration of RRT.

Cumulative percent fluid overload may be calculated as follows:

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fluid input (liters) - fluid output (liters) / ICU admission weight (kg) * 100
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Evaluation for RRT should occur in the setting of >10% cumulative fluid overload. Initiation of RRT is advised if cumulative fluid overload is >20% [29].

Critically ill pediatric patients presenting with euvolemia but oliguria that is unresponsive to fluid resuscitation may have intrinsic AKI. In this population, continued fluid resuscitation may be detrimental if the patient remains oliguric; thus, guidelines suggest restricting fluid to insensible losses plus replacement of urine output plus extra renal losses [30]. However, nutrition needs may require early RRT to prevent volume overload and electrolyte disturbances.

Electrolyte Abnormalities

Electrolyte abnormalities occur commonly in AKI and may develop rapidly, necessitating vigilant monitoring. Hyperkalemia is common, particularly in oliguric patients, and is potentially life-threatening due to ventricular tachycardia and fibrillation. Medical management of hyperkalemia is directed towards removal of potassium from the body (sodium polystyrene sulfonate, loop diuretic if responsive) and preventing arrhythmias via driving potassium into the cells (albuterol, sodium bicarbonate, insulin + glucose) and stabilizing the cardiac membrane (calcium), as summarized in Table 52.1. Dialysis may be necessary if moderate to severe hyperkalemia is refractory to medical management (Table 52.1).

Hyponatremia occurs more often than hypernatremia in AKI [30]. Hyponatremia is often due to water retention and may be exacerbated by intake of hypotonic fluids. Hyponatremia due to water retention secondary to volume depletion may respond to isotonic fluid. In contrast, fluid

Table 52.1 Medical agents utilized in the management of hyperkalemia

Agent(s)	Mechanism	Dose	Onset	Complications
Sodium bicarbonate	Shifts K+ into cells	1 mEq/kg IV over 10–30 min	15–30 min	Hypernatremia, change in ionized calcium
Albuterol	Shifts K+ into cells	400 μg by nebulizer	30 min	Tachycardia, hypertension
Glucose and insulin	Shifts K+ into cells	Glucose 0.5 g/kg, insulin 0.1 U/kg IV over 30 min	30–120 min	Hypoglycemia
Calcium gluconate 10%	Stabilizes membrane	0.5–1 mL/kg IV over 5–15 min	Immediate	Bradycardia, arrhythmias, hypercalcemia
Calcium chloride	Stabilizes membrane	10 mg/kg IV over 5–15 min	Immediate	Bradycardia, arrhythmias, hypercalcemia
Sodium Polystyrene Sulfonate (Kayexalate®, Concordia Pharmaceuticals, Oakville, Ontario, Canada)	Exchanges Na^+ for K^+ across the colonic mucosa	1 g/kg orally or PR in sorbitol	30–60 min	Hypernatremia, constipation, colonic membrane irritation if given PR

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K^+ potassium, *IV* intravenous, *Na^+* sodium, *PR* per rectum

restriction is effective if due to free water excess. RRT may be necessary if renal dysfunction prevents excretion of excess free water. Sodium levels less than <120 mEq/L are associated with a high risk for cerebral edema and seizures. Sodium correction with hypertonic saline solution over several hours should be initiated. Further correction of hyponatremia can be achieved by free water restriction.

Hyperphosphatemia in AKI is secondary to reduction in glomerular filtration rate (GFR). Hyperphosphatemia may be treated with phosphate binders and dietary phosphorus restriction. Selection of a phosphate binder should consider the patient's calcium level. In patients with low ionized calcium, a calcium-containing phosphate binder (e.g. calcium carbonate) should be used; by contrast, in patients with hypercalcemia, a non-calcium phosphate binder, such as sevelamer, is recommended.

Hypocalcemia occurs in AKI secondary to high serum phosphorus. Both total and ionized calcium levels should be monitored since total calcium may be inaccurate due to decreased total calcium secondary to hypoalbuminemia and changes in calcium binding to albumin based on the patient's acid-base status. Initial treatment for mild hypocalcemia in AKI and phosphate retention is correction of hyperphosphatemia, typically using oral phosphate binders. Symptomatic hypocalcemia requires correction with IV calcium. This replacement should be provided with caution in the severely hyperphosphatemic patient given the possibility of systemic calcium phosphate precipitation, potentially worsening existing AKI with calcium phosphate deposition in the renal tubules. Inability to correct hypocalcemia in a symptomatic patient (e.g. tetany and/or seizures) secondary to severe hyperphosphatemia is an indication for dialysis.

For children who are not receiving RRT, several measures can prevent severe metabolic and electrolyte disturbances. First, no supplemental phosphorus or potassium should be provided to the patient unless symptomatic or there is significant hypophosphatemia or hypokalemia. Second, to prevent worsening hypertension and fluid overload, sodium should be restricted to 2–3 mEq/kg/day. Third, parenteral or enteral nutrition should be considered early in the patient's course, as described below, to replete electrolyte abnormalities. If adequate nutrition cannot be provided due to fluid overload, RRT should be initiated. Serum electrolytes, phosphorus, calcium, and albumin should be regularly monitored as dictated by the patient's clinical status.

Acid-Base Disturbances

Metabolic acidosis in AKI is due to renal dysfunction and systemic disease (e.g. sepsis, trauma, burns). Severe acidosis can be treated with IV or oral sodium bicarbonate, with careful monitoring for fluid overload and worsening of hypertension due to the sodium load. Patients with AKI who develop severe, refractory metabolic acidosis may require dialysis therapy, especially in the setting of oligoanuria. It is important to measure the serum total and ionized calcium prior to bicarbonate treatment due to potential for symptomatic hypocalcemia given increased pH-dependent binding of calcium to proteins, which decreases the ionized calcium.

Nutritional Interventions

Patients with AKI are in a hypercatabolic state and should have nutritional support to ensure full calorie, protein, and micronutrient delivery. Nutritional goals include preservation of lean body mass and avoidance of metabolic derangements. Potential benefits include improved wound healing, immune function, and scavenging of oxygen free radicals, with the goal of decreasing patient mortality [32].

Delivery

Adequate nutrition may require supplemental enteral and/or parenteral nutrition if oral intake cannot meet nutritional requirements. Enteral nutrition is preferred if the gastrointestinal tract is functioning given the ease of administration and lower rates of infection. Conversely, parenteral

nutrition should be employed when the gastrointestinal tract cannot be utilized and/or enteral feeding cannot provide sufficient nutrition [33]. See Fig. 52.1 for a decision tree illustrating mode of nutritional support in AKI [34].

Protein

Current evidence suggests that patients with AKI require increased protein, particularly when receiving RRT [35]. Protein-energy wasting (PEW) is loss of lean body mass and fat mass and may occur in patients with AKI d [36]. PEW is an independent predictor for patient mortality and is also directly associated with the length of hospital stay and risk of complications [37]. The adult literature recommends an increased protein intake goal of 1.5–2 g/kg per day for hypercatabolic patients or for patients receiving RRT that utilizes high-flux and/or highly efficient filters, which are associated with amino acid losses [38].

Calories and Lipid

General guidelines suggest that critically ill children should receive from 1 to 1.3 times basal metabolic needs in calories [39]; however, additional calories for hypercatabolic states should be provided given the patient's pre-existing state of nutrition. To optimize enteral nutrition, "renal-specific" formulas are an option for patients being medically managed with AKI or on RRT. These formulas may be beneficial given high caloric and protein density with low electrolyte levels [40]. Adequate caloric intake is needed to prevent catabolism, to promote protein synthesis and to offset heat-dependent caloric losses [41]. Parenteral nutrition, including IV lipids, is necessary if enteral nutrition cannot be provided [38, 41].

Nutrients

Levels of vitamin C and water-soluble vitamins, including thiamine and folic acid, may be low in patients with AKI [42]. Use of RRT can exacerbate nutrient and trace element losses due to the very efficient removal of small molecular weight substances [43]. Appropriate replacement is indicated.

Glycemic Control

Stress hyperglycemia is a notable feature of critical illness [44]. Several studies have investigated the impact of conservative versus intensive insulin therapy on patient mortality, with secondary analyses investigating impact of glycemic control on incidence of AKI [45–47]. Tight glycemic control has decreased the incidence of AKI in adults [48]. Insulin is recommended to correct hyperglycemia in AKI, with typical glucose goals between 110 and 150 mg/dL [49].

Avoidance of Nephrotoxins

Many commonly used medications are metabolized and/or excreted by the kidneys. Nephrotoxic medications in the ICU contribute to nearly 25%

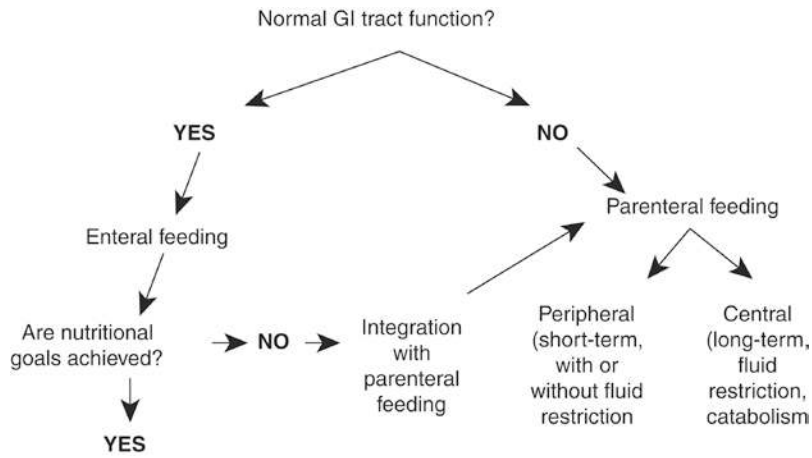

Fig. 52.1 Decision tree for nutritional support in AKI patients with protein energy wasting (PEW) or at risk of PEW. (Used with permission of John Wiley and Sons from Fiaccadori et al. [34])

of AKI cases [50, 51]. Prevention of drug-induced AKI is more effective than any available therapy; recognition of high-risk patients is therefore necessary. The dose and frequency of administration of any potentially nephrotoxic medication should be adjusted (or avoided) based on the patient's GFR.

Common nephrotoxins include aminoglycosides, nonsteroidal anti-inflammatory drugs (NSAIDs), contrast agents, and chemotherapeutic and immunosuppressant medications [52]. AKI induced by drugs occurs by two predominant mechanisms: direct toxicity to renal tubular epithelium, as is seen with aminoglycosides and amphotericin, and interference with autoregulatory mechanisms, leading to unrestricted vasoconstriction and reduced renal blood flow, as is seen with NSAID toxicity. NSAIDs are a common cause of AKI in children, even when ingested at recommended doses; the incidence of AKI may be underestimated both in the inpatient and outpatient settings [53]. Aminoglycosides are widely utilized in pediatric patients. Repeat administration of these agents may lead to renal interstitial and tubular epithelial cell accumulation, and as such, recommendations have been made to administer aminoglycosides every 24 h (or less) to minimize toxicity, with drug levels obtained daily [7].

Contrast-induced AKI (CI-AKI) in adults is associated with an increased risk of mortality in the year following the episode of AKI [54]. The incidence in children is not well-characterized. Patients with chronic kidney disease or diabetes are at increased risk of CI-AKI [54]. Volume and osmolality of contrast administered is directly associated with risk of AKI [55, 56], and non-ionic agents are thought to be safer, especially in patients with chronic kidney disease [57]. The pathophysiology of CI-AKI is still largely unknown. Severe vasoconstriction following contrast administration has been implicated [58, 59], as has direct cytotoxicity via oxygen free radical generation. Serum creatinine rises 1–2 days after the imaging procedure and is usually not accompanied by oligoanuria [60]. Dialysis is required in a minority of patients. No treatment exists other than support if CI-AKI occurs. However, in recent years, attention has focused on prevention [61, 62]. A meta-analysis of prevention strategies recommends pre- and post-contrast IV volume expansion with bicarbonate-containing fluids and use of low or iso-osmolar contrast agents in the smallest volume possible in patients with pre-existing kidney disease who are at increased risk. N-acetylcysteine and ascorbic acid have also been suggested for use as free radical scavengers in the higher risk populations [57]. N-acetylcysteine is indicated for the prevention of contrast-induced AKI per the 2012 KDIGO guidelines [7]. While older studies have suggested a benefit [63], controversy regarding its use remains as the effect of N-acetylcysteine to prevent AKI can be variable, and several studies have failed to show a significant benefit. There is no significant benefit of either N-acetylcysteine or bicarbonate infusion for prevention of AKI, even among populations at high risk for kidney complications [64]. There have been no randomized trials in children.

Other Medical Therapies

Growth Factors

Renal tubular injury is a major component in the pathogenesis of AKI, and renal tubular repair is a required step for recovery. Growth factors play an important role in the regeneration of epithelial cells. In animal models, several growth factors have been shown to accelerate recovery from renal injury such as erythropoietin, insulin-like growth factor, hepatocyte growth factor, and epidermal growth factor [65]. In cellular and animal models, erythropoietin appears to reduce necrosis and apoptosis in renal epithelial cells while also promoting cell proliferation [66]; however, human studies have failed to yield AKI-related benefit from use of erythropoietin for primary AKI prevention [67]. Similarly, hepatocyte growth factor and insulin-like growth factor-1 appear to limit apoptosis in animal models of renal injury [68, 69], but again, preliminary clinical trials with IGF-1 have not shown benefit to patients. The KDIGO work group recommends against use of IGF-1 to prevent or treat AKI [7].

Adenosine Receptor Antagonists

Theophylline is a non-selective adenosine receptor antagonist that is thought to increase renal blood flow at the level of the afferent arteriole [70]. KDIGO recommendations suggest a single dose of theophylline in high-risk neonates with perinatal asphyxia at risk of AKI [7]. Research to date supports an initial renoprotective effect following theophylline in asphyxiated infants; however, long-term impact on renal function is unclear [71, 72].

Renal Replacement Therapies in AKI

The lack of evidence-based guidelines regarding the definition of pediatric AKI (see Chap. 46) has resulted in much uncertainty and discussion regarding the indications and timing for initiation of RRT as well as the optimal modality to employ. Retrospective reviews in critically ill children demonstrate that those who develop AKI early in a hospital course have greater morbidity and mortality [73, 74]. Use of RRT may prevent and correct life-threatening complications of AKI refractory to medical management. Indications for initiation of RRT in pediatric AKI have traditionally been those used for end-stage renal disease (and are not necessarily easily juxtaposed in the acute setting), including metabolic/electrolyte abnormalities refractory to medical therapy, symptomatic fluid overload, and/or symptomatic uremia; however, metabolic derangement and fluid overload are often late findings in severe renal injury [75]. In the acute setting, consideration of RRT must account for the patient's clinical situation in the context of the aforementioned laboratory abnormalities.

Adult literature suggests that earlier initiation of RRT, for example before the appearance of florid metabolic derangements and symptomatic fluid overload, may yield better patient outcomes, including decreased risk of death, shorter duration of RRT, and shorter hospital stays [76, 77]. For example, volume overload in the setting of AKI refractory to medical management is an indication for RRT even without significant azotemia or elevation in creatinine. Additionally, the presence of multisystem organ dysfunction in the presence of AKI refractory to medical management is a strong indication for initiation of RRT.

More recently, the concept of renal angina [78] has been suggested whereby likelihood of developing AKI is informed by baseline and contextual factors as well as objective evidence to identify those patients at greatest risk for renal injury. Additionally, renal angina criteria stratify patients into moderate-, high-, and very-high risk categories for AKI based on their underlying clinical condition (Table 52.2). Renal angina can be thought of mathematically as "signs of injury" (i.e., fluid overload, estimated creatinine clearance) multiplied by presence of AKI and is comparable to assessment of risk for a myocardial infarction in an adult patient presenting with chest pain. Given that there are currently no reliable biomarkers for establishing the severity or

Table 52.2 Pediatric renal angina criteria

Hazard tranche	Renal angina threshold
Moderate-risk patients	Doubling of SCr
Patients admitted to PICU	OR
	eCrCl decrease >50%
	OR
	ICU fluid overload >15%
High-risk patients	Serum Cr increase ≥0.3 mg/dL
Acute decompensated heart failure	OR
Stem-cell-transplant recipient	eCrCl decrease 25–50%
	OR
	ICU fluid overload >10%
Very-high-risk patients	Any serum Cr increase
Receiving mechanical ventilation and one or more vasoactive medications	OR
	eCrCl decrease >25%
	OR
	ICU fluid overload >5%

Used with permission of Springer Science + Business Media from Basu et al. [78]
PICU pediatric intensive care unit, *SCr* serum creatinine, *OR* odds ratio, *eCrCl* estimated creatinine clearance, *ICU* intensive care unit

prognosis of a patient's AKI, renal angina criteria may help the clinician to predict risk for AKI early in the clinical course and intervene, if needed, with RRT before reaching a state of fluid and metabolic derangement. In one study, the renal angina index obtained on Day 0 of pediatric ICU admission was predictive for progression to AKI on ICU Day 3 [79].

Greater than 20% fluid overload is a significant independent risk factor for increased morbidity and mortality [80]. Other less concrete indications for initiation of RRT include oliguria not responsive to diuretics, escalating ventilatory requirements (especially if pulmonary edema is secondary to fluid overload), need for a large volume of medications/blood products in a currently fluid overloaded patient (>10% overload), and/or when ability to provide adequate nutrition is compromised by fluid restriction secondary to fluid overload and oliguria.

Literature exists regarding when to stop RRT in the patient with AKI. In stopping RRT, the nephrologist may decrease the frequency of therapy from daily to every other day or change modality (e.g. conversion from CRRT to acute hemodialysis [HD]). The decision to stop should be based on evidence for improvement in the underlying disease pathology that led to RRT initiation and improvement in renal function (i.e., increased urine output, diminished azotemia, and decreased fluid overload).

Modality Choice

When initiating RRT for AKI, the clinician should first identify the goals of dialytic therapy. The patient's size, hemodynamic stability, and potential for vascular access also inform modality selection. Choice of therapy will also depend on clinician preference, available resources (e.g. dialysis equipment, nursing staff), and even capabilities for placement of dialysis access.

Acute Peritoneal Dialysis

Acute peritoneal dialysis (PD) provides gradual solute and water clearance through both convective and diffusion-based mechanisms. Although the use of continuous renal replacement therapy (CRRT) has dramatically increased in the past decade, there remains an important role for acute PD in preterm neonates with limited vascular access and patients admitted to the ICU following surgery for congenital heart defects [81, 82]. Acute PD is still the modality of choice in many countries, especially in the developing world [83–85] as it is a relatively inexpensive form of dialysis that does not require sophisticated technical expertise or equipment. It avoids the risks and complications associated with extracorporeal perfusion, including the possible need for blood-product exposure and systemic anticoagulation. Additionally, large volumes of fluid can be removed slowly over a prolonged period, maintaining hemodynamic stability. Due to the relatively slower solute clearance, including that of nitrogenous waste products, it is not associated with dialysis disequilibrium, which may occur in acute intermittent HD.

Initiation

An in-depth discussion regarding PD access is provided in Chap. 63. In brief, PD does not require vascular access, which can be a challenge in infants and small children, and provides a means for critically ill patients to be dialyzed with preservation of vascular access, thus allowing for rapid institution of therapy even in the less hemodynamically stable patient. Access can be obtained using semi-rigid stylet catheters requiring a trochar and canula method of insertion, the main advantage of which is the ease of bedside insertion by the pediatric nephrologist without surgical intervention and general anesthesia [86]. In patients who can tolerate a surgical procedure, placement of a tunneled permanent catheter is preferred to semi-rigid, temporary catheters to reduce technical complications such as leaks and catheter obstruction [87]. Newer acute placement techniques are performed with soft catheters, often where a Seldinger technique is utilized to insert the catheter over a guidewire (Fig. 52.2). This technique can be well-utilized in infants as it carries a minimal risk of dialysate leakage since no incision is required for the catheter insertion. Consequently, the risk of peritonitis is less, and

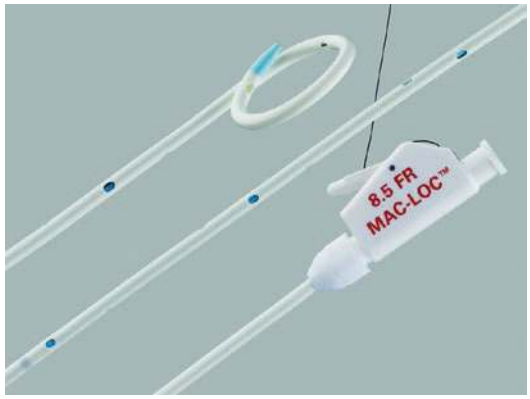

Fig. 52.2 Peritoneal dialysis catheter using Seldinger technique for insertion (8.5FR Mac-Loc™)

these catheters can be kept for up to 5 days without complications [87].

Prescription and Technique

The dialysis prescription for acute PD comprises four major components: the exchange volume, dialysate composition, individual cycle time, consisting of fill, dwell and drain, and total length of the dialysis session. For acute PD, the target exchange fill volume for adequate dialysis in terms of fluid and solute clearance, without the risk of leakage, is 30 mL/kg. However, smaller initial volumes of 10 mL/kg may be used for at least 24–48 h, if there is a risk of leakage from a wide incision site or if a tunneled cuffed catheter is used. Initially, short dwells (e.g. 30 min or hourly exchanges) for 48–72 h are required to remove accumulated solutes and excess fluid. Volume and metabolic control may best be achieved with exchanges performed around-the-clock. Subsequently, in maintenance dialysis, the dwell times can be extended, and total daily therapy time may be reduced, similar to chronic PD, with increasing volumes up to 40–45 mL/kg if a cuffed catheter has been used. PD should be continued until urine output improves, indicating recovering renal function.

Commercial dialysate solutions are available differing in osmolality, osmotic agent, and buffer. The osmotic agent is typically dextrose in a variety of pre-prepared concentrations. The hypertonic dextrose solutions utilized in PD provide a source of additional calories that may be beneficial in the critically ill child where IV access for nutrition and maintenance of glycemia may be limited; conversely, hyperglycemia may result from dextrose used in dialysate and insulin therapy may be required [88].

Complications

PD is contraindicated in patients with diaphragmatic defects, omphalocele, gastroschisis, and bladder exstrophy [89]. Complications with PD include catheter malfunction, peritonitis, and poor ultrafiltration. Neonates may have poor drainage due to catheter malposition or kinking, omental wrapping or a fibrin clot, which is exacerbated by the relatively small-bore peritoneal catheters required in the smallest patients. Inadequate drainage may also occur due to constipation. Bedside PD catheter placement with a semi-rigid trochar is associated with a risk of viscus perforation, especially in neonates, both at the time of insertion and with increasing dialysis duration. Severe abdominal pain and shock may occur, and the catheter must be removed for bowel repair and treatment of sepsis. The incidence of peritonitis is highest with the semi-rigid catheter, particularly if it has been kept in place for longer than 72 h [86]. In some cases, patients may develop a diaphragmatic pleuroperitoneal communication following cardiothoracic procedures, which results in a large pleural effusion once PD is initiated. Hypothermia is a complication of PD, particularly in neonates and small children, if dialysate is not warmed prior to infusion into the peritoneal cavity.

Poor ultrafiltration may occur, especially in critically ill infants due to the low fill volume with inadequate fluid reservoir intraperitoneally. Often it is difficult to increase the dwell to the desired volume in the setting of infant acute respiratory distress and low lung volumes as the increasing peritoneal fluid fill volumes results in splinting of the diaphragm. In some cases, during the inflow phase, critically ill infants may desaturate, and require transient compensation in ventilatory pressures. As a result, there is poor ultrafiltration, which increases the fluid retention, thus worsening the respiratory distress. These ill patients are often hypotensive requiring multi-agent inotropic and pressor support. The resultant

decrease in bowel perfusion due to vasoconstriction of the mesenteric vessels secondary to pressor support may also contribute to poor ultrafiltration. Additionally, in neonates there is a decrease in the osmotic gradient, because of increased absorption of dextrose from the dialysate, resulting in poor ultrafiltration. With the use of higher dialysate dextrose concentrations to facilitate ultrafiltration, hyperglycemia may occur and necessitate insulin administration.

Electrolyte abnormalities may occur with PD. Hypokalemia may occur, and if noted, potassium should preferably be added to the IV fluid if the patient is not feeding, rather than adding to the dialysate, to avoid frequent bag changes due to changing orders. Potassium can be added to the dialysate if the hypokalemia is severe enough such that the maximum safe concentration of potassium infusion will be exceeded.

Acute Intermittent Hemodialysis

In many countries, acute intermittent HD is the mainstay of dialysis for AKI, particularly in older children. Its main advantage is rapid ultrafiltration and solute removal. It is therefore indicated in AKI that requires rapid fluid removal (acute fluid overload) or rapid solute removal such as hyperkalemia, tumor lysis syndrome, toxic poisonings and other profound metabolic abnormalities. Acute intermittent HD is ideal for hemodynamically stable patients who can tolerate rapid fluid shifts. It is a versatile modality as it allows for ultrafiltration without solute removal, as well as adjustment of the dialysate bath to treat electrolyte abnormalities such as hypernatremia. Moreover, because of the intermittent nature of the dialysis, patients can be mobilized for other procedures. Systematic reviews have suggested that in hemodynamically stable adult patients the continuous forms of RRT do not appear to have a survival advantage over intermittent HD [90–92].

Initiation

When initiating acute HD, one must consider vascular access, HD prescription, and type of dialyzer membrane. Other factors include the patient's ability to tolerate rapid fluid shifts, the need for vasoactive substances to maintain blood pressure, and total fluid removal goals.

Vascular access for HD is discussed in detail in Chap. 65. As with all extracorporeal therapies, treatment success is dependent on the quality of the vascular access. Adequate blood flow (Qb) is essential to providing optimal therapy with minimal interruption. In pediatric patients, the choice of vascular access, catheter size, and insertion site is critical. Short, large bore catheters provide improved performance due to lower resistance to flow [93]; conversely, longer, smaller-bore catheters (e.g. Broviac catheters) are unsuitable due to their high flow resistance.

The HD prescription in AKI must be individualized to provide adequate solute clearance and fluid removal. The prescription for acute intermittent HD is comprised of the dialysis dose delivered per session and the frequency of the sessions. Additional factors affecting the individual HD prescription include the extracorporeal circuit volume, the dialyzer size, blood flow rate, dialysate flow rate, ultrafiltration required, dialysate composition, anticoagulation, and length of session. Blood flow rate is determined by vascular access and determines solute clearance, with higher blood flow increasing solute clearance by optimizing diffusion and convection. Dialysate flow rate is also a determinant of solute clearance [94]. Dialysate flow rate should be at least 1.5 times greater than the blood flow rate to maximize diffusion gradients of solutes. In pediatric patients, HD equipment and prescription require modifications for smaller children (i.e., infants less than 3 kg).

When initiating acute HD for AKI, dialysis dose delivered may change frequently with a need for greater renal support during initial therapy for AKI, as compared to the relatively stable initial dialysis doses provided in initiating chronic HD for end-stage renal disease [95]. Similarly, in patients receiving chronic HD, urea kinetic modeling (Kt/V) is utilized as a measure of dialysis adequacy; however, due to the rapidity of fluid shifts with therapy and frequently changing renal function, Kt/V may not be as reliable in the patient with AKI receiving HD [96]. A review of dialysis dosage in adults concluded that in AKI

a Kt/Vurea greater than 1.2 from thrice weekly intermittent HD is associated with improved survival in patients with intermediate severity of illness but does not influence outcomes in more severely ill patients [97]. Increased acute HD treatment frequency may be required despite reaching "adequate" Kt/V to achieve daily fluid removal goals. Although Kt/V recommendations exist, dialysis dosage must be individualized and higher doses of therapy for various metabolic derangements may be required in AKI [97].

The total volume of the extracorporeal circuit includes the volume of the tubing and the dialyzer and should not exceed more than 10% of the patient's blood volume, calculated as 75 mL/kg for older children and 80 mL/kg for infants. If the extracorporeal blood volume exceeds 10–15% of the patient's total blood volume, or the patient has a low hematocrit and/or hemodynamic instability, a blood prime is recommended [98]. When initiating a blood prime, using buffered packed red blood cells (PRBC) or transfusing the PRBC post-membrane in conjunction with a saline prime have been shown to reduce risk of "bradykinin release syndrome" (BRS) [99]. BRS, characterized by a precipitous decline in blood pressure 5–10 min after initiating both acute HD and continuous RRT, has been associated with the use of the AN-69 polyacrylonitrile membrane [99, 100]. Exposure of the primed blood to the negatively charged AN-69 membrane co-activates pre-kallikrein and Hageman factor, resulting in the release of bradykinin, a potent vasodilator. The reaction is potentiated by exposure to blood with an acid pH, which is typical of banked blood used for blood priming the circuit. Thus, the use of a blood prime with an AN-69 membrane can result in profound hypotension. PRBCs to be used in a blood prime should be diluted with normal saline to produce a hematocrit of approximately 35–40%. Buffering the banked blood for priming to physiologic pH prior to priming the circuit or infusing the blood post-filter at the same rate as a saline prime have been shown to be effective in minimizing the BRS, as has avoidance of the AN69 membrane [101, 102].

Dialyzer selection considers the type of membrane desired, the need for a blood prime, the dialyzer membrane surface area, and the ultrafiltration coefficient [103]. The membrane properties of the dialyzer, such as membrane thickness, pore size, and pore density affect dialysis efficiency, with varying clearances for small and middle molecular weight solutes. Dialyzer membrane biocompatibility should be considered when initiating acute HD. See Chap. 66 for additional information on this topic. The use of biocompatible synthetic membranes does not appear to confer any significant clinical advantage either in terms of mortality or AKI recovery when compared to substituted cellulosic membranes [104, 105]. High-flux membranes have larger pores resulting in greater clearances of higher molecular weight solutes but have the risk of back transport from the dialysate of water-borne solute contaminants. In a systematic review comparing the use of high-flux and low-flux membranes in AKI in adults, there was no difference in the risk of mortality or dialysis dependence in survivors [105]. However, in another meta-analysis, there appeared to be a significantly improved renal function recovery with the use of high-flux membranes [104]. High-cut-off-point membranes made from polyamide/polyarylethersulfone, polysulfone, or cellulose triacetate, have greater cytokine clearance and enhanced adsorption properties than conventional high-flux dialyzers [106], and have been developed for use in septic patients with AKI [107, 108]. Treatment using high-cut-off-point membranes has been shown in animal models of sepsis to have beneficial effects on immune cell function and survival [109]. Preliminary clinical studies show that use of these membranes in adult patients with AKI was associated with decreased need for vasopressor therapy, with no reports of serious adverse effects [108].

The smaller blood volumes in infants and young children place them at risk for blood loss due to clotting of the dialyzer; thus, anticoagulation is required with acute intermittent HD for pediatric AKI. Heparin is the most commonly used anticoagulant for intermittent HD. A loading dose of heparin may be given at the start of dialysis followed by intermittent bolus heparin doses. To monitor therapy, the activated partial

thromboplastin time (aPTT) or activated clotting time (ACT) may be used. The aPTT should be kept at 1.2–1.5 times the baseline, and the ACT between 120 and 180 s. When heparin is utilized, platelet count should be monitored frequently to assess for development of heparin-induced thrombocytopenia [110]. In coagulopathic patients, heparin-free dialysis can be performed by intermittently flushing the circuit with 0.9% saline; unfortunately, this method increases the ultrafiltration target and decreases dialysis efficiency [111–113]. When using saline anticoagulation, the filter pressure should be monitored, and dialyzer inspected for early clot formation. Regional citrate anticoagulation is also an alternative to systemic heparin anticoagulation in the coagulopathic patient [114] requiring HD for AKI.

Complications

HD in young children can be challenging due to the smaller patient blood volume. This problem is accentuated in the critically ill child, where pressor infusion may be required to support the systemic blood pressure. Moreover, these children may have acute respiratory distress syndrome with hypoxemia or other associated clinical problems such as congestive heart failure or cerebral edema. Therefore, maintenance of an adequate blood pressure in these children is critical to alleviate tissue hypoxia.

Rapid HD using dialyzers with larger surface areas in patients with very high plasma blood urea nitrogen (BUN) concentrations may result in the dialysis disequilibrium syndrome, characterized by neurological symptoms such as fatigue, headache, nausea, vomiting, altered consciousness, convulsions, and coma [115]. Patients with AKI may be at increased risk due to catabolism (high BUN) and pre-existing neurological compromise related to the acute illness. Measures to prevent the disequilibrium syndrome include decreasing the initial dialysis dose, increasing dialysate sodium concentration (143–146 mmol/L), and administration of osmotically active substances such as IV mannitol (0.5–1 g/kg) to prevent rapid osmolar shifts that can cause cerebral edema [116].

Hypotension is one of the most common complications with acute intermittent HD and occurs in part due to rapid fluid and solute removal. Technical advances in the delivery of HD have dramatically reduced the propensity for intradialytic hypotension. The use of volume-controlled dialysis machines and biocompatible synthetic dialysis membranes helped decrease the incidence of intradialytic hypotension. In adult studies, it has been demonstrated that priming the circuit with isotonic saline, discontinuing vasodilator therapy, keeping the dialysate sodium greater than 145 mmol/L and setting the dialysate temperature to below 37 °C result in lesser hemodynamic instability and better outcomes [117]. See Table 52.3 for a summary of recommendations to minimize hemodynamic instability with acute HD. Additionally, use of in-line noninvasive blood volume monitoring to minimize abrupt changes in extracellular volume is useful in young children with hemodynamic instability where large acute changes in extracellular volume are not well tolerated [118]. This method of performing intradialytic noninvasive blood volume monitoring indicates intravascular blood volume change during the dialysis session (Fig. 52.3).

Catheter site complications are possible in acute HD and include infection at the catheter exit site, catheter malfunction, and risk of

Table 52.3 Techniques to improve hemodynamic tolerance of intermittent hemodialysis

All patients:
Use only synthetic or modified cellulose membrane
Connect both lines of the circuit filled with 0.9% saline simultaneously to the central venous catheter
Set dialysate sodium concentration ≥145 mmol/L
Limit maximal blood flow to 150 mL/min with a minimal 4 h session
Set dialysate temperature at ≤37 °C
Advice for hemodynamically unstable patients:
Cool dialysate to 35 °C
Start session with dialysis and continue with ultrafiltration alone
Additional recommendations:
Stop vasodilator therapy

Used with permission of the American Thoracic Society from Schortgen et al. [117]

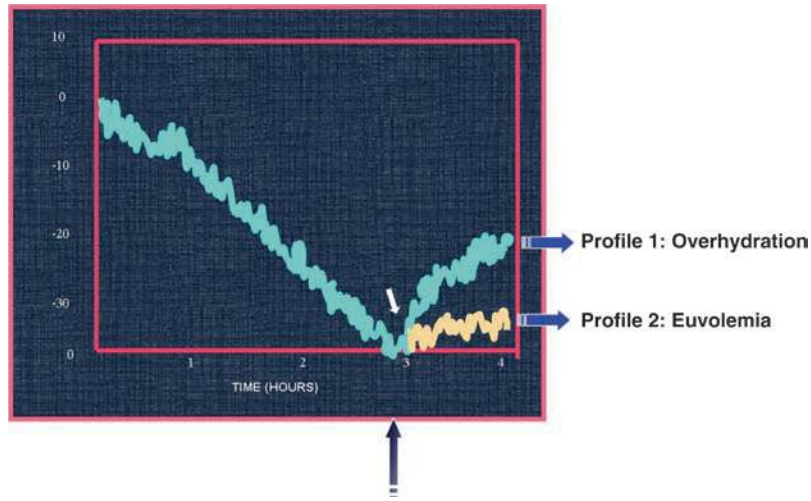

Fig. 52.3 Intradialytic noninvasive blood volume monitoring: Profile 1: overhydration. Profile 2: euvolemia

hematologic disturbance (e.g. bleeding, clot). In the event of signs of infection such as fever, line blood cultures should be obtained, and empiric antibiotics started. There is also risk for clotting of the extracorporeal system in acute HD; this can place patients at risk for acute blood loss if the blood is unable to be returned to the patient.

Continuous Renal Replacement Therapies

CRRT is now widely available in pediatric centers throughout the world, and in some has become the preferred method of RRT. CRRT in AKI offers several advantages over traditional dialysis methods when used in critically ill, unstable patients. Because CRRT is continuous, removal of solutes and modification of the volume and composition of the extracellular fluid occur gradually. Unstable patients, who are often intolerant of the abrupt fluid volume and solute concentration changes that accompany standard HD treatments, can be successfully treated with CRRT. The precision and stability with which fluid and electrolyte balance can be maintained using CRRT is unmatched by any currently available dialysis therapies, except perhaps the extended HD techniques mentioned in the following section.

The basic principles of CRRT are similar for adults and children. However, the application of these modalities in children requires attention to several important details unique to therapy in pediatric patients. For example, extracorporeal blood volume may be large compared to the patient blood volume, necessitating blood circuit priming in the very small child as described in the section on acute HD (see "acute intermittent hemodialysis," above). The most demanding considerations arising in pediatric CRRT are related to the need to adapt equipment and prescriptions designed for adult-size patients in order to meet the special needs of the smallest of pediatric patients requiring renal support.

Initiation

The indications for initiating CRRT in children and adults are similar and most often involve the treatment of AKI and fluid overload in a critically ill patient [20, 119]. CRRT may be combined with extracorporeal membrane oxygenation (ECMO) and plasmapheresis circuits.

CRRT refers to a variety of modalities that use one or both solute clearance mechanisms. In continuous venovenous hemofiltration (CVVH), blood flows through the hemofilter, generating large volumes of ultrafiltrate, which is replaced by a physiologic "replacement fluid," either before (pre-dilution) or after (post-dilution) the hemofilter (Fig. 52.4a). Clearance is thus exclu-

sively convective. If a dialysate is infused into the hemofilter, clearance is primarily diffusive, as in HD. Hence, this CRRT modality is called continuous venovenous HD (CVVHD, Fig. 52.4b). When both replacement fluid and dialysate are used, permitting convective and diffusive clearance, the therapy is known as continuous venovenous hemodiafiltration (CVVHDF, Fig. 52.4c).

Initiation of CRRT also requires adequate vascular access, as discussed previously and in Chap. 65.

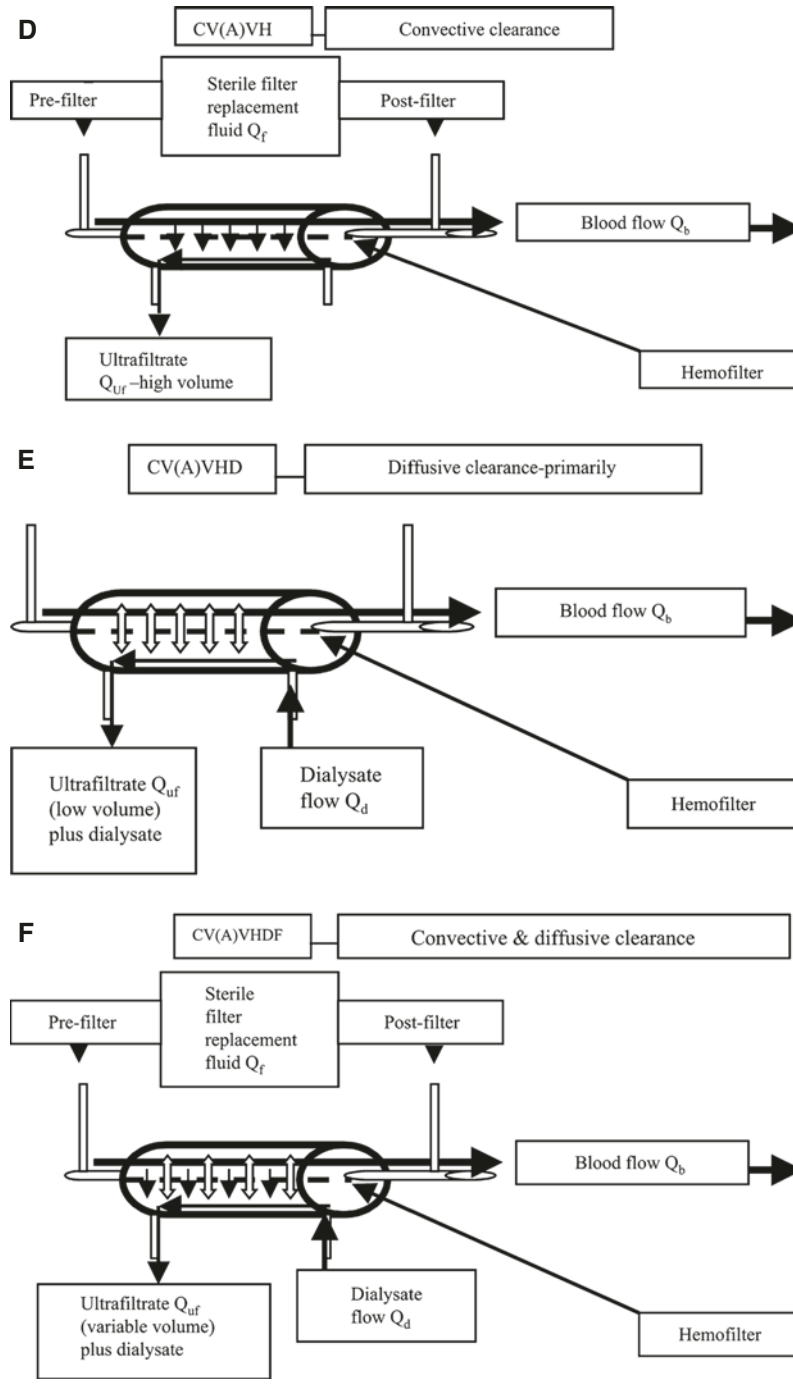

Fig. 52.4 (**a**) Diagram of a convective based (CVVH) continuous renal replacement therapy (RRT). Note use of either pre- or post-filter replacement fluid rather than dialysate. (**b**) Diagram of diffusion based (CVVHD) continuous RRT. Note use of dialysate rather than replacement fluid. Dialysate flow is countercurrent to blood flow. (**c**) Diagram of combined convective and diffusive based (CVVHDF) continuous RRT. Note use of both dialysate and replacement fluids

Blood Flow Rates

With a well-functioning vascular access, it is possible to adjust Qb based on to the size of the child and the clinical setting. Higher Qb may support longer filter life by reducing the likelihood of filter fiber clotting. Higher Qb also facilitates increased patient fluid removal by providing greater filter plasma flow rates and reduces the loss of clearance efficiency from pre-dilution mode CVVH or CVVHDF. However, not all patients will tolerate a higher Qb, especially at initiation of CRRT. Hence, we suggest initiating CRRT with lower Qb and advance to the targeted rate over the first 30 min of therapy as tolerated. In contrast to pediatric HD where initial Qb can be readily extrapolated from patient body weight, there are no true "body-weight" recommendations for Qb in any form of pediatric CRRT. The Qb chosen should provide adequate clearance for the size of the patient, with consideration of access limitations and device requirements. Recommendations for Qb range from 4 to 10 mL/kg/min; consequently, Qb may vary widely in CRRT. Depending on the patency of the access, the Qb may need to be higher to maintain flow. For example, 10–12 mL/kg/min may be necessary to accommodate technical requirements (access) and clearance in extremely low birth-weight neonates while 2–4 mL/kg/min may be appropriate in larger adolescents.

Solutions

The tolerability of CRRT has been greatly improved with the introduction of bicarbonate-based CRRT solutions. In the past, with lactate as the buffer, worsening lactic acidosis was common, leading to hypotension and depression of cardiac function [120]. A series of comparative clinical trials of lactate- and bicarbonate-based CRRT fluids in adults [121, 122] and children [123] have demonstrated the superiority of bicarbonate as a buffer; consequently, bicarbonate-based CRRT solutions are now the standard of care, although trace amounts of lactate may be used in solutions to maintain stability.

CRRT solutions also contain sodium, potassium, chloride, glucose, calcium, phosphate, and magnesium. Bicarbonate-based CRRT solutions are available from several manufacturers in a wide array of electrolyte formulations. Most hospital pharmacies stock only a single brand and in only a few formulations. A feature of CRRT, especially in small patients, is the tendency over time for the composition of the CRRT fluids to determine the electrolyte composition of the patient. A fluid low in potassium, phosphorous and magnesium may be appropriate at initiation of CRRT when concentrations of these electrolytes in AKI patients are often elevated. However, depending on the CRRT prescription, within a short time the patient may become deficient in these electrolytes, which can complicate management. Thus, while a "starter" fluid with reduced potassium, phosphorous and magnesium is needed, a fluid that includes these electrolytes in physiologic concentrations should follow. Rather than stocking multiple formulations, some pharmacies may prefer to add potassium, phosphorous, magnesium and even additional bicarbonate to the "starter" solutions as needed, a practice that may add the risk of pharmacy errors and increase costs. Calcium is always left out of solutions when phosphate is present to avoid precipitation. Calcium has usually, but not always, been left out of CRRT solutions used with citrate anticoagulation, as will be discussed below.

Prescription

The optimal "dose" of RRT is not known. Adult AKI studies by Ronco and colleagues using CVVH established a total convective clearance (replacement fluid plus patient fluid removal) target of 35 mL/kg/h as a threshold below which survival was significantly worse [124]. In a subset of these patients with sepsis, there was a trend in favor of improved survival with total convective clearance ≥45 mL/kg/h. Despite theoretical considerations that seemed to favor high clearance targets in cytokine-driven illnesses like sepsis [125] and preliminary results in septic adults treated with very high flow CRRT [126], available evidence does not support the use of clearance targets above 20–35 mL/kg/h. For pediatric patients, this translates to 2–3 L/1.73 m^2/h, rates that are reasonably easy to achieve.

Anticoagulation

Effective CRRT requires optimal anticoagulation. Activation of the clotting cascade occurs in CRRT circuits due to contact of the circulating blood with artificial surfaces. Low blood flow rates, turbulent flow, small catheters and high hematocrits hasten clotting. Anticoagulation regimens using mixed molecular weight heparin or sodium citrate are the most commonly used in pediatric CRRT, and either can be effective. An early comparison in pediatric centers showed equal filter life span with heparin and citrate, but more hemorrhagic events in the heparin group [127].

Heparin has been the mainstay of HD anticoagulation for decades. Many pediatric CRRT programs continue to rely on heparin. Heparin is infused in the CRRT circuit pre-filter and titrated to achieve a targeted post-filter activated aPTT 1.5–2 times normal, or an ACT between 180 and 220 s. This is usually accomplished by giving an initial heparin bolus of 20–30 units/kg, followed by a continuous infusion of 10–20 units/kg/h. Alternatively, the circuit may be rinsed and primed with 1–2 L of normal saline to which has been added 2500–5000 units/L of heparin, followed by the pre-filter heparin infusion.

Sodium citrate anticoagulation is widely used in pediatric CRRT programs due to its ease of administration and decreased bleeding risk [128]. By infusing citrate into the arterial limb of the CRRT tubing as it leaves the catheter, calcium ions are bound to the citrate, reducing available calcium and thereby inhibiting coagulation within the circuit, since normal coagulation is calcium-dependent. Systemic hypocalcemia is prevented by infusion of either calcium gluconate or calcium chloride into the patient at a central site. Thus, citrate anticoagulation achieves regional anticoagulation by affecting only the circuit, thereby eliminating the increased risk of bleeding with heparin. Since the original citrate protocol employing 4% trisodium citrate (440 mEq/L sodium), newer modifications utilize anticoagulant citrate dextrose "A" (ACD-A), a less-concentrated formulation, which is also commonly used as the anticoagulant in apheresis procedures.

Adverse effects of citrate anticoagulation include acid-base disturbances, citrate excess and hyperglycemia in infants when ACD-A is used. Patients receiving citrate anticoagulation may develop metabolic alkalosis; fortunately, citrate is readily cleared by dialysis [129]. Citrate excess may be diagnosed by monitoring the ratio of the total calcium to the ionized calcium levels [130]. If hepatic metabolism of citrate is insufficient, citrate accumulates; thus, patients with diminished liver function are at increased risk for citrate excess. Citrate excess can occur when citrate clearance is less than citrate delivery. Citrate is not inherently toxic, but citrate excess causes systemic hypocalcemia. Total calcium levels rise and the ratio of total calcium to systemic ionized calcium levels rises precipitously. As citrate accumulation progresses, it becomes more difficult to maintain the declining systemic ionized calcium levels within normal ranges. Monitoring of ionized calcium is the most sensitive way to detect citrate accumulation [131]. Treatment often requires increasing the removal of citrate by increasing clearance within the circuit (i.e., increased dialysate and/or replacement fluid rate); this assures ongoing anticoagulation while balancing the build-up of citrate in the patient. An initial citrate infusion rate of 50–70% of the usual rate is also recommended in patients with hepatic insufficiency who are at increased risk for citrate toxicity. A pediatric citrate anticoagulation protocol using bicarbonate dialysate has been published [132].

It is also possible in certain situations to use no anticoagulation, relying on periodic saline flushes of the circuit. This approach is typically considered in larger patients with evidence of a sustained coagulopathy due to disseminated intravascular coagulopathy or hepatic failure. However, many of these patients are receiving periodic fresh frozen plasma and platelet infusions to correct the underlying coagulopathy; these infusions will clot a CRRT system when no anticoagulation is used. Moreover, patients with hepatic failure may have a paradoxical hypercoagulable state. An uncontrolled study demonstrated that the no coagulation/saline flushes approach was associated with an inferior circuit life span compared to heparin or citrate anticoagulation [127].

CRRT Use in Combination Therapies

Extra-corporeal Membrane Oxygenation

The widespread use of ECMO in neonatal and pediatric critical care units along with the common occurrence of AKI in these patients with multi-organ dysfunction has led to the need to incorporate CRRT into the ECMO circuit. Fluid overload at CRRT initiation has been shown to be a consistent factor associated negatively with survival in ECMO and preventing the development of significant fluid overload at the outset of ECMO may be more clinically effective than attempting fluid removal later in therapy [133].

The ECMO circuit is fully heparinized, eliminating the need for anticoagulation of the CRRT circuit. Blood flow in the ECMO circuit is often 20–30 times that required for optimal CRRT. Newer ECMO circuits with multiple access phalanges allow the insertion of the CRRT circuit in an entirely pre-oxygenator location, avoiding shunt of oxygenated blood from the patient when the CRRT circuit is placed in a post- to pre-oxygenator position. Close collaboration between CRRT and ECMO teams is required to find the best location for the CRRT circuit and to coordinate therapy goals [134].

Plasma Therapy

Patients with AKI secondary to immune complex–mediated disease and sepsis-associated thrombotic microangiopathy may require both CRRT and plasma therapies (i.e., plasmapheresis, plasma exchange) [135]. CRRT is readily combined concurrently with plasma therapy procedures without interrupting the CRRT circuit. The placement of a three-way stopcock at both arterial and venous limbs of the CRRT circuit at the connection to the double lumen catheter allows diversion of blood through the centrifugation plasmapheresis machine [136].

Plasma exchange removes inflammatory mediators and replaces the volume with fresh frozen plasma in attempt to correct underlying homeostatic abnormalities; conversely, plasmapheresis removes plasma with inflammatory mediators, but replaces the volume with a non-plasma solution (usually albumin) [137]. Additionally, CRRT is believed to have an immunomodulatory effect on inflammatory cytokines in sepsis; however, the impact on patient outcome in sepsis remains unclear and the primary role remains management of fluid overload [138]. CRRT may downregulate the inflammatory response through nonselective extracorporeal removal, mainly by absorption, of cytokines and other mediators, restoring hemodynamic and immunologic homeostasis [139]. One retrospective study demonstrated benefit of isovolemic hemofiltration followed by conventional continuous venovenous hemofiltration in patients with septic shock and oliguric AKI with subsequent improvement in oxygenation and mean arterial pressure as well as significant improvement in survival at 28 days versus those receiving conventional supportive therapy [140].

Complications

CRRT requires the patient to remain relatively immobilized while connected to the CRRT circuit for prolonged periods. As a result, small children typically require sedation and occasionally even pharmacological paralysis to prevent small movements that may disrupt flow in the CRRT circuit. Additionally, a relatively large fraction of total circulating blood volume is in the extracorporeal circuit, placing the child at substantial risk for hypothermia during CRRT. Careful temperature monitoring is required during RRT, particularly when combination therapies are utilized. In-line fluid warmers can be used but increase priming volume. Line warmers that can be applied to the return line offer the best results.

Outcome

The Prospective Pediatric CRRT (ppCRRT) Registry reports an overall pediatric CRRT survival rate of 58% [80]. Survival of pediatric patients treated with CRRT has been reported in single center studies to vary widely by disease and modality [20, 141, 142]. A single center study initially demonstrated that the degree of fluid overload was an independent determinant of outcome in pediatric patients treated with CRRT [20], and that was confirmed by a large multicenter study from the ppCRRT Registry [80].

Patient survival was inversely correlated with percentage fluid overload at initiation of CRRT: survivors had a mean fluid overload of 14.2% while in non-survivors had a mean fluid overload of 25.4%, a difference that was highly significant and independent of diagnosis or severity of illness [20]. Further analysis of the ppCRRT Registry data demonstrated that 20% fluid overload was associated with four times the mortality of pediatric patients receiving CRRT when compared to patients with less than 10% fluid overload at initiation of CRRT [80]. These data suggest that earlier initiation of measures to control fluid accumulation, including CRRT, may improve survival.

Extended Hemodialysis Techniques

Extended hemodialysis techniques, also known as hybrid therapies, utilize intermittent hemodialysis machine technology while providing the slower solute and fluid removal associated with continuous RRT for use in less stable patients with AKI [143, 144]. The terms for these modalities include sustained low-efficiency daily dialysis (SLEDD) or extended daily dialysis (EDD) or slow continuous dialysis (SCD) [145].

SLEDD is a dialytic modality that allows for flexible options in treatment duration, prolonged or even continuous treatments, using conventional dialysis machines with varying pump speeds for 6–18 h daily [146]. Variants such as sustained low-efficiency daily diafiltration (SLEDD-f), aimed at improving clearance of middle molecular inflammatory mediators of the systemic inflammatory response associated with sepsis, have been developed for clinical use [92, 147]. Advantages of SLEDD-f over CVVHDF include faster clearance of small solutes and fluid removal yet maintaining hemodynamic stability [148]. It allows flexible therapeutic schedules so that patients are accessible and can be mobilized for other medical treatments.

Hybrid therapies also have lower heparin requirement than CRRT, but less frequent clotting. The reported incidence of clotting is 17–26% with heparin, while the reported incidence of circuit clotting without anticoagulation is 24–26% using single pass machines and lower using batch systems [149]. Hybrid therapies, with the high diffusive capacity for solutes, are able to correct alkalosis or hypernatremia, while at the same time removing the calcium chelated citrate complexes in citrate anticoagulation, an advantage in patients with liver failure [150]. With hybrid therapies, phosphate removal can be very extensive. Hypophosphatemia and metabolic alkalosis is easily induced in a critically ill patient, especially those on prolonged parenteral nutrition; therefore, preemptive fluid correction with phosphorous and reduction in dialysate bicarbonate are often warranted.

Conclusion

AKI is common in hospitalized children and associated with high risk of mortality and long-term morbidity. Recent advances in the understanding of the pathophysiology of AKI have pointed to newer diagnostic and therapeutic strategies that focus on early recognition and treatment. Exciting developments in technology have made RRT more accessible and more easily applied in the pediatric setting. Yet, despite these advances, mortality rates among children with AKI remain disturbingly high. Hopefully, future developments will improve outcomes for children with AKI.

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Neonatal Kidney Dysfunction

53

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Kidney Function in Neonates

At birth, the kidney replaces the placenta as the major homeostatic organ, maintaining fluid and electrolyte balance and removing harmful waste products. In a healthy term neonate, dynamic changes to renal blood flow occur which lead to alterations in glomerular filtration rate (GFR) over the first few months of life. Tubular development is intact such that conservation or elimination of electrolytes and water are efficient and adequate. Alternatively, the glomerular, tubular and vascular regulation of the kidney in premature infant are abnormal compared to the healthy term counterpart. Describing the "normal" renal physiology in preterm neonates is difficult (as one can argue they are all abnormal); understanding how a term neonate maintains renal blood flow, glomerular filtration and tubular function is critical to extrapolation of how the premature infant's underdeveloped/immature kidneys function. As we describe neonatal homeostasis, we will contrast the physiology of the "normal" healthy term infant to those of premature infants, understanding that the degree of immaturity and the neonatal course will affect the ability of premature infants "normal" homeostasis.

Healthy term infants are ready to maintain homeostasis of water, electrolytes, and acid/base. In addition, their kidneys function to metabolized drugs/toxins, and eliminate waste products. As the clinician has an integral role in prescribing fluids, electrolytes, and nutrition, proper homeostasis in sick term/near term newborns and premature infants depends on the clinicians' ability to appropriately prescribe fluids, nutrition and electrolytes. Infants who lack ability to remove uremic toxins and maintain appropriate electrolyte/fluid balance with medical management rely on nephrology teams to support the neonate with dialytic therapies designed to maintain homeostasis.

Renal Blood Flow in Newborns

Starting with delivery and umbilical cord clamping, major hemodynamic changes occur in renal blood flow which drive changes in neonatal glomerular filtration rate. The proportion of cardiac output distributed to the kidney changes abruptly. Fetal kidneys receive approximately 2.5–5% of cardiac output at birth [1], which increases to 6%

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by 24 h of life [2, 3], steadily escalates to 10% at 1 week of life, and attains 15–18% at 6 weeks of life [4] as it approaches the fractional cardiac output to the kidneys observed in adults (approximately 20–30%). These dynamic changes in renal blood flow are driven by both increased systemic blood pressure and a substantial decrease in renal vascular resistance. The abrupt and significant drop in renal vascular resistance is due to a redistribution of renal blood flow within the kidney, changes in the number of vascular channels and changes in glomerular arterial resistance [5]. Initially, renal blood flow primarily extends to the outer cortex which gradually distributes to medullary sections over the first few months of life [6].

The auto-regulatory mechanisms which control renal blood flow are driven by both the myogenic reflex of vascular smooth muscles and the tubule-glomerular feedback system. These reflexes aim to maintain constant blood flow by sensing vascular endothelial stretch and distal tubular fluid flow respectively. Nitric oxide, angiotensin II, adenosine, bradykinins, and endothelin play a central role in maintaining adequate blood flow.

The renin angiotensin system is active throughout fetal development and contributes to normal fetal maturation. Congenital abnormalities (i.e., defects to angiotensin II receptors) and secondary abnormalities (i.e., maternal use of angiotensin converting enzyme inhibitor) can significantly affect kidney development. Prostaglandins (potent vasodilators) also serve to increase renal blood flow by active vasodilation of afferent arterioles. These agents are increased in times of stress and help counteract vaso-constrictive effects of angiotensin II and catecholamines. Prostaglandins and inhibitors of prostaglandins are commonly prescribed in neonatal medicine to maintain patency, or electively close patent ductus arteriosus; respectively. Such dynamic changes in renal blood flow, alterations in hemodynamics, and medications to promote vasoconstriction/ vasodilation will greatly affect glomerular filtration rate in neonates.

Glomerulogenesis

Kidney development commences during the 5th week of gestation with partially functional temporary organs (the pronephros and metanephros). The first nephrons are formed by about the 8th week of gestation and increase over time. Four glomerular generations are present after 14 weeks and 12–13 generations of glomeruli after 36 weeks of gestation [7]. The juxtamedullary nephrons develop initially, with superficial ones following. Nephrogenesis continues until 36 weeks of gestation at which time the number of nephrons, 1.6–2.4 million, approximates that of an adult [8]. Autopsy studies suggest that the extra-uterine environment is not amenable to neo-glomerulogenesis, leading to a low nephron number in premature infants [9]. Thus, the premature infant may be programmed for low nephron endowment, and subsequent chronic kidney disease.

In individuals with a lower number of nephrons, single-nephron hyperfiltration can increase total GFR to a similar level as attained by those with normal numbers of glomeruli. However, the impact of low nephron endowment may become problematic over time as single nephron hyperfiltration may cause glomerulosclerosis and ultimately progressive loss of kidney function, especially in the context of other risk factors for CKD such as acute kidney injury, hypertension, diabetes, and other primary kidney diseases.

Glomerular Function Rate (GFR)

GFR is the most useful measurement of kidney function. GFR is measured indirectly through the concept of clearance (the equivalent volume of plasma from which a substance would have to be totally removed to account for its rate of excretion in urine per unit time). Clearance is calculated by dividing the excretion rate of a substance by its plasma concentration ($C_x = U_x \times V$); where U_x and P_x are urine and plasma concentrations of substance x and V is urine flow rate. C_x is expressed as milliliters per minute and is usually normalized to 1.73 m^2, the idealized adult body surface area [10]. GFR is the best clinical test to estimate functional renal mass, which can assist the clinician in prescribing fluids/electrolytes, determine disease progression, and appropriately prescribe medications excreted by the kidney.

The gold standard method for GFR measurement is inulin clearance. Tables 53.1 and 53.2 summarize studies conducted to estimate GFR (measured by inulin clearance) in healthy term and preterm infants, respectively. Dependent on degree of prematurity, GFR steadily improves from 10–20 mL/min/1.73 m^2 during the first week of life to 30–40 mL/min/1.73 m^2 by 2 weeks after birth concomitant with alterations in renal blood flow. Thereafter GFR improves steadily over the first few months of life [11, 12]. Serum creatinine (SCr) is the most common method to estimate GFR and monitor kidney function but has significant shortcomings (see Chap. 3).

Table 53.1 Formal GFR measurements for term neonates during the first 2 years of life

Term infants	
Age	mL/min/1.3 m^2
1–3 days	21 ± 5
4–14 days	37 ± 7
1–3 months	85 + 35
4–6 months	87 ± 22
7–12 months	96 + 2
1–2 years	105 ± 17

Data from Schwartz and Furth [11]

Table 53.2 Formal GFR measurements for preterm neonates during the first 4 months of life

Premature infants	
Age	mL/min/1.73 m^2
1–3 days	14.0 ± 5.0
1–7 days	18.7 ± 5.5
4–8 days	44.3 ± 9.3
3–13 days	47.8 ± 10.7
1.5–4 months	68.4 ± 16.6

Data from Schwartz and Furth [11]

There are several specific problems with using SCr in neonates: SCr in the first few days of life reflects mother's and not the infant's kidney function. Moreover, GFR in term and preterm infants is generally very low and normal serum creatinine values vary greatly dependent on level of prematurity and age [13] (Fig. 53.1). Finally, bilirubin levels in premature infants rise in the first several days and return to normal after a few weeks. When the colorimetric Jaffe method of SCr is used this may impact SCr interpretation [14].

Attempts to estimate GFR using SCr in neonates have suggested the following formula for children who are <1 year of age [15]: Height (cm) * *k*/serum creatinine (mg/dL); where $k = 0.33$ for low birth weight and 0.45 for normal birth weight infants. However, caution should be used when applying this equation in clinical practice for several reasons. At best the formula represents a mean estimate and the true GFR may be off in either direction by 20% or more. In addition, the k coefficients were derived using the

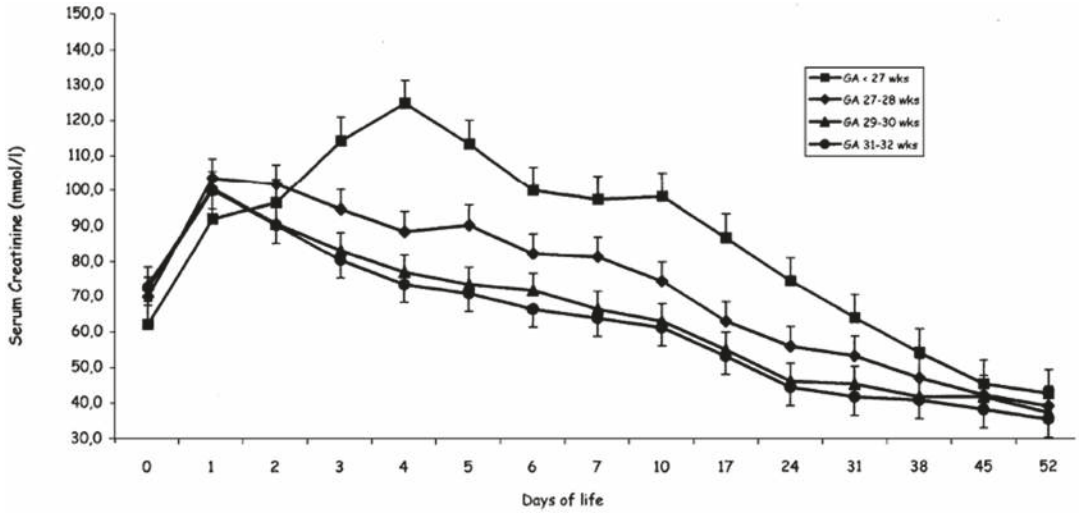

Fig. 53.1 Serum creatinine values over time by gestational age categories. (Used with permission from Gallini et al. [13])

Jaffe calorimetric method to measure SCr. As most hospitals now only use the enzymatic equation, the coefficients may no longer be applicable.

Cystatin C has been extensively studied as a measure of GFR and a marker of acute kidney injury. Since Cystatin C is not influenced by the maternal serum level and is highest at birth, it may be better suited than SCr to monitor kidney function in infants. Cystatin C concentrations significantly decrease during the first three days of life and are independent of gestational age, birth weight and maternal kidney function status in very low birth weight infants [16]. Cystatin C does not differ between males and females and is not influenced by gestational age. Thus, cystatin C seems to have many properties of an ideal marker of kidney function in this age group [17]. However, as only a few studies on this topic have been conducted so far and no studies have linked cystatin C levels with short and long-term outcomes in this population, further research is needed before adopting Cystatin C as a primary marker of kidney function in neonates.

Water, Electrolyte and Acid-Base Disorders in Neonates

Metabolic Acidosis

Neonates typically show a certain degree of acidosis depending on gestational and postnatal age [18]. In full-term neonates, after completion of nephrogenesis at the 34th week of gestation [19], the mechanisms devoted to maintain acid base (AB) equilibrium are still immature. This immaturity concerns both the capacity of H^+ excretion and the HCO_3^- reabsorption threshold [20, 21]. Moreover, the ability of excreting large amounts of acid through ammoniagenesis is impaired in the newborn baby, due to the decreased presence of enzymes necessary for ammoniagenesis, like glutaminase [22]. Finally, neonatal nutrition involves a two to three times higher protein load than older children with consequently higher acid production. In premature infants, this unfavorable condition is even worsened by the inability to efficiently acidify urine with further acid retention and consequently increased risk of metabolic acidosis. In fact, in preterm infants, plasma bicarbonate levels are lower than in full term neonates for the first 3 weeks of life due to the lower renal threshold for bicarbonate (see Chap. 36). This predisposes preterm neonates to condition known as late metabolic acidosis, which is further promoted by milk formulas containing casein, by parenteral nutrition (especially in TPN containing arginine HCl), and by withdrawal of milk alimentation (and consequently alkali intake), e.g., during episodes of diarrhea.

The acid-base disturbances seen in the NICU occur in an organism with immature homeostatic mechanisms. According to the classic metabolic acidosis classification based on the anion gap, we can take into account a number of clinical situations leading to metabolic acidosis. The anion gap attests the balance (or unbalance) between acid accumulation and loss of base equivalents. It is calculated by: $Na^+ - (Cl^- + HCO_3^-)$ in mEq/L. Metabolic acidosis with a normal anion gap (<16 mEq/L) is seen in neonates with intestinal or renal HCO_3^- losses, which can be due to either proximal or distal renal tubular acidosis (see Chap. 36). Also, the use of the carbonic anhydrase inhibitor acetazolamide in pregnancy has been associated with metabolic acidosis in preterm neonates [23, 24].

The most common conditions causing an anion gap >16 mEq/L in neonates are kidney failure, inborn errors of metabolism (IEM) or lactic acidosis [25]. In kidney failure, the impairment of acid load elimination increases HCO_3^- consumption while in IEM (in particular in organic acidurias [26]. HCO_3^- stores are depleted by the increased production of organic acids. Small bowel drainage following surgical procedures may also induce large HCO_3^- losses. In necrotizing enterocolitis (NEC) acidosis is associated with progressive systemic shock and lactic acidosis [27]. In VLBW infants, metabolic acidosis on the first day of illness is more common in infants with perforated NEC compared to infants without perforation [23].

The treatment of metabolic acidosis in neonates firstly relies on the diagnosis and treatment

of the underlying cause. The treatment of renal tubular acidosis with $NaHCO_3$ in critically ill children is controversial [28]. Only one randomized trial examining potential benefits of $NaHCO_3$ in asphyxiated newborn infants is available - it showed no influence on the outcome [29]. No benefit of bicarbonate supplementation has also been found in adult patients. In cardiac arrest, the administration of $NaHCO_3$ has been widely utilized in an attempt to correct acid overproduction caused by decreased tissue oxygenation. There are no available randomized trials demonstrating positive effects of $NaHCO_3$ administration in cardiac arrest in either adult and pediatric patients, including neonates. On the contrary, $NaHCO_3$ may induce CO_2 accumulation in poorly oxygenated districts and hypercarbia [30].

In 2013, a survey in Canadian NICUs showed that $NaHCO_3$ is most frequently administered in septic shock whereas its use is much less frequent in cardiac arrest [31]. With an analogous mechanism, CO_2 accumulation may occur also in ARDS if its elimination by the lungs is insufficient. In this case also, the addition of bicarbonate may induce only a transient rise of pH, usually followed by hypercarbia [32]. A different situation occurs when a net $HCO3^-$ loss or its excessive consumption take place. Examples are gastrointestinal losses and kidney failure. The ability of the neonatal kidney to reabsorb $HCO3^-$ is about one third of that of adult individuals [33] and a mature ammoniagenesis mechanism is lacking [20].

Potential hazards of $NaHCO_3$ supplementation in infants include risk of sodium overload and hypernatremia, hypokalemia and hypocalcemia. Moreover, the use of $NaHCO_3$ in infants has been associated with a number of adverse events like intracranial hemorrhage, deterioration of cardiac function and myocardial injury [34, 35]. In neonates $NaHCO_3$ should be given only after establishment of adequate ventilation and circulation and should be restricted to selected situations like severe acidosis with life-threatening hyperkalemia, massive GI losses, and tubulopathies. In neonatal resuscitation, a dose of 1–2 mmol/kg of a 0.5 mmol/mL solution may be given by slow intravenous push (over at least 2 min) after adequate ventilation and perfusion have been established [36]. In infants with kidney failure, titration of acid/base balance using sodium citrate is necessary to maintain appropriate metabolic control and growth.

Metabolic Alkalosis

Metabolic alkalosis is almost invariably accompanied by hypokalemia and can be classified on the base of chloride urine concentration. In critical infants, low urinary Cl^- concentration (expressed as $Cl^- < 10$ mmol/L or, more precisely, as Cl^-/Creatinine ratio < 10 in mol/mol) suggests that the kidneys are avidly holding on to chloride, thus, ruling out chloride loss from the kidneys as the etiology. Low urine chloride metabolic alkalosis may be present as a consequence of loss of gastric secretions (vomiting, nasogastric suction), secretory diarrhea or after rapid correction of hypercapnia, and chronic diuretic which cause low total body chloride). In contrast, metabolic alkalosis with high urine chloride suggests inappropriate losses of chloride from the kidney from primary chloride losing tubulopathies (Bartter syndrome, Gitelman syndrome) and, more frequently, the use of diuretics. If metabolic hypochloruric alkalosis is present in context of systemic hypertension, defects in regulation of aldosterone and renin should be sought (Fig. 53.2).

A peculiar low-chloride associated clinical situation is that of infants after cardiopulmonary bypass surgery. In these patients, metabolic alkalosis is associated with younger age, preoperative ductal dependency and hemodilution [37]. Volume and chloride depletion have been advocated as possible causes [38]. Metabolic alkalosis is also reported in up to 18% of infants referred to surgery for pyloric stenosis [39].

Hyperkalemia

Hyperkalemia may represent a life-threatening condition in the NICU. It is the most common electrolyte disorder associated to heart conduction problems [40]. Non-oliguric hyperkalemia

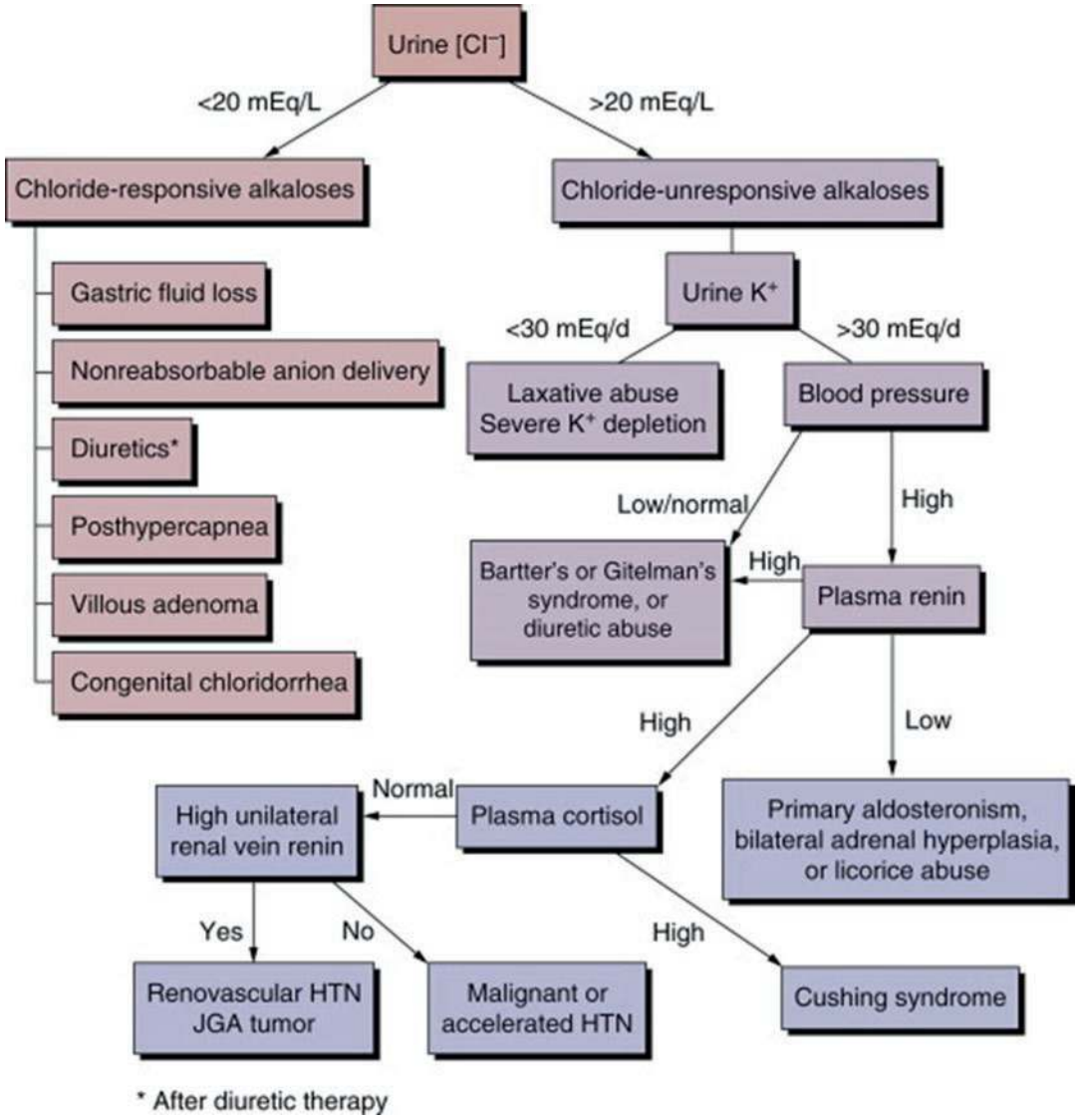

Fig. 53.2 Clinical decision tree diagram for metabolic alkalosis [1] used with permission

of the newborn is defined as a plasma potassium level >6.5 mmol/L in the absence of acute kidney failure [41]. In neonates, spurious hyperkalemia has to be initially excluded since mechanical trauma to red blood cells during venipuncture with consequent release of intracellular potassium is particularly frequent in neonates and prematures. The hemolytic Index is a measure of red color of serum which is due almost exclusively due to hemoglobin. This index (reported as 0 to 4 +) assist in detecting spurious hyperkalemia in infants [42]. Once spurious hyperkalemia is ruled out, a number of causes of true hyperkalemia have to be considered. Hyperkalemia may be induced by increased intake, intra-extracellular potassium redistribution and decreased elimination. Increased intake in critical infants may be seen with acute potassium load and blood transfusion. Acute load is infrequent in non-oliguric infants and it is usu-

ally consequent to dosing errors or the administration rate of intravenous potassium containing solutions. Potassium concentration in transfused blood may be as high as 50 mmol/L, so that even small amounts may induce severe hyperkalemia in small infants. This renders the use of fresh blood in newborns mandatory [43]. The most common cause of hyperkalemia due to intra-extracellular potassium redistribution in critically ill infants is metabolic acidosis. In this condition, potassium moves from the intracellular compartment in order to maintain electroneutrality after H^+ ions have accumulated in the intracellular space.

Hyperkalemia is frequent in preterm neonates. It presents as a sudden rise of serum potassium in the first 72 h of life of preterm neonates with a gestational age <28 weeks and may cause heart conduction impairment that may result in sudden death. This condition results from potassium loss from the intracellular space, together with the immature renal excretion of potassium and aldosterone unresponsiveness. Management of hyperkalemia is mandatory when symptoms and/or EKG alterations are present. The latter include tall, peaked T waves with narrow base, prolonged PR interval, decreased or disappearing P wave, widening of QRS, amplified R wave, ventricular fibrillation or asystole. There are three main approaches to the treatment of hyperkalemia: (1) antagonizing the membrane actions of hyperkalemia, (2) driving potassium into cells, and (3) removing potassium from the body. First, stabilization of myocardial function may be obtained by Ca^{2+} infusion. Calcium inactivates sodium channels and increases membrane excitability in 2–3 min. Its effect lasts for 30–60 min, so that an alternative therapy is required after that time. Calcium is given by slow intravenous injection over 5–10 min: 0.11 mmol/kg (0.5 mL/kg of calcium gluconate 10%). Potassium can be driven into intracellular space by insulin in exchange with sodium. I.v. insulin is used with glucose for emergency treatment of hyperkalemia at the dose of intravenous solution of insulin (0.1–0.6 units/kg/h in neonates) with glucose infusion of 0.5–1 g/kg/h (5–10 mL/kg/h of glucose 10%). The effect starts in 15 min and can last for hours. Blood glucose levels must be carefully monitored to avoid both hypo-and hyperglycemia [43]. Intravenous sodium bicarbonate reverses potassium ions from the extra- to the intracellular compartment to maintain electro-neutrality. A half correction of the base excess (0.3 × weight × BE) over 10–15 min can be administered and the rest given in the next 12–24 h. The main constraint of sodium bicarbonate use is sodium and volume overload, especially when kidney impairment is present. β-2 adrenergic agonists increase sodium-potassium ATPase activity and potassium is driven back into cells. Salbutamol or albuterol can be nebulised or given by intravenous infusions [44, 45]. Finally, removal of potassium is generally obtained by diuretics and cation exchange resins. Furosemide can be administered in 1 mg/kg dosage and repeated in case of need. Since the efficacy of diuretics depends on GFR, higher dosage may be required in kidney failure. Calcium or sodium polystyrene sulfonate (Kayexalate®, Concordia Pharmaceuticals, Oakville, Ontario, Canada) binds potassium by exchanging it with sodium in GI tract so that potassium is eliminated in the stool. In adult patients with life-threatening hyperkalemia, the role of exchange resins has been questioned [46]. In neonates, while potassium elimination can be enhanced by ion resins, gastro-intestinal obstruction and/or perforation can occur following oral or rectal administration of exchange resins [47]. Moreover, in a comparative study in hyperkalemic preterm neonates cation-exchange resin did not lead to a better outcome regarding all-cause mortality than glucose and insulin [48]. Given the above considerations, combined insulin/glucose infusion should be preferred over treatment with rectal cation-resin for acute hyperkalemia in preterm infants [49]. Low potassium formulas are available for children who have poor potassium elimination. In addition, premixing formula with sodium polystyrene sulfonate (Kayexalate®), allowing the resin to settle and providing the supernatant for nutrition, efficiently lowers the oral potassium load without risking bowel obstruction/perforation.

Hypokalemia

True, not alkalosis induced hypokalemia (<3.5 mmol/L) in critically ill infants may develop as a consequence of potassium loss due to intestinal problems (vomiting, nasogastric suction, diarrhea), kidney conditions (diuretic use, recovery from acute kidney injury) or insufficient potassium intake, mainly coming from unbalanced parenteral nutrition. Congenital conditions if untreated can be lethal during the first weeks or months of life. These include congenital chloride diarrhea (a rare autosomal recessive disease characterized by chronic secretory diarrhea), and some inherited conditions like Bartter syndrome, Gitelman syndrome, and related syndromes (see Chap. 33).

In neonates, potassium replacement must be managed with extreme caution, given the rapid change of kalemia induced by small amounts of potassium. Intravenous potassium treatment should only be given for immediately life-threatening emergencies over several minutes while severe hypokalemia may be treated with an infusion of 0.2–0.5 mmol/kg/h to a maximum of 1 mmol/kg. Non-emergencies are best treated using oral supplements if possible, otherwise as small dosages as low as 0.03–0.07 mmol/kg by slow injection. During potassium administration, very frequent monitoring of plasma levels has to be established as well as continuous EKG monitoring while correction occurs [50].

Hypernatremia

Changes in sodium concentration are common in critical neonates, due to the small patient volume and the body fluid changes occurring in the perinatal period. Hypernatremia (>145 mmol/L) must always be considered in relation to the water content and is traditionally classified as hypo-, normo- or hypervolemic. Hypovolemic hypernatremia is often seen when fluid restriction is required and water loss exceeds that of sodium. Other frequent causes of hypovolemic hypernatremia are losses through the gut or the kidney (like watery diarrhea, water loss in post-obstructive polyuria). Hypernatremia is more frequent in VLBW infants in which water loss through lungs and the immature skin may reach 150 mL/kg/day, thus exposing the preterm to hypernatremia due to free water deficit, especially in the first hours of life. Moreover, renal sodium handling is inversely related to creatinine clearance in the first 2 weeks of life. After 4–5 days from birth sodium balance becomes negative with a sudden decrease of fractional excretion, thus enabling the neonatal kidney to spare sodium. This occurs also in preterm babies [51]. A particular case is that of critically ill infants with severe hypoperfusion and acidosis requiring large amounts of sodium bicarbonate. In this case, often a capillary leak syndrome is present with leak of albumin, sodium and water to the interstitium in a mixed hypovolemic-sodium retaining situation in which sodium is in a third space. Hypervolemic hypernatremia in the NICU is frequently induced by administration of large amounts of sodium with drugs and blood products.

Management of hypernatremia in critically ill infants is usually an urgent treatment. Respiratory distress, necrotizing enterocolitis, and patent ductus arteriosus are associated with hypernatremia and volume expansion [52, 53]. Correction of hypernatremia basically consists in free water administration with correction velocity being the crucial issue. There is evidence that plasma both sodium changes and velocity of these changes are associated with neurological outcome [54]. The rule is to reduce natremia at a speed not >0.5–1.0 mmol/L/h. If plasma sodium is very high (>160 mmol/L), it is advisable to administer a 0.9% saline solution in order to reduce natremia slowly.

Hyponatremia

Hyponatremia (<130 mmol/L) is associated with cerebral edema and permanent neurologic sequelae especially in preterm neonates [55, 56]. In critical neonates, hyponatremia is most frequently seen as a consequence of diuretic use, surgical procedures, diarrhea/vomiting and third

space loss. A particular neonatal issue is hyponatremia during therapeutic hypothermia. In these patients, hyponatremia has been related to water loss as a consequence of cooling induced skin vasoconstriction [57]. Total body fluid overload will also cause hyponatremia.

Diuretics are commonly used to treat infants with oxygen-dependent chronic lung disease [58] and congenital heart defect [59]. In acute situations, high-dose diuretics may be required and this may cause hyponatremia which, in turn, hampers the response to the diuretic. Hypokalemia, alkalosis, and calcium wasting can be part of this picture. During surgery, standard neonatal intensive care guidelines recommending hypotonic i.v. infusions containing 20–40 mmol of sodium are often followed [60]. However, these guidelines may not meet metabolic and volume needs in the perioperative period and hyponatremia may result in up to 60% of patients [55, 60, 61]. Careful monitoring of sodium levels and the use of balanced sodium solutions are mandatory in these patients [61]. During neonatal sepsis, capillary leak may take place and large amounts of sodium together with water and albumin are displaced into the interstitium with severe edema poorly responding to diuretics [62].

Replacement of sodium loss in volume depletion must take into account the variation of sodium plasma levels since, if a patient is seizing with a serum sodium <120 mol/L, 3% saline can be given. In patients that are clinically stable, use of isotonic saline can improve sodium concentrations. Like in hypernatremia, correction velocity must not be >1 mmol/kg/h. Careful attention to fluid shifts and serial electrolyte monitoring is essential.

Hypocalcemia

Normal levels of serum calcium are normally achieved during the second week of life when PTH secretion from parathyroid glands can efficiently respond to hypocalcemic stimuli [63]. Before then, normal neonates spontaneously lean towards hypocalcemia. Actually, a physiologic fall in serum calcium concentration, occurs in the first 24 h of life due to the sudden stop of calcium supply from the placenta. PTH is then stimulated but its action becomes valid from 2 to 3 days of life onward [64] . The kidney plays a key role in calcium homeostasis and, although the timing of the action of PTH on renal calcium excretion in neonates is not certain, calciuria increases after the second week of life [65].

NICU infants may develop hypocalcemia (<8.8 mg/dL or ionized calcium <4.9 mg/dL) for a number of reasons. When PTH secretion from immature parathyroid glands is insufficient, a prolongation or a worsening of hypocalcemia occurs (early onset neonatal hypocalcemia) [63]. Under these circumstances, hypocalcemia is rarely symptomatic but EKG alterations (Q-T prolongation) may be present [63]. Preterm infants and children of diabetic mothers [66] are more exposed to the risk of hypocalcemia. Approximately 50% of infants of mothers who have diabetes show hypocalcemia [54].

The etiology of neonatal hypocalcemia is multifactorial. It is probably due to loss of calcium and magnesium with urine, resulting in reduced placental transfer and decreased neonatal secretion of PTH. Another risk factor for early onset hypocalcemia is maternal calcium ingestion during pregnancy, inducing inhibition of the neonate's PTH response and consequent hypocalcemia [67]. Hypocalcemia starting after 5–10 days of age is due to resistance of renal tubule cells to PTH leading to renal retention of phosphorus and hypocalcemia (late onset neonatal hypocalcemia) [63].

Overt hypoparathyroidism in neonates occurs in case of dysgenesis of the parathyroid glands. The most common cause is the DiGeorge syndrome. The phenotype is characterized by hypocalcemia caused by parathyroid gland hypoplasia, defective T-lymphocyte function and impaired cell-mediated immunity caused by impaired thymic differentiation and conotruncal defects of the heart or aortic arch. The syndrome is associated with microdeletions of chromosome 22q11.2. Some neonates may have isolated hypoparathyroidism. Also in the CATCH 22 syndrome (cardiac anomaly, abnormal facies, thymic aplasia, cleft palate, hypocalcemia), haploinsufficiency

for genes located in the 22q11 region is associated with contiguous gene deletion syndromes that include not only the DiGeorge syndrome but also the overlapping conotruncal anomaly and velocardiofacial syndromes [63, 68].

In NICU patients, acquired hypocalcemia is frequently drug induced. Aminoglycosides, often used in NICU, can increase renal calcium loss and induce hypocalcemia in neonates [69]. Anticonvulsants such as phenytoin or phenobarbital are potential inducers of cytochrome P450 (CYP450), causing increased vitamin D degradation. Also, the prolonged use of anticonvulsant in the mother during pregnancy can induce hypocalcemia in the newborn [70]. Renal excretion of calcium is notably enhanced during treatment with loop diuretics. This concerns particular populations like infants with heart problems or after cardiosurgery. Ionized calcium also can be reduced in infants treated with sodium bicarbonate, which increases calcium binding to albumin.

Urgent treatment of neonatal hypocalcemia is based on i.v. calcium supply. Calcium gluconate and calcium chloride are both available at 10% concentration. Both preparations have to be administered via a central vein. Although there is no proven superiority of one form over the other for the treatment of ionized hypocalcemia [71], calcium chloride appears to be more irritating for vessels and gluconate should be preferred in neonates. Calcium chloride contains three times more elemental calcium than gluconate (272 vs. 90 mg in 10 mL at 10%, respectively). Serum calcium levels should be corrected by continuous intravenous infusion of calcium (at 1–3 mg of elemental calcium/kg body weight per hour) under strict monitoring of ionized calcium levels, in order to avoid complications as such as bradycardia and arrhythmia or vessel necrosis.

Hypercalcemia

Neonatal hypercalcemia is much less frequent than hypocalcemia. Infants normally show higher total (8.8–11.3 mg/dL) as well as ionized calcium levels (1.19–1.40 mmol/L) than older children or [61]. Hypercalcemia is often asymptomatic. Hypercalcemic infants can show irritability, dizziness and arterial hypertension. It is not infrequent that hypercalcemia is discovered after diagnosis of nephrocalcinosis or lithiasis.

Hypercalcemia in NICU infants is almost always iatrogenic [63]. Vitamin D and calcium supplementation are a frequent cause of hypercalcemia. Hypophosphatemia is frequently seen in preterm neonates as a consequence of poor intake. Low phosphate levels stimulate PTH secretion which in turn increases intestinal calcium absorption and calcium resorption from the skeleton. Children on Extracorporeal Membrane Oxygenation (ECMO) experience hypercalcemia up to 30% of patients, probably due to aberrant vitamin D-PTH regulation [72].

Rare congenital conditions must be considered in the presence of neonatal hypercalcemia. The calcium-sensing receptor (CASR) is expressed in the PTH producing chief cells of the parathyroid gland and the cells lining the kidney tubule. Inherited abnormalities of the *CASR* gene can cause either hypercalcemia or hypocalcemia. This autosomal recessive condition affects neonates and induces neonatal severe hyperparathyroidism (NSHPT) [73]. Hypercalcemia is usually severe and can be life-threatening. Typically, PTH levels are normal to high and calcium urinary excretion is low.

Subcutaneous fat necrosis (SFN) can be the consequence of a difficult delivery and is characterized by necrosis of fat and a local macrophagic reaction to the necrotic fat. Hypercalcemia derives from the excess of calcitriol produced by macrophages and is associated with a 15% mortality rate [74]. Of interest, SFN with hypercalcemia has been recently associated with neonatal therapeutic hypothermia [75, 76]. Given the growing use of this therapy, blood calcium levels should be monitored in children undergoing therapeutic hypothermia.

Initial treatment of severe hypercalcemia in critical infants relies on hydration and loop diuretics. Calciuria can rapidly increase, which can worsen kidney function and/or nephrocalcinosis. Withdrawal of hypercalcemic agents such as calcium supplements or vitamin D supplements is mandatory. Treatment of neonatal hyperparathyroidism is an urgent requirement.

Steroids and bisphosphonates have been used with success [77]. More recently, the calcimimetic agent cinacalcet has been used successfully in neonatal hyperparathyroidism in combination with bisphosphonates [78].

Acute Kidney Injury (AKI) in Neonates

Definition and Epidemiology

Previously referred to as acute renal failure, acute kidney injury (AKI) is characterized by a sudden impairment in kidney function, which results in retention of nitrogenous waste products and altered regulation of extracellular fluid volume, electrolytes and acid-base homeostasis. The term "acute kidney injury" has replaced "acute renal failure" by most critical care and nephrology societies, primarily to highlight the importance of recognizing this process at the time of "injury" as opposed to waiting until "failure" has occurred [79].

Despite its limitations (as outlined above and in Chap. 46), SCr is the most commonly used measure to evaluate glomerular filtration in the clinical setting and is more specific than blood urea nitrogen (BUN). BUN is an insensitive measure of glomerular filtration rate (GFR) because it can be increased out of proportion to changes in GFR secondary to high dietary protein intake, gastrointestinal bleed, use of steroids and hypercatabolic states. If the BUN:SCr ratio exceeds 20, increased urea production or increased renal urea reabsorption that occurs in pre-renal azotemia should be suspected [80].

Historically the most common SCr cutpoint used to define neonatal AKI was set at an arbitrary cutoff of 1.5 mg/dL or greater, independent of day of life and regardless of the rate of urine output [81]. In 2009, categorical definition based on a rise in SCr to diagnose and define different stages of AKI [77] was applied to neonates [81]. Since then, we and others have used AKI definitions similar to those published by the 2007 AKIN and 2012 KDIGO guidelines [82–92]. A neonatal AKI definition has been adapted from the KDIGO definition (Table 53.3).

Table 53.3 Neonatal AKI definition

Stage	Serum creatinine (SCr) criteria	Urine output (UOP criteria)
0	No change or rise <0.3 mg/dL	UOP >1 cc/kg/h (over previous 24 h)
1	↑ SCr of ≥0.3 mg/dL or ↑ SCr to 150–199% × baseline	UOP >0.5 cc/kg/h and ≤ 1 cc/kg/h (over previous 24 h)
2	↑ SCr to 200–299% × baseline	UOP >0.1 cc/kg/h and ≤ 0.5 cc/kg/h (over previous 24 h)
3	↑ SCr to ≥300% × baseline or SCr ≥2.5 mg/dL or Receipt of dialysis	UOP ≤0.1 cc/kg/h (over previous 24 h)

Baseline SCr will be defined as the lowest previous SCr value

Table 53.4 Incidence and outcomes of neonates with AKI

Population	Incidence (%)	Mortality	Ref.
		AKI v no AKI	
VLBW[a]	18	55% vs. 5%	[82]
ELBW[b]	12.5	70% vs. 22%	[89]
Sick near-term/term	18	22% vs. 0%	[78]
Sepsis	26	70% vs. 25%	[90]
Asphyxiated Newborn	38	14% vs. 2%	[91]
ECMO[c]	71	72.7% vs. 20%[d]	[92]

[a]Very low birth weight (VLBW) infants <1500 g
[b]Extremely low birth weight (ELBS) infants <1000 g
[c]Extra Corporeal Membrane Oxygenation (ECMO)
[d]In group with highest stage of AKI

The following modifications have been made to account for specific neonatal issues:

- Because SCr normally declines over the first week of life [13], each SCr is compared to lowest previous value.
- As SCr of 2.5 mg/dL represents glomerular filtration rate <10 mL/min/1.73 m^2 in neonates, this cutoff is used to define Stage 3 AKI (as opposed to 4.0 mg/dL in adults).
- UOP criteria have been adapted to levels obtained over 24 h.

Table 53.4 shows the incidence and outcomes of neonatal AKI in different neonatal populations

using category AKI definitions similar to that presented here. However, more research is needed to develop, and validate this definition against hard clinical endpoints.

Risk Factors and Etiology

AKI is a common clinical condition in the NICU [93–96]. Up until 2013, limited data coming from small single-center studies identified premature infants, sick near-term or term infants, and infants who undergo cardiopulmonary bypass or ECMO at particular risk for neonatal AKI [88, 97–100]. Also, since there was a wide variation in the incidence of AKI according to the AKI definition used and the number of SCr values measured, a uniform understanding of the risk factors for AKI was difficult to ascertain [101–103]. In 2014, the Neonatal Kidney Collaborative was established and designed a multicenter study to evaluate AKI among critically ill neonates (the Assessment of Worldwide Acute Kidney Injury Epidemiology in Neonates (AWAKEN)) [104]. In AWAKEN, several risk factors have been evaluated according to gestational age and postnatal age [104]. In particular, perinatal risk factors are associated with early neonatal AKI (first perinatal week) and cardiac and kidney anomalies, surgery and nephrotoxin medications rather than perinatal factors are risk factors of late AKI (after the postnatal week) (Table 53.6) [105, 106]. Furthermore, nearly one third of neonates with late AKI had an episode of early AKI increasing the risk for CKD.

These conditions are important in identifying neonates at risk for development of AKI and because some of them are potentially preventable [107, 108]. The causes of AKI in newborns are typically divided into three groups: pre-renal failure, intrinsic renal failure and post-renal failure with pre-renal and intrinsic being the most frequently reported [109].

Pre-renal and intrinsic renal failure both include congenital and acquired causes of AKI (Table 53.7). The most common form of AKI in neonates is pre-renal failure which accounts for more than 80% of cases.

In numerous studies, **perinatal asphyxia and sepsis** are the most frequent associated conditions [110]. Asphyxia causes renal dysfunction secondary to redistribution of blood flow to more vital organs such as heart and brain at the expense of the others, especially kidneys and gut. The reported incidence of renal failure in asphyxiated neonates varies between 50% and 70% in infants not treated with therapeutic hypothermia [111–113] and 30–40% after introduction of therapeutic hypothermia for the treatment of perinatal asphyxia [114–116]. In asphyxiated newborns, renal outcome correlates with oliguria but also with clinical markers of the severity of asphyxia. In particular, Apgar score at 5 min <6, initial and 12-hour lactate concentrations, and hypoxic ischemic encephalopathy were much better predictors of adverse outcome than kidney function tests [113, 116]. The incidence of AKI among term and near-term neonates with encephalopathy in the AWAKEN cohort was more than 40% and analysis of data including antenatal risk factors selected among factors associated with AKI

Table 53.5 Time to dialysis start, fraction of patients reaching a negative fluid balance and survival rate of infants treated with PD after cardiopulmonary bypass surgery. Only in studies where all patients reached negative fluid balance survival exceeded 50%

Reference	*n*	Time to PD start	Patients with negative fluid balance (%)	Survivors (%)
Lowrie [179]	17	NA	35	24
Fleming [180]	21	2.5 days (1–6) after surgery	36	38
Golej [181]	116	NA, but 43% of pts. started on PD when CVP >10 mmHg	53	47
Werner [182]	23	2.6 ± 0.6 days	100	53
Dittrich [183]	27	In the OR or first hrs in ICU	100	73
Santos [184]	23	4.8 ± 16.8 h	100	57
Sorof [185]	20	22 h	100	80
Chien [186]	7	1.2 ± 0.4 days after AKI onset	NA	57

Table 53.6 Available filters for neonatal CKRT

Filter	Manufacturer	Surface (m^2)	Membrane	Priming (mL)
Miniflow 10	Gambro-Lundia	0.045	AN69	3.5
Minifilter	Minntech	0.07	Polysulfone	6
Carpediem 1	Bellco	0.075	Polysulfone	27.2
Carpediem 3	Bellco	0.245	Polysulfone	41.5
HF20	Gambro-Lundia	0.20	AN69	60
Aquadex UF500	Nuwellis	0.12	Polysulfone	33
FX paed	Fresenius	0.20	Polysulfone	18

Table 53.7 Monitors for pediatric and neonatal CKRT

Monitor	Manufacturer	Pediatric lines	Neonatal lines	Blood pump range (mL/min)	Blood flow steps (mL/min)	Fluid management range (mL/h)
Prismaflex[a]	Gambro-Lundia	Yes	–	20–100	2	50–2500
Multifiltrate	Fresenius	Yes	–	10–100	2	10–7000
Aquarius	Baxter	Yes	–	10–200	2	50–11,000
Plasauto Sigma	Asahi	Yes	Yes	1–400	1	10–12,000
Carpediem	Bellco	–	Yes	2–50	1	10–300
Aqudex	Nuwellis	No	No	5–40	5	10–500

[a]Prismaflex equipped with HF20 circuit

being outborn, low admission temperature, intrauterine growth restriction and the presence of meconium-stained amniotic fluid (MSAF) [117].

Among the neonatal population, **premature infants and low birth-weight** are particularly sensitive to asphyxia and hypoperfusion [97, 118, 119]. Very low birth weight (VLBW) and extremely low birth weight (ELBW) children are at increased risk for AKI because of prenatal fetal distress secondary to intrauterine growth retardation, placental insufficiency and maternal medications and a postnatal course frequently complicated by hypotension and hypoxia and the need for cardio-pulmonary support [97, 119, 120]. Studies focused on AKI in VLBW/ELBW reported an incidence of 12.5% and 18%, respectively [119, 120]. VLBW infants with AKI were more likely to have low birth weight, low gestational age and low Apgar scores and they frequently required umbilical arterial catheters, assisted ventilation and inotropic support. In another study, infants with AKI had a higher mean airway pressure, a lower mean arterial blood pressure and higher exposure to cefotaxime than non-AKI controls [119].

Given the high vulnerability of the neonatal kidney to the effects of hypoperfusion, **acute tubular necrosis** (ATN) commonly occurs. Pre-renal failure is due to renal hypoperfusion or ischemia in the presence of a normal kidney. Hence, irrespective of whether pre-renal failure is caused by total body volume depletion or decreased effective blood volume, renal function quickly returns to normal if perfusion is rapidly restored. Conversely, if the insult is severe and prolonged, acute tubular necrosis can occur [101]. During renal hypoperfusion many compensatory mechanisms are activated; in particular intrarenal vasodilatory prostaglandins are released. In order to help differentiate pre-renal failure from ATN, urinary indices have been proposed, in particular urine sodium concentration and the fractional excretion of sodium (FENa) [121] (Table 53.7). The assumption is that renal tubules work properly in pre-renal failure and are able to reabsorb solute and water while they are damaged and do not adequately conserve sodium in ATN. The urine samples for measuring indices must be obtained prior to a fluid and diuretic challenge. This could be difficult in oliguric neonates. Urine sodium less than 20 mEq/L and more than 50 mEq/L is suggestive of prerenal and intrinsic renal failure, respectively. FENa is calculated as urine sodium factored by serum sodium divided by urine creatinine factored by serum creatinine:

$$\text{FENa} = \text{urine sodium} \times \text{serum creatinine} / \text{serum sodium} \times \text{urine creatinine}$$

In term infants, a FENa above 3% indicates intrinsic renal failure. Preterm babies physiologically lose more sodium than term infants and a FENa of more than 6% can be used to define intrinsic AKI in infants between 29 and 32 weeks of gestation [121, 122].

Sepsis is the second most common condition associated with AKI after perinatal asphyxia [110]. AKI develops in 20–30% of neonates with sepsis [123–126]. In a series of 203 neonates with sepsis, 40 (20%) developed AKI. Increased baseline serum creatinine, vasopressor days, history of necrotizing enterocolitis (NEC) and ECMO requirement were good predictors of AKI in septic neonates. Most AKI episodes were detected within 2 days after sepsis evaluation; 23% of sepsis cases with AKI did not return to their serum creatinine baseline and all of them died [126]. In developing countries with limited resources, sepsis is more common than in developed countries [127, 128] and is the most common cause of AKI in neonates, occurring in 31% of newborns with a mortality rate of 65% [129]. The pathogenesis of AKI in sepsis is multifactorial, including shock, disseminated intravascular coagulation, haemorrhage, cardiac failure, and nephrotoxic drugs. All these conditions cause renal' hypoperfusion with resultant ischemic-reperfusion injury and cytokine- and oxidant-mediated kidney injury.

Administration of **nonsteroidal anti-inflammatory drugs** (NSAID) (for instance, indomethacin for patent ductus arteriosus closure) can inhibit this compensatory mechanism and precipitate AKI during renal hypoperfusion. In a multicenter Italian Study, ibuprofen treatment was identified as a risk factor correlated with impaired kidney function. Interestingly, maternal consumption of NSAID during pregnancy negatively influenced neonatal kidney function as well [130]. The risk of NSAID therapy-associated AKI is even higher when neonates concomitantly receive additional nephrotoxic drugs. Among neonates with patent ductus arteriosus who received gentamicin and NSAID, the rate of AKI was 14.8% compared to 9.1% for those not exposed to NSAID [131].

Antimicrobial agents are another major class of drugs associated with the development of AKI in preterm infants. Nephrotoxic AKI has been reported in association with aminoglycosides, vancomycin, piperacillin-tazobactam, acyclovir and amphotericin B [132]. In particular, aminoglycosides are widely used in pediatric patients. They accumulate in renal tubular cortical cells and exert nephrotoxicity causing damage to the proximal tubular epithelial cells secondary to lysosomal dysfunction [101, 133, 134]. Several studies have been performed to better understand the risk of nephrotoxicity associated with aminoglycoside therapy. A metanalysis of 16 trials involving 823 neonates comparing once daily aminoglycoside dosing with multiple doses per day found no difference in nephrotoxicity [135]. Similarly, in a Cochrane analysis of 11 studies and 574 neonates once daily dosing did not lead to more nephrotoxicity than twice-daily dosing, at comparable efficacy [136].

In a study that included 281 consecutive cases of AKI in preterm infants in NICU, multivariate logistic regression analysis showed that ceftazidime administration was associated with a greater risk of AKI compared to the other variables selected from univariate analysis including ampicillin, ibuprofen and furosemide [130]. Cephalosporin antibiotics have also been implicated in a case-control study involving 46 matched pairs of infants with and without AKI. Infants who developed AKI had a significantly higher prior exposure to cefotaxime, benzodiazepines, diuretics, and dopamine/dobutamine [119]. Globally, the AKI risk appears to increase with the number of nephrotoxic drugs [137] and the extended use of combination therapies [138].

Conservative Management of Neonatal AKI

Neonatal AKI is associated with a high morbidity and mortality [93]. Unfortunately, very few trials designed to test interventions have been per-

formed in the neonatal population. Management of AKI in newborns is therefore basically supportive and based on maintaining homeostasis until recovery of renal function [110, 122, 139, 140]. The first approach should be to carefully assess risk factors and to precisely define the cause of AKI. In particular, conditions that result in poor renal perfusion such as hypovolemia and sepsis should be promptly recognized and corrected [139, 140].

When and how to implement a fluid challenge continues to be an area of controversy and active investigation. In the resuscitative phase, hypovolemia can be initially corrected by the administration of a **fluid challenge** of 10–20 mL/kg of normal saline in bolus [141]. After active resuscitation has occurred, the goals of therapy should be to limit the degree of fluid overload, meanwhile providing adequate nutrition and medications necessary to promote recovery. Abnormalities in fluid balance are common among neonates and may impact patient outcome even in the absence of AKI. In a cohort of 645 critically ill neonates, a positive fluid balance was reported in more than 60% of patients during the first postnatal week [142]. Furthermore, the degree of fluid overload is an independent predictor of mortality [143, 144]. In a prospective study conducted on 58 neonates, those with AKI had more marked fluid overload and higher mortality rates over the first few days of life [143]. In a study of 154 newborns with AKI, fluid overload in excess of 7% was independently associated with a 13-fold mortality risk [141]. To prevent fluid overload, daily fluid input should not exceed insensible water losses (30 mL/kg/day) plus urinary losses. To guarantee adequate energy and nutrient intake while maintaining restricted fluid intake, concentrated solutions should be used. The volume required to apply drugs should be minimized by administration of pure or highly concentrated infusion volumes [139]. Body weight should be checked twice daily and the estimated fluid overload should be carefully assessed and tracked. Weight monitoring rather than cumulative fluid balance recording is, in fact, considered the most accurate method for measuring fluid balance in neonates and overcomes the problem of missed in and out measurements [142–145].

In order to maintain fluid balance and allow nutrition and drug infusions, diuretics are commonly used in patients with AKI. Studies in adult patients have not provided any evidence that diuretics improve survival or modify the course of AKI [146]. In young infants with AKI, furosemide use was an independent predictor of poor renal outcome [147] whereas data from AWAKEN suggested that diuretic administration was associated with a decreased risk of early-onset AKI [105]. Although controlled study evidence is lacking, furosemide has been used in neonates with AKI to facilitate clinical management by converting oliguric into non-oliguric AKI. Intravenous furosemide boluses (1 mg/kg) have been adopted for the treatment of oliguria in this setting given the challenges and risks associated with kidney replacement therapies in this difficult group of patients [139]. In addition to furosemide also bumetanide, a potent loop diuretic with similar pharmacologic characteristics as furosemide, was employed to increase urine output in preterm infants with oliguric AKI; while effectively increasing urine output the drug also caused a transient increase in serum creatinine levels, highlighting the nephrotoxic potential of loop diuretics in this vulnerable population [148, 149].

Low-dose **dopamine** has also been utilized to improve urine output in critically ill term and preterm neonates [110]. Dopamine is an endogenous catecholamine that influences different catecholamine receptors in a dose-dependent manner, and, in particular, has been claimed to induce selective renal vasodilation when administered at low dose. Dopamine administration is associated with increased cerebral blood flow and has been shown to be safe and effective for treating hypotension in preterm infants. In oliguric, non-hypotensive neonates, GFR and urine output increased significantly with dopamine infused at a rate of 2.5 μg/kg per min. Moreover, dopamine induced renal and mesenteric vasodilation without an effect on cerebral blood flow when started precociously in preterm neonates treated with indomethacin for the presence of a patent ductus

arteriosus. However, an assessment of dopamine use in 19 NICUs and PICUs together with a literature review failed to demonstrate an improvement in renal function and urine output in neonates and pediatric intensive care patients. Moreover, evidence emerged that dopamine may have detrimental effects by worsening renal perfusion in critically ill patients with AKI. More recently, **Fenoldopam**, a selective dopamine A1 receptor agonist that decreases vascular resistance and increases renal blood flow, improved urine output in neonates requiring cardiac surgery with positive fluid balance despite diuretics [150–156]. However, in 40 infants undergoing cardiac surgery with cardiopulmonary bypass, Fenoldopam infused at a low dose (0.1 μg/Kg/min) for 72 h soon after anesthesia did not exert any effects on urine output, fluid balance or AKI incidence [157]. The same authors treated 40 infants undergoing cardiac surgery with a higher dose of Fenoldopam (1 μg/kg/min) during cardiopulmonary bypass. They observed decreased urinary NGAL and cystatin C levels, but no difference in plasma creatinine and urine output between subjects receiving fenoldopam and placebo [158].

Perinatal asphyxia is the primary cause of AKI in neonates. During hypoxia and ischemia, adenosine is released and acts as a vasoconstrictive agent in the kidney contributing to a fall in glomerular filtration rate. In this setting, methylxanthines such as aminophylline and theophylline (non-specific **adenosine receptor antagonists**) can inhibit the vasoconstriction induced by adenosine [159]. Three independent randomized trials in severe asphyxiated term infants and one randomized trial involving preterm neonates have shown that a single dose of theophylline, given early after birth, was associated with decrease in serum creatinine and improved urine output [159–162]. Based on these findings, the KDIGO guidelines recommend a single dose of aminophylline for asphyxiated infants at risk for AKI [163]. More recently, a meta-analysis including 6 randomized trials enrolling 436 neonates treated with prophylactic theophylline as compared to placebo further confirmed the reno-protective effect of theophylline. A 60% reduction in the incidence of AKI and a decrease in serum creatinine over days 2–5 without significant difference in complications was reported [164]. However, because theophylline has some potentially harmful neurologic effects and because therapeutic hypothermia is now standard of care in these infants, further comparative trials are needed to determine whether these agents improve short-term and long-term renal and neurodevelopmental outcomes [165]. Recently, another methylxanthine, caffeine citrate, administered in the first 7 postnatal days, was associated with a reduced incidence and severity of AKI both in preterm neonates and in premature infants diagnosed with NEC [166, 167].

Few other studies have specifically addressed therapies for AKI in neonates. In an uncontrolled retrospective study of 7 infants with hyperuricemia secondary to AKI treated with intravenous **rasburicase** administration of a single bolus determined a significant decrease of uric acid and creatinine and an increase of urine output within 24 h [168].

As described above, drugs are common causes of AKI in neonates [130–138]. In the setting of AKI, an **evaluation of all medications** should be performed to eliminate nephrotoxic agents and to determine the proper dose of other medications in the context of reduced kidney function and drug clearance. Moreover, whenever possible blood levels should be measured in order to maintain the levels in the therapeutic range and reduce the risk of nephrotoxicity [140]. Involvement of a pharmacist is highly advised especially in those infants receiving nephrotoxic combination therapy for extended periods of time [138–169].

Electrolyte disorders and acidosis are common in neonates with AKI and may complicate the clinical course after AKI. For the management of electrolyte disorders we refer to the above sections of this chapter.

Finally, efforts should be made to provide adequate **nutrition** in NICU patients with AKI [122–170]. Trials in neonates are lacking but extrapolating data from critically ill children,

underfeeding is common in AKI and it is important to ensure adequate caloric intake in order to prevent catabolism [171]. Hyperglycemia should be avoided and may require treatment with insulin [137]. Protein intake should be adjusted to meet at least the basal growth requirements (1–2 g/kg/day). These goals could be challenging in oligo/anuric neonates and the risk of fluid overload should be carefully weighed against the risk of malnutrition. If nutrition cannot be provided due to risk of further fluid accumulation, initiation of kidney replacement therapy should be considered.

Dialysis in Newborns

Over the past decades, technological advances and increased expertise have made it possible to support very small infants with dialysis. Nowadays, infants with AKI can receive peritoneal dialysis, hemodialysis or continuous kidney support therapy (CKST) [143, 172, 173]. The **choice of dialysis modality** in critically ill neonates relies on the clinical goals, patient characteristics, and local expertise. Local expertise and facilities affect the dialysis modality choice. Different programs provide neonatal dialysis in one or more location(s) - neonatal ICU, pediatric ICU and/or cardiac ICU.

The **indications** for the different kidney support therapies for neonates and young infants generally resemble those in older children. Whatever the clinical context, general indications for therapy include severe hyperkalemia, intractable acidosis, uremia, fluid overload, prevention of fluid overload, inability to provide adequate nutrition due to concerns of fluid accumulation.

Although most programs that offer dialysis in neonates use peritoneal dialysis, it may not be possible to perform in certain situations; thus HD and/or CKST complements PD. CKST has increased as a form of treatment for children and neonates with AKI in the US [174] and Europe.

In the largest reported multicentric, multinational cohort of neonates with AKI (AWAKEN study), kidney replacement therapy (KRT) was required in 25/605 patients (4.1%). Eleven out of 25 of these patients needed CKRT + ECMO, 9 PD, 4 CKRT and 1 PD + CKRT. No patient was treated with intermittent HD. Although the survival of infants with AKI treated with KRT was higher than that previously reported (76% vs. 44%), the use of KRT was significantly associated with survival (76% vs. 90,9% in AKI patients not treated with KRT; $p < 0.01$) [175].

Peritoneal Dialysis

Peritoneal dialysis (PD) has been extensively used in infants given its simplicity, ability to perform without sophisticated machines, and its slow, continuous action. It is usually well tolerated in small infants [176]. PD has been successfully performed also in very low-birth weight and even extremely low-birth weight neonates, where extracorporeal dialysis faces anatomical barriers.

PD is performed after placement of straight or curled infant catheters. This can happen by percutaneous placement or by surgery. Although surgical implantation is considered the gold standard for PD placement in children, percutaneous placement is a valid alternative and may become essential in low income countries where surgery may not be available. The catheter can be placed by a trocar or, more safely, by Seldinger technique [177].

The most frequent complications include: leakage from catheter entrance, peritonitis, catheter obstruction, bleeding at catheter insertion, exit site infection, hyperglycemia and bowel perforation are the main PD complications [177].

PD prescription in critical neonates is based on frequent, continuous exchanges, with low volumes of dialysate. Regardless whether the catheter is placed percutaneously or surgically, initial loads should be in a low range of 10–20 mL/kg of body weight since larger loads may cause dialysate leakage and diaphragm lifting with respiratory complications [177]. Starting with a standard glucose concentration (1.5 g/dL) is recommended to avoid initial hyperglycemia. Subsequently ultrafiltration rate must be monitored and dialysate glucose concentration adapted according to ultrafiltration needs [177].

Dwell times are usually short, down to 30–40 min in neonates. When large volumes of ultrafiltration are obtained, excessive sodium loss in the ultrafiltrate may occur. This loss is related to patient size and is most marked in neonates. Oral, intravenous or intraperitoneal sodium supplementation may be needed [177].

Outcome data in neonates who undergo PD after cardiopulmonary bypass surgery suggest that early PD improves outcomes. Data on 146 infants who underwent cardiopulmonary bypass surgery, significantly better survival at 30 and 90 days was observed with "early" PD (started at the end of surgery or day after surgery) as compared with controls starting PD after the second day after surgery. The impact of fluid overload on outcome was analyzed in a prospective trial comparing two interventions in infants undergoing cardiac surgery. In the first study period, ascites was passively drained through a PD catheter placed at the time of surgery. In a second series of patients PD was initiated within 2 h of arriving at the cardiac ICU. The infants receiving active PD had significantly more negative net fluid balance, lower mean inotrope score, lower serum cytokine concentrations and earlier sternal closure compared to the infants who had only their peritoneal fluid drained [178].

Further analysis of these and other studies in infants undergoing cardiopulmonary bypass surgery confirmed that attainment of a negative fluid balance is associated with improved survival and other clinical outcomes. Taken together, early start of dialysis, avoidance of fluid overload and its consequent correction appear to favor a good outcome in critically ill infants and may be more important than the choice of treatment modality.

Hemodialysis (HD) can be performed in infants with good results, although poor vascular tolerance and the large extra-corporeal volume provide additional challenges [173].

Hemodialysis

Hemodialysis (HD) can be performed in infants with good results, although poor vascular tolerance and the large extra-corporeal volume provide additional challenges [173]. Although **Extracorporeal Dialysis** has been performed in neonates in the past five decades the main problems that have been limiting its widespread use are vascular access and cardiovascular tolerance. HD has mainly been used in metabolic crisis and intoxications, when urgent toxic compound removal is needed. There are few reports on the use of *intermittent hemodialysis* (*HD*) in neonates with AKI. In 1994, Sadowski described 33 acute infants weighing less than 5 kg treated with intermittent HD. A high rate of hypotensive episodes was reported (64%), and a 52% survival rate [173].

Blood flow is mainly dependent on vascular access performance. It is usually prescribed as 5–10 mL/kg body weight; in patients needing a high clearance rate (e.g. hyperammonemic crisis) the maximally achievable flow rate should be used and will be rate limiting for the efficacy of purification. Standard or even lower dialysate flows (300–500 mL/min) combined with low neonatal blood flows usually provide maximal solute extraction. Dialysate warming systems are provided by any HD monitor and preserve neonatal thermoregulation. Vascular access for extracorporeal dialysis is often troublesome in neonates due to the mismatch between vessel size and the diameter of the catheters minimally required to obtain adequate blood flow.

Continuous Kidney Replacement Therapy (CKRT)

Continuous Kidney Replacement Therapy (*CKRT*) may allow for achievement of the goals of therapy better than HD in neonates. Because it allows for 24 h metabolic and fluid control as opposed to the transient correction and shifts that occur between intermitten HD sessions.

The most commonly used catheters in neonates are dual-lumen, low resistance catheters. 6.5–8 F caliber. The length of the catheter will be depending on whether the catheter is tunneled or not, and whether the catheter is placed in the right internal jugular, left internal jugular, or femoral approach. As an alternative, two smaller single-lumen catheters have been used successfully [187].

Vascular access in children and infants on CKRT can be challenging. Results from the

ppCRRT Registry, 5-French catheters showed the poorest function, none of them lasting more than 20 h [188]. Catheterization of the internal jugular vein and the use of CVVHD was found to be associated with the best catheter survival introduction of latest-generation CKRT machines might change the choice of vascular access in neonates (see below).

Until recently, CKRT was performed using tubing, filters and consoles originally conceived for adults [189]. However, problems related to the fluid accuracy and large extracorporeal volumes compared to patient blood volume pose to the smallest neonates. The larger the extracorporeal volume the higher the risk of hemodynamic instability during start, hypocalcemia, acidosis and dilution effect of hemoglobin, platelets and coagulation factors. Blood prime procedures have been reported to minimize the blood exposures.

The creation of monitors and circuits specifically dedicated to neonates has profoundly changed the approach to the extracorporeal treatment of newborns with AKI. In Tables 53.6 and 53.7 list of filters and monitors available for neonates in 2022.

In 2012, Ronco et al. described the creation of the first CKRT monitor for infants <10 kg BW [190] and in 2014 the first patient was treated with this specific device [191]. This machine was approved by the US FDA in April 2020. It provides extraordinary accuracy in blood pump and fluid balance with a blood priming volume ranging 27–42 mL. The machine provides either CVVH and CVVHD (but not both). Blood flow can be set at 1–50 mL/min and dialysis flow from 1 to 10 mL/min, with a maximum effluent flow (net ultrafiltration + dialysate) of 20 mL/min. The maximum fluid removal is 1000 mL per day, with an accuracy of 1 mL per hour. Carpediem® works with such small catheters thanks to a three-roller blood pump that reduces the dead space of the pump tubing segment and the pulsatile profile of the blood flow as compared to conventional two-roller pump systems. This allows continuous aspiration even at very low blood flows through very small catheters [190]. 5F double lumen or combinations of 3.5F and 5F single lumen catheters were used in a series of 26 neonates treated with Carpediem® [192]. 25 of the children survived the CKRT period and 18 survived the NICU stay.

In 2014, Coulthard et al. showed the ability of a syringe-driven machine, the Newcastle Infant Dialysis and Ultrafiltration System (NIDUS®, Allmed, London, UK) to provide better clearance and more accurate ultrafiltration than peritoneal dialysis [193]. The device withdraws 5–12.5 mL aliquots of blood from a single-lumen central venous line, runs it across a dialysis filter and returns it back through a syringe pump. The I-KID study, a randomized clustered wedge study was conducted in England. Study results are expected to be shared in 2022 [194].

Askenazi et al. adapted the Aquadex® (CHF Solutions, Eden Prairie, MN) ultrafiltration system to provide CVVH to neonates [195]. Aquadex® was originally conceived for the treatment of edema in adult cardiac patients but its small priming volume was exploited to create a hemofiltration system in infants. The machine provides a blood flow of 10–40 mL/min; it is equipped with a 0.12 m^2 polysulfone membrane and a second pump that produces up to 500 mL/h ultrafiltrate in 10 mL/h increments. Reinfusion is provided by an infusion pump external to the circuit. In a 3-center retrospective study among 117 children treated with the Aquadex® system, 72 were under 10 kg body weight and 23 of these survived to discharge. Complications during therapy were seen in 15% of treatments and most were vascular access–related [196].

CKST Prescription for Neonates

No official recommendations for *adequacy* in critically ill infants exist. In neonates with AKI or congenital kidney failure, the daily clearance is potentially much higher than intermitten HD because clearance can occur continuously. The broader aims of metabolic and fluid control, hemodynamic stability, vascular access preservation, nutritional adequacy, and appropriate levels of essential drugs (i.e. anti-microbials) may be more important than waste product removal rates. Some programs aim for filtration rate of 2000 mL/h/1.73 m^2 others aim for a filtration rate of 20–25 mL/kg/h.

Given the immaturity of neonatal thermoregulation, CKRT induced heat loss could hamper the thermal protection adopted in NICU, especially in low birth weight neonates, with unpredictable consequences on vascular stability. Warming of fluids is needed and different approaches are available. Neonates are at an intrinsically high risk of hemorrhage, and extracorporeal treatment modalities are clearly fraught with an increased risk of hemorrhage. Safe and effective anticoagulation protocols are critical to the success of CKRT.

In the ppCRRT registry, small children <10 kg were more likely to receive heparin anticoagulation compared to citrate regional anticoagulation. In the overall registry, the rates of circuit survival was similar between groups, but the heparin group had higher rates of bleeding than the citrate group [197]. In those receiving heparin anticoagulation, some programs monitor aPTT (goal range 50–70) other target ACT rates of 160–180, while others target anti-Xa levels of 0.3–0.5. The use of citrate regional anticoagulation in smaller children can be more challenging for several reasons. First, traditional CKRT machines require a minimum blood flow, and the blood flow rate per kg is inversely related to body weight. Because the citrate dose depends on blood flow, the amount of citrate used in small children is therefore higher and may require higher clearance rates to achieve target levels. Furthermore, premature infants are born with immature liver function.

In critically ill children with a low body weight.

Management of Hyperammonemia with Kidney Support Therapy

A peculiar indication to KRT in the neonatal age group with metabolic crisis due to Inborn Errors of Metabolism (IEM), most often manifesting as neonatal hyperammonemia or leucinosis. Hyperammonemia is a severe clinical condition characterized by high ammonium levels, excess glutamine accumulation in astrocytes inducing cell swelling and brain edema. In most cases, it presents in full-term neonates with anorexia, seizures, lethargy, coma and death. Most frequently, it is caused by urea cycle defects (UCD) and organic acidurias (OA). The initial management of an undiagnosed hyperammonemia includes stopping protein intake, intravenous glucose, and initiation of first-line medications including L-arginine, nitrogen scavengers, carbamylglutamate, carnitine, vitamin B12, and biotin. When conservative treatment fails and whenever there is severe symptomatic hyperammonemia, dialysis has to be rapidly established in order to avoid permanent neurological sequelae or death [198]. In leucinosis (maple syrup urine disease; MSUD), deficiency in branched chain ketoacid dehydrogenase leads to the accumulation of branched chains aminoacids (BCAA) leucine, isoleucine, and valine in cells and body fluids. Given the poor renal clearance of BCAA, their accumulation can cause neurologic damage. Medical treatment consists in incorporation of BCAA into protein synthesis with nutritional support but, like in hyperammonemia, it may not be successful and dialysis must be started to clear excess BCAA [199]. In both cases, extracorporeal dialysis provides higher clearances than PD [198, 200]. HD provides highest ammonium clearance and it has been recommended as gold standard of therapy [201], although high volume CKRT (initially at 8 L/h/1.73 m^2) has been proposed as an alternative [198, 200, 202]. The practical application of CKRT in neonatal metabolic decompensation differs from that of AKI since it has been demonstrated in vitro that the clearance of ammonium and leucine achieved with CVVHD depend on dialysate flow rate, being substantially higher with increasing flow rates [200]. However, as in the case of AKI, there is no definite demonstrated association between a specific dialysis modality or of dialysis efficiency with survival. In the study of Schaefer et al. [200], out of nine patients, the five with fastest depuration survived with no or mild neurological impairment while the other four died or survived with severe sequelae. By contrast, in the study of Pela et al. [203], four out of seven neonates with organic acidurias treated with PD survived with no or mild neurological impairment. In a series of 12 neonates with metabolic crises from different IEM, all survivors showed plasma ammonia less than 300 μmol/L

after 8 h of KRT and significantly lower ammonia concentration than non-survivors at the same time of treatment. This difference was not related to dialysis modality [180]. In a systematic review of 90 reports on the impact of ammonium levels and dialysis on outcome in 202 infants with UCDs, a higher ammonium level was a determinant for starting dialysis but no significant influence of this treatment on outcome was observed [181]. In a series of 45 hyperammonemic newborns treated with KRT, predialysis ammonium levels were significantly associated with a composite end-point of death or neurological sequelae while the outcome was not related with dialysis modality. Interestingly, while the patients treated with HD had a shorter ammonium decay time compared with all other patients ($p < 0.05$), no significant difference in ammonium reduction rate was observed between patients treated with PD, CAVHD or CVVHD.

The above considerations raise the question if it is correct to evaluate dialysis efficiency by decay time. Under these circumstances, ammonium concentration is strongly dependent on the metabolic state and the majority of patients start dialysis with a medical therapy already under way. Dialysis is part of an overall treatment setting which includes variably efficacious pharmacological support and variably timed initiation of medical treatment and dialysis, with a major modifying influence of the type of underlying metabolic defect on final outcome.

In summary, while ammonium and leucine are small, unbound molecules and behave as such during dialysis (i.e.: best clearance by diffusion, most rapid depuration by HD, less rapid depuration by CKRT and by PD), the dialysis modality does not determine the outcome. A practical approach to the treatment of hyperammonemia in infants has been recently proposed [204].

Outcomes of Neonatal AKI

AKI is associated with significant mortality in critically ill children [205, 206] and adults [207–211], even after controlling for medical co-morbidities, severity of illness scores, and patient demographics. Epidemiological studies in several high-risk groups of neonates have been performed, including very low birth weight infants, near term/term infants with perinatal depression, neonates with severe asphyxia undergoing hypothermia, neonates receiving extra-corporeal membrane oxygenation and infants with cardio-pulmonary bypass—associated AKI. Using a categorical AKI frameworks the RIFLE classification systems [205, 207] allow for improved diagnosis and staging of AKI by severity, these studies have provided some evidence that AKI is independently associated with mortality in neonates and young infants even when controlling for potentially confounding demographics, co-morbidities, and interventions.

Critically Ill Neonates

In the multinational retrospective observational Assessment of Worldwide Acute Kidney injury Epidemiology in Neonates (AWAKEN) study using the KDIGO neonatal AKI definition, the incidence of AKI in NICU patients was about 30%, with gestational age following a "U"-shaped distribution (AKI incidence: < 29 week: 45%; ≥29–36 week: 14%; ≥36 week: 41%). Even after controlling for multiple potential confounders, neonates with AKI had 4.6 times higher adjusted odds of death and 8.8 more adjusted hospital days [175]. These associations held true for all gestational age groups [175]. A full account of all studies conducted in this population is beyond the scope of this chapter. Review of outcome studies in premature neonates [212–214], neonates with perinatal asphyxia (REF), those who receive extra-corporeal membrane oxygenation [215] those who undergo cardio-pulmonary bypass surgery have been recently published [216].

Outcomes of Neonates Treated with Kidney Support Therapy (KST) for AKI

The interpretation of outcome of infants treated with dialysis is hampered by several problems. First, the reasons to start KSTt for AKI or not based on AKI diagnosis/criteria. Rather, most pediatric patients initiate kidney support for fluid

overload or a combination of fluid overload and AKI. Secondly, the timing of support and the treatment choices are made according to local availability, clinician preference and expertise. Thirdly, the modality, dose and approach are set to institutional practice. Finally, programs will provide kidney support (or choose not to support) neonates based on the severity of illness. Programs that provide kidney support for neonates of higher severity of illness would be expected to have higher mortality rates. Furthermore, most of the studies are retrospective studies at risk for reporting bias.

With this in mind, the overall survival rates of critical ill neonates treated with dialysis are generally poorer than those reported for older children. In the Prospective Pediatric Continuous Renal Replacement Therapy (ppCRRT) Registry, the cohort of children <10 kg (n = 84) had lower survival rates than children >10 kg (n = 166) (43% vs 64%). Not differently from older children, fluid overload, high PRISM2 score and urine output at dialysis initiation were found associated with mortality. In particular, patients with fluid overload >20% at the time of CKRT initiation had an almost five times higher odds ratio of death than those who initiated CKRT with <10% fluid excess. Survival was better in neonates who were able to achieve dry weight during CKRT. This suggests that fluid overload predisposes to a poor outcome but its correction may reverse this association [143]. Recent studies using novel and adapted machines with small extra-corporeal volumes suggest better outcomes [196, 217].

Long-term Effects of Prematurity and Neonatal AKI on Chronic Kidney Disease

Links between prematurity/low birth weight and chronic kidney disease in adulthood become increasingly apparent [218]. Figure 53.3 depicts the possible etiology of chronic kidney disease in premature and low birthweight infants. Pre-term delivery disrupts nephrogenesis, which is not complete until around 34–36 weeks gestation. A small number of autopsy studies have suggested that nephrogenesis continues for only a short time after birth [9, 219, 220]. The remaining nephrons hypertrophy to compensate for decreased nephron mass and, according to Brenner's hypothesis, the resultant "hyperfiltration" eventually becomes deleterious and leads to glomerulosclerosis with sodium retention, systemic hypertension, proteinuria and progressive chronic kidney disease [221]. Thus, premature birth may 'prime' infants for kidney injury and chronic kidney disease. Indeed, premature infants with a birthweight less than 2500 grams have nearly twice the odds of having low glomerular filtration rate, microalbuminuria, end-stage kidney disease and hypertension than their term counterparts [222].

Moreover, the impact of additional AKI events in the NICU on long-term kidney and health outcomes is not yet known. Previously, it was assumed that subjects who survive an episode of AKI would recover kidney function without long-term sequelae; however, recent data from animals [223], critically ill children [224, 225] and adults [226–238] suggest that AKI survivors are indeed at risk for development of CKD. A meta-analysis in adults with AKI showed an

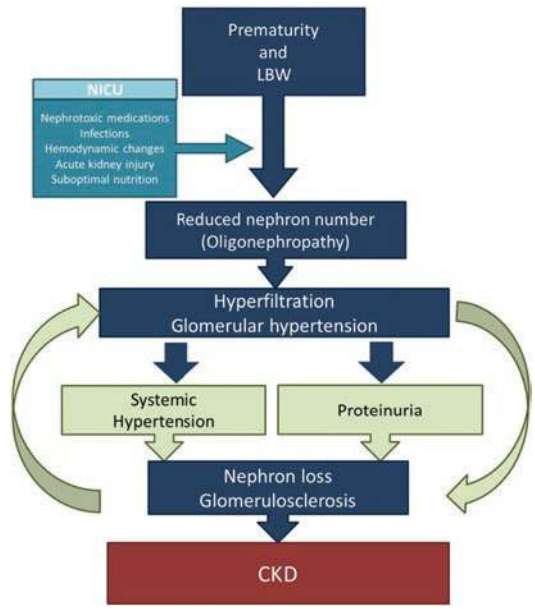

Fig. 53.3 Possible etiology of chronic kidney disease in premature and low birthweight Infants [218] (from Carmody et al. Pediatrics 2013)

almost ninefold risk to develop incident CKD, a threefold risk to progress to end-stage kidney disease and a doubled mortality risk compared to patients without AKI [239].

The role that AKI plays in the development of CKD in the infant population is still unknown. Several small single center retrospective reports have suggested that CKD can develop in infants who had AKI [101, 240]. Human autopsy and animal studies suggest that AKI affects post-natal nephron development. Premature infants who suffer AKI were shown to have fewer layers of nephrons and abnormally configured glomeruli compared to term neonates [9, 220]. Large prospective cohort studies designed to determine risk factors for long-term CKD are needed to define the most appropriate surveillance protocols and identify the subjects at greatest risk.

Future Directions in Neonatal AKI

Our understanding of neonatal AKI has improved and we now have clear epidemiological evidence suggesting that AKI is common and associated with mortality in this age group. New AKI definitions based on SCr, urine output, Cystatin C and emergent urinary biomarkers promise to improve our ability to reliably define neonatal AKI. Studies in VLBW neonates [241, 242], infants undergoing cardiopulmonary bypass surgery [243–251], and other sick NICU patients suggest that these biomarkers can predict a subsequent rise in SCr as well as mortality [84]. However, large comparative studies will be required to determine which functional and kidney injury biomarkers can best predict hard clinical outcomes. Importantly, the normal ranges of urine biomarkers in premature neonates will require careful validation since excretion varies by gestational age, probably due tubular immaturity [241]. With better definitions and earlier indicators of AKI, we will be able to better understand the risk factors, develop preventive strategies, and timely apply appropriate management to improve outcomes in those at risk for AKI.

Another emerging breakthrough regards the provision of extracorporeal treatments for neonates. Thanks to major technological advances, dialysis machines miniaturized for the specific needs of neonates have become available. Machines such as the CARPEDIEM, Aquadex and NIDUS are making important differences in the ability to provide safe and effective therapies in small infants. The main advantage of these machines is the low extra-corporeal volume which drastically lowers the rates of hemodynamic instability at circuit initiation. Furthermore, smaller circuits allow for smaller catheters to be used, enabling extracorporeal therapy even in very small neonates. Maximizing the efficiency and safety of kidney support therapies with these devices will change our approach to the neonate with AKI. One day soon, any infant who could benefit from kidney support therapy will no longer be left without an appropriate and safe treatment option.

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Part XII

Chronic Kidney Disease

Demographics of CKD and ESRD in Children

54

Julien Hogan and Karlijn J. van Stralen

Introduction

Irreversible kidney damage or so-called chronic kidney disease (CKD) has become a major public health problem worldwide. The adult population has been the subject of extensive epidemiological research [1, 2] but fewer data are available about CKD in children [3]. Despite major scientific advances resulting in substantial improvement in the care of children with CKD, some will still progress and require kidney replacement therapy (KRT). ESKD is a devastating disorder causing substantial mortality and morbidity (most notably cardiovascular, cancer and infection), but this is compounded by specific problems which occur in children such as impaired growth and psychosocial adjustment [4], all of which severely impact upon quality of life [5]. Understanding of the epidemiology of CKD in children is required in order to make a precise and early diagnosis, identify preventable or reversible causes of progression, predict prognosis, and aid the counseling of children and their families.

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Part I: CKD (Stages I–4)

Definition of CKD

Precise data on the epidemiology of CKD in children allowing the evaluation of the incidence and prevalence of CKD and the comparison between countries is lacking. This was in part due to the lack of a universal definition of CKD. For example, the ItalKid Project and North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS) defined CKD as having a glomerular filtration rate (GFR) of below 75 mL/min/1.73 m^2 [6, 7]. Others based their definition on serum creatinine levels themselves or on other thresholds of GFR [8–10]. In 2002, the National Kidney Foundation's Kidney Disease Outcomes Quality Initiative (NKF-K/DOQI) published a classification of CKD applicable to children [11]. CKD was defined by the persistence for more than 3 months of morphological, histological or biological abnormalities of the kidneys and/or a glomerular filtration rate (GFR) below 90 mL/min/1.73 m^2. This classification grades CKD in five stages from stage 1 with normal GFR to end-stage kidney disease (ESKD, stage 5). The K/DOQI classification was revised in 2012 by the KDIGO (Kidney Disease: Improving Global outcomes) to reflect the risk of progression to ESKD and is based on both GFR and albuminuria [12]. Of note, some pediatric specificities need to be considered when using this classification: a) the criteria for duration >3 months

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does not apply to newborns or infants <3 months of age, (b) the criteria of a GFR <90 mL/min/1.73 m^2 does not apply to children <2 years of age as neonates are born with lower GFR, which increases to normal values in the first 2 years of life, (c) a urinary total protein or albumin excretion rate above the normal value for age may be substituted for albuminuria ≥30 mg/24 h. A similar classification based on the same GFR cut-offs and on the urine protein-creatinine ratio and specifically validated in two large pediatric cohorts (CKID in the USA and ESCAPE in Europe) has been developed recently (Fig. 54.1; Table 54.1) [13].

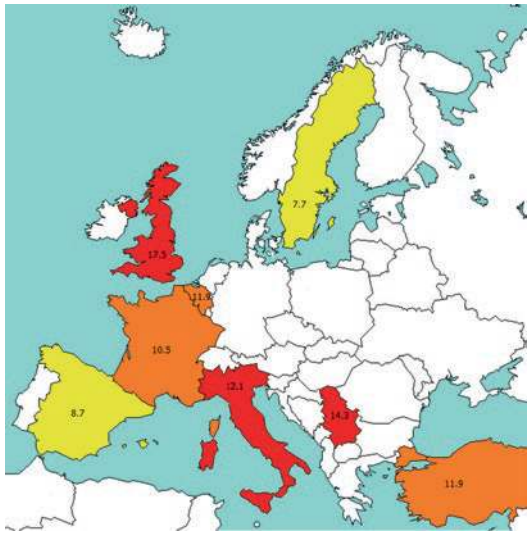

Fig. 54.1 Reported incidence (pmarp) of CKD in children in Europe

The new classification was widely adopted after its introduction; however, its limitations and possible modifications have been a matter of extensive discussions. Moreover, despite efforts to standardize creatinine measurement (by using enzymatic methods instead of colorimetric methods) and GFR estimation, there are still ongoing debates on which eGFR equation should be used in various clinical conditions particularly in early stages of kidney injury [14–17]. In 2009, the bedside Schwartz formula using height and serum creatinine and a unique k coefficient was developed and remains frequently used in clinical practice [18]. Since then many equations have been published using serum creatinine or cystatine C or a combination of both. Recently, papers focused on developing formulas that perform consistently over the whole range of GFR from infants to young adults (FAS [19], CKID U25 [20]).

Screening for CKD

CKD screening and surveillance in adults, either population-based or targeted at risk populations, has become widely advocated and implemented in many countries worldwide, in an attempt to prevent ESKD and the progression of CKD. However, the benefit of screening for early-stage CKD is uncertain [21]. The benefit of such programs in children is even more uncertain [22]. Tests used for CKD screening in children

Table 54.1 CKD classification and estimated risk of progression adapted from (Furth et al. AJKD)

			Baseline UPCR		
			<0.5	[0.5, 2.0]	>2.0
Baseline GFR	I	≥ 90	IR = 2.3 (0.7, 7.0) per 100p-y	–	–
	II	[60–90)	IR = 1.5 (0.8, 2.6) per 100p-y	IR = 8.1 (4.8, 13.8) per 100p-y	IR = 14.2 (6.4, 31.6) per 100p-y
	IIIa	[45–60)	IR = 3.6 (2.5, 5.0) per 100p-y	IR = 6.4 (4.5, 9.1) per 100p-y	IR = 22.8 (13.7, 37.8) per 100p-y
	IIIb	[30–45)	IR = 5.9 (4.4, 7.8) per 100p-y	IR = 10.7 (8.1, 14.1) per 100p-y	IR = 32.0 (23.5, 43.6) per 100p-y
	IV	[15–30)	IR = 17.4 (12.8, 23.6) per 100p-y	IR = 24.8 (19.5, 31.6) per 100p-y	IR = 58.4 (44.5, 76.6) per 100p-y
	V	<15			

Based on baseline GFR and UPCR patients are classified in 6 groups (colors) based on their risk of progression. Incidence rates (IR) of 50% GFR decline or GFR < 15 mL/min/1.73 m^2 are reported

are usually limited to urinary dipstick protein instead of urine albumin/creatinine ratio or on creatinine-based calculation of estimated GFR as recommended for adults. There is also a large variation in the methods used and approaches taken by the different countries, and the findings have shown poor reproducibility [22].

The main studies about screening for CKD in children are summarized in Table 54.2 [23–34]. Mass screening programs to detect CKD in children have been undertaken for many years in several Asian countries such as Japan, Taiwan and Korea [23–25]. Conversely, screening programs have not been adopted in Europe or Australia but screening using urine dipsticks have routinely been performed in healthy children for decades in the United States. In 2000, the recommendations from the American Academy of Pediatrics were to screen the urine of preschool children and adolescents [35]. This policy has been revised in 2007 and this practice is no longer recommended [36]. Although a decrease in the incidence of ESKD has been observed in Japan and Taiwan, there is only limited evidence that early detection of kidney injury in children may lead to effective interventions to slow progression of CKD and further reduce the risk of developing ESKD [22]. Furthermore, some studies suggest that a urine dipstick is not a cost-effective strategy for screening in children [37] given the high prevalence of transient proteinuria in this population. Although some population-based studies assessing CKD epidemiology by GFR estima-

Table 54.2 Results from studies reporting screening programs from chronic kidney disease in children

Country [Reference]	Study period	Study population	Screening criteria	Main findings
Japan [23]	1974–2002	Population-based 6–14 years old	2 positive urine dipsticks	Prevalence of Pu: 0.07% in 6–11 years 0.35% in 12/14 years old
Taiwan [24]	1992–1996	Population-based 6–15 years old	Pu > 100 mg/dL CKD (SCr >1.7 mg/dL)	Prevalence Pu 0.06% Prevalence CKD 0.002%
Korea [25]	1998–2004	Population-based 6–18 years old	2 positive urine dipsticks	Prevalence Pu 0.2%
Australia [26]	2004–2008	Population-based (57% Arboriginal) 4–14 years old	Single assessment Urine dipsticks uACR≥3.4 mg/mmol	Prevalence of albuminuria at baseline 11.5%
India [27]	2013–2016	Population-based 8–18 years old	Single assessment Urine dipsticks	Prevalence of Pu 1.9% Prevalence of hematuria 5.2%
USA [28]	2009–2014	Population-based 12–18 years old	Albuminuria (uACR) eGFR (bedside Schwartz)	Prevalence of albuminuria: 13.7% Prevalence of persistent albuminuria 3.29% Prevalence of eGFR<60: 0.91%
Singapore [29]	1999–2000	Population-based 12 years old	Urine dipstick	Prevalence of Pu 1.3%
China [30]	2003–2005	Selected population School age children	2 positive urine dipsticks	Prevalence of Pu and/or Hu in 2 specimens: ~1%
Finland [31]	NA	Selected population 8–15 years old	4 urine samples (2 morning, 2 evening. Dipstick and measured Pu	Prevalence of Pu: 10.7% on at least 1 specimen 2.5% on 2 specimens 0.1% on 4 specimens
Iran [32]	NA	Selected population 6–7 years old	Urine dipsticks	Prevalence of Pu: 3.6% Prevalence of persistent Pu: 1.3%
Mexico [33]	2006–2007	Selected population 0–18 years old	Single assessment urine dipstick eGFR (Schwartz)	Prevalence of Pu 16.1% Prevalence of hematuria 17.5% Prevalence of eGFR<60: 1.7%
UK [34]	1967–1969	Selected population 5–16 years old	2 positive urine dipsticks	Prevalence of persistent proteinuria 0.8% on 2 specimens

tion have been performed and indicate that a certain proportion of asymptomatic children have CKD, no systematic national screening program based on GFR assessment in children is currently ongoing.

Demographics of CKD

There is limited information on the epidemiology of early stages of CKD in children. As CKD is usually asymptomatic in its early stages, providing precise epidemiological data is difficult so CKD in children is likely to be underestimated and underreported. Although some pediatric CKD registries using the K/DOQI classification are beginning to emerge, only a few reports on the epidemiology of CKD stages 2–5 in children are available. Due to lack of resources and national renal registries, we know even less about the incidence and prevalence in low income countries. For these countries, data are mostly obtained from reports of major tertiary care referral centers, but the validity of this data is variable.

Europe

The largest population-based study in Europe on the epidemiology of pediatric CKD is the ItalKid project. This study in Italy has been collecting data since 1990 on the epidemiology of childhood CKD, describing the natural history of the disease, and identifying factors that influence its course [6]. So far, nearly 1198 patients have been registered. Other nation-wide European studies are the Serbian CKD registry [38], collecting data on over 336 patients since 2000, the Belgium CKD registry which started in 2001 and has over 143 patients [39], and the data from the Swedish Pediatric Nephrology Association [40]. Also regional studies have taken place in Spain [41], the South-East of the UK [42] and Lorraine in France [8].

Several pediatric nephrology societies from European countries have provided data on the early stages of CKD. Even though age categories and definition of CKD differed between countries, incidence in Europe was consistent, ranging from 8 to 14 per million age-related population (pmarp) for CKD stages 2–5, and being around 8 pmarp for CKD stage 4–5 (Fig. 54.1). The incidence was highest (17.5 pmarp) in a report from the United Kingdom but the study was hospital-based leading to potential referral filter bias and there may be some uncertainty about the covered geographical area [42].

While an increase in incidence since the 1970s was seen in France [8], this was not seen when comparing two time periods in Sweden [9, 40]. Two studies from Serbia and the UK also suggested an increase in incidence in the past 10 years [38, 42]. Prevalence ranged from about 55–60 to 90–95 pmarp in Spain, Italy, UK and Serbia, depending on the clinical definition of CKD that was used in each study.

In Turkey, the CREDIT study reported a prevalence of CKD stage 3–5 of 2600 pmarp in children aged 5–18 years old in 2007 [43].

North America

In Northern America most of the information on CKD in children derives from two large sources of information namely the North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS) [44] and the Chronic Kidney Disease in Children Prospective Cohort Study (CKiD) [45]. Both studies are collecting data on a voluntary basis and are not population based.

Population-based data are available in adolescents since the NHANES study investigated albuminuria and GFR in a nationally representative sample of the US population including 9225 adolescents aged 12–18 years over 3 study periods. They found a prevalence of 0.91% [95% CI 0.58–1.42%] for CKD stage 3–5 and a prevalence of persistent albuminuria of 3.29% [95% CI 1.94–4.63%] in the 2009–2014 survey [28].

Latin America and the Caribbean

In Chile, a national survey of pediatric nephrologists estimated an incidence of CKD (GFR <30 mL/min/1.73 m^2) in children aged less than 18 years of 5.7 pmarp and a prevalence of 42.5 pmarp in 1996 [46]. Among these patients, half were on conservative treatment and the others were on KRT. Very similar results were found in Argentina, with an incidence of 6.5 pmarp, but with a lower prevalence

(15.4 pmarp) [47]. Fifty-eight percent of this population had ESKD and started with dialysis. In Jamaica, the estimated incidence of CKD was 4.6 pmarp and 28% of them were already in ESKD, without having access to KRT [48].

A study on the epidemiology of CKD conducted in several Latin American countries (Argentina, Brazil, Chile, Colombia, Mexico, Uruguay, and Venezuela) has shown a wide variation in incidence that ranged from 2.8 to 15.8 new cases pmarp [49]. Also an indirect estimation of the incidence of CKD in Mexico suggested a very high incidence, between 24 and 39 per million inhabitants, for which the differences within Mexico were explained by the level of social deprivation [50].

Asia

The estimated prevalence of CKD stage 3–5 among Japanese children in 2010 was 29.8 pmarp [51]. This lower prevalence of pre-dialysis CKD in Japan than in Europe was consistent with the lower prevalence of pediatric ESKD in Japan. Two reports from Vietnam and one from Thailand have suggested an annual incidence of hospitalization for CKD around 5 pmarp, most of patients had already reached ESKD [52–54]. Very little is known about pediatric CKD epidemiology in India and China. A survey conducted in 91 Chinese hospitals found a total of 1658 children aged <15 years with CKD stage 3–5 between 1990 and 2002 which suggests a very low incidence of treated CKD <0.5 pmarp [55]. Patients were referred late with advanced CKD or ESKD in 80% and in-hospital mortality was as high as 72%. Similarly, in India 58% of children had ESKD at the time of CKD diagnosis suggesting that children with CKD are underdiagnosed and referred late [55].

Middle East

The referral center for pediatric kidney diseases in Kuwait provided data on children aged 0–15 years with a GFR <50 mL/min/1.73 m^2 [10]. The mean incidence was found to be as high as 38 pmarp whereas the prevalence increased from 188 in 1996 to a rate as high as 329 pmarp in 2003. The marked difference in incidence between Kuwaiti children and non-Kuwaiti residents suggested the role of genetic factors. An incidence of 12 pmarp was found in a Turkish survey including children with a GFR <75 mL/min/1.73 m^2 [56]. An incidence of 11 pmarp and a prevalence of 51 pmarp have been reported in Jordanian children [57].

Africa

Single center studies from sub-Saharan Africa showed very low incidence of CKD estimated at 1–4 pmarp in Nigeria, Sudan, and South Africa [58–61]. Another single center report from Nigeria, however, found an annual incidence of CKD stage 1–5 of 11 pmarp and a prevalence of 48 pmarp [62], which was much higher than the 1.7 pmarp reported in 2004 [63].

Causes of CKD

Type 2 diabetes and hypertension are the leading causes of CKD in adults. The distribution of the causes of CKD in children are very different with major variations between countries. Indeed, congenital abnormalities of the kidney and the urinary tracts (CAKUT) account for 50–60% of CKD cases in children in Europe [6, 38, 39, 42], Japan [51] and the USA [44]. In Turkey and in the Middle East, CAKUT remains the first cause of CKD with often a higher proportion of hereditary nephropathies related to higher rates of consanguinity [10, 56, 57, 64]. Higher proportions of glomerular diseases are found in developing countries such as India, Southeast Asia [52–54], Latin America [48, 49] and Sub-Saharan Africa [58]. The latter may be related to the high prevalence of bacterial, viral and fungal infections in these regions.

Whereas CAKUT predominates in younger patients, glomerulonephritis is the leading cause in children older than 12 years of age. Causes of CKD vary across races, for example, focal segmental glomerulosclerosis, the main cause of glomerular disease, is three times more common in blacks than in whites (19 compared with 6%) and especially among black adolescents (35%) [65].

In general, there is a predominance of male gender (male/female ratio ranging from 1.3 to 3.0). This partly reflects the higher incidence of CAKUT in boys than in girls, but has also been reported in the regions with a high rate of glomerulonephritis.

Part II: KRT (CKD Stage 5D and 5 T)

Epidemiological data on ESKD treated by dialysis or transplantation is more robust thanks to the development of several national and international registries. Unfortunately not every country has such a registry, not all children are reported to the relevant registry, and some countries with registries do not regularly publish reports. Also, as KRT is expensive not all countries are able to offer KRT to children with ESKD. Approximately 80% of the children on KRT live in Europe, Japan or the United States. Dialysis and transplant registries only collect data on treated ESKD; untreated children with ESKD are not captured. However, at least in the developed world, the proportion of children with ESKD who do not receive KRT is likely to be very low [1].

Incidence

The incidence of KRT in children varies greatly between countries but can be estimated between 5 and 10 pmarp [66–70] with extreme values ranging from 0 (Malta) to 17 pmarp (Kuwait) [71]. However, given that pediatric KRT is extremely rare, numbers in smaller countries are subject to random error. Moreover, variations in incidence may reflect variations in the incidence of CKD, differences in pre-ESKD care or differences in access to KRT. Among large countries with universal access to KRT, the US incidence is consistently high at around 12.9 per million population [72]. In Japan, incidence of pediatric KRT (4.0 pmarp) was consistently much lower than in other high income countries (Table 54.3).

Among lower income countries the incidence is typically lower, as was shown for the Eastern European countries in the ESPN/ERA-EDTA registry [73]. In developing countries where KRT is unaffordable for all but the very wealthy, incidence rates are either not available or were extremely low (<1 pmarp in Bangladesh and Nepal). Some of the variation in incidence may be due to differences in the timing of KRT initiation. In Europe, KRT was generally started at a median GFR of 10.4 mL/min/1.73 m^2 whereas mean GFR ranged from 11.3 to 13.6 mL/min/1.73 m^2 in the United States [72, 74] (Fig. 54.2).

Within-country variations occur by racial group. For example African American in the US, arboriginal children in Australia and New Zealand or children from South Asian origin in the UK [72, 75, 76] have a significantly higher incidence of ESKD than their white counterparts, although differences in the prevalence of other ESKD risk factors such as obesity or disparities in access to medical care may account for at least

Table 54.3 Incidence, prevalence and KRT modality distribution among children with ESKD

	Countries	Year	Age	Incidence/ prevalence	Treatment modality distribution		
					HD	PD	Transplantation
ESPN/ERA-EDTA	36 European countries	2018	0–14	4.9/29.8	43.8 15.9	37.9 17.9	17.8 66.2
USRDS	USA	2017	0–21	12.9/98.7	51.3 16.6	27.8 10	20.9 73.4
CORR	Canada	2014	0–19	7.7/65.1	29.5 10	16.4 7	54.1 83
JSPN Pediatric survey 2012	Japan	2006–2011	0–19	4/–	16 –	61.7 –	22.3 –
ANZDATA	Australia and New Zealand	2018	0–17	6–9/25–100	25 6	45 12	30 82

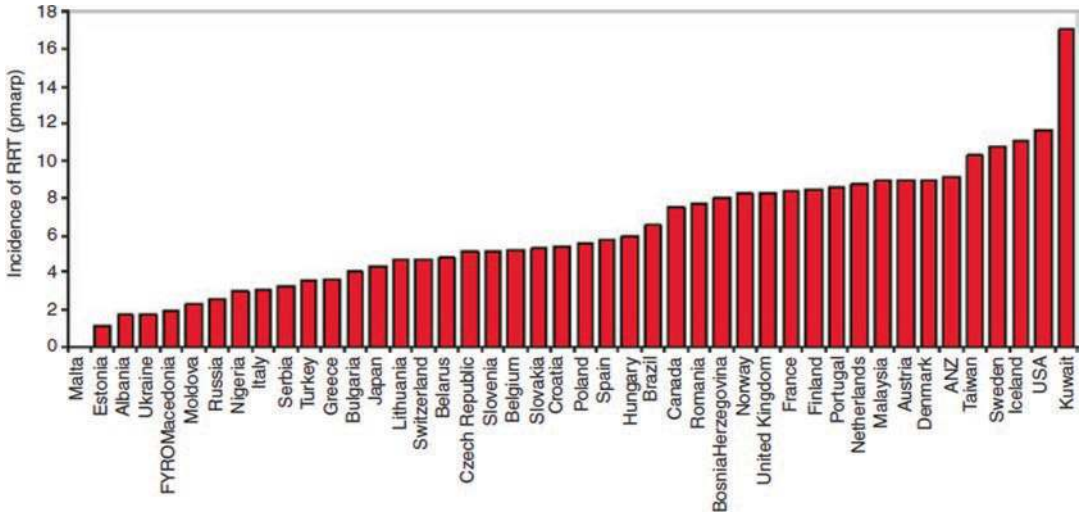

Fig. 54.2 Incidence of KRT in children aged 0–14 between 2008 and 2014

part of these differences. There are also large differences between age groups. The incidence has a typical U-shape distribution, with the highest incidence in the preschool children and in adolescents. Therefore, registries that include patients up to 20 years of age report higher incidence and prevalence data compared with registries excluding those over the age of 15.

Around 20% of patients receive a pre-emptive kidney transplant. In patients starting on dialysis, dialysis modality is strongly dependent on age; while peritoneal dialysis is the treatment of choice in the majority of young children, this pattern decreases with age, with typically higher rates of HD from the age of 10 onwards [75]. Finally, the relative proportion of HD and PD is quite variable between countries and between centers presumably reflecting differences in clinician preference and funding models [77].

Prevalence

The prevalence of treated ESKD is completely dependent on access to KRT in each country. In countries with available data on KRT, the IPNA global registry reports prevalences ranging from less than 1% in some African and Asian countries to 98.7 pmarp in the United States [72, 78]. Indeed, 80% of prevalent ESKD patients live in Europe, North America and Japan, while the prevalence of treated ESKD remains very low in many countries with the highest CKD burden [79]. Within Europe, there are also large differences, with high income countries reporting prevalence rates over 55 pmarp similar to Australia/New Zealand with 56.7 cases per million population [69], while middle income European countries report prevalences around 40 pmarp (Fig. 54.3).

In many countries the prevalence is rising due to the combination of a fairly steady incidence and improved patient survival on KRT. In the United States, the adjusted annual incidence of ESKD in the pediatric population rose slowly during the 1980s then increased marginally from 14 to 15 pmarp between 1990 and 2011 [80]. In contrast, the adjusted prevalence increased from 60 to 85 in between 1990 and 2011. Similar trends were observed in Australia and New Zealand, where the incidence has remained constant at about 8 pmarp over the past 25 years, while the prevalence of KRT increased from approximately 30–50 pmarp [69]. A report from the ERA-EDTA registry on patients aged 0–19 years starting KRT between 1980 and 2000 in 12 Western European countries showed that the incidence of KRT rose from 7 pmarp in 1980–1984 to 10 pmarp in 1985–1989 and remained stable thereafter [81], while the preva-

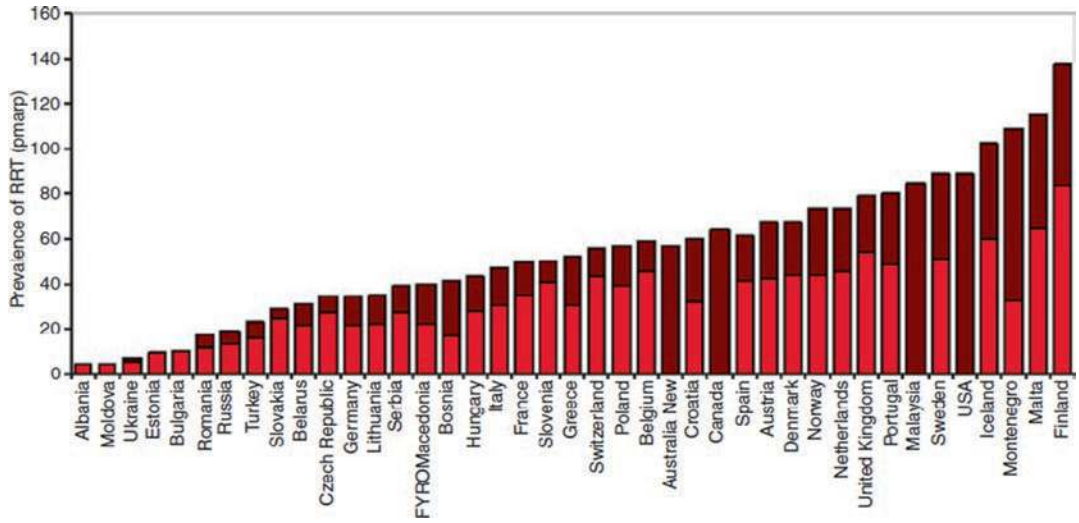

Fig. 54.3 Prevalence of KRT in children on 31st of December 2011 (2012 for Australia and New Zealand and Malaysia). The light bar corresponds to the prevalence in children aged 0–14 years, the sum of the light and the dark bars corresponds to the prevalence in children aged 0–19 years

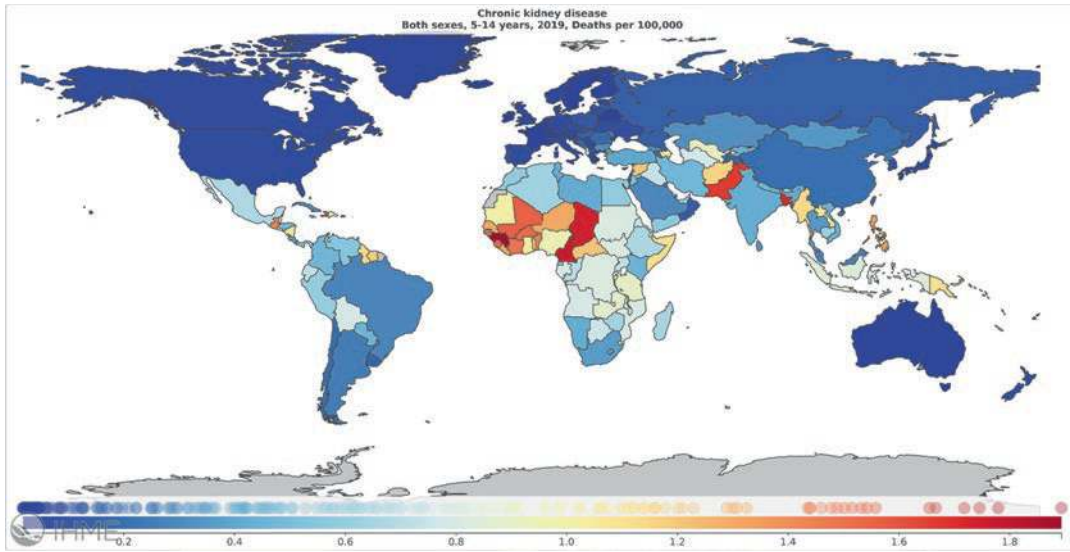

Fig. 54.4 CKD-related death rates in children aged 5–14 years (Global Burden of Disease [83])

lence increased from 22.9 pmarp in 1980 to 62 pmarp in 2000. The increases in prevalence were explained by improved survival and treatment of younger children, while the prevalence was relatively constant for the pubertal age groups.

In developing countries, a lower prevalence of children with ESKD is explained by a low access to KRT [78] and by lower patient survival. Figure 54.4 presents the death rates per country caused by CKD in children aged 5–14 years old in 2019. As expected, this map perfectly matches maps reporting access to KRT by countries [82].

Transplantation is by far the most common treatment modality in most countries, accounting for 60–80% of patients receiving KRT (Table 54.3). Here again, differences among

countries are substantial. For example, fewer than 10% of children on KRT are maintained with a kidney transplant in Belarus, compared with over 90% in Japan and Finland [84]. Recent data show that differences among countries were explained by factors such as the deceased donor rate, the pediatric priority from deceased donor programs, the living donation rate, and healthcare funding models [85]. Compared to adults, children are much more likely to be treated by transplantation due to a combination of fewer comorbidities, higher availability of living donors and, in some cases, preferential allocation of deceased donor kidneys.

Causes of ESKD

the distribution of primary kidney diseases in children reaching ESKD is different than the distribution in children with CKD. Although CAKUT is the most prevalent cause also in children with ESKD, a relatively higher proportion of ESKD cases is caused by glomerular diseases reflecting the faster progression and higher risk of ESKD of this disease group. However, a recent large cohort study from Israel demonstrated that young adults (16–25 years old) with a medical history of kidney disease in childhood but with normal serum creatinine, blood pressure and no proteinuria presented a four-fold increased risk of ESKD over a 30 year follow-up [86]. This underlines the impact of kidney diseases in childhood on ESKD in adulthood and supports long-term follow up of these patients.

There are also very specific local factors. For example, congenital nephrotic syndrome of the Finnish type, explains the very high prevalence of childhood KRT in Finland. Finally, the difference in the distribution of the causes of ESKD in children vs. adults and especially the absence of diabetes or hypertension induced ESKD explains the moderate increase in the prevalence of ESKD in children (+16.6%) in contrast with the major growth experienced by the entire ESKD population (+77%) between 2000 and 2017 in the US [72].

Conclusion

CKD and ESKD in children is a significant public health burden worldwide. Despite significant effort to collect data on children with CKD, the incidence and prevalence of CKD are underestimated in many parts of the world and further studies aiming at improving early CKD diagnosis and at developing effective strategies to slow down CKD progression are needed. For children reaching ESKD, the main challenge is the access to KRT and especially transplantation that remain unavailable to the majority of children with ESKD worldwide.

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55 Progression of Chronic Kidney Disease and Nephroprotective Therapy

Elke Wühl and Franz Schaefer

Progression of kidney malfunction towards end-stage kidney disease (ESKD) is common in patients with chronic kidney disease (CKD), and once significant impairment of kidney function has occurred CKD tends to progress almost irrespectively of the underlying kidney disorder.

It was first shown in the 1930s that removal of three quarters of the kidney mass in rats leads to progressive glomerulosclerosis and functional deterioration of the remaining nephrons [1]. The glomerular lesions of the remnant kidney were associated with abnormal glomerular permeability and proteinuria. At that time, proteinuria was considered a mere marker of the extent of glomerular damage, despite the findings of Volhard and Fahr in 1914 [2] and von Mollendorf and Stohr [3] in 1924 demonstrating that kidney damage was globally related to pathological amounts of protein excreted in the urine. In 1954 Oliver et al. recognized protein droplets in the cytoplasm of tubular cells. It was proposed that proteinuria could lead to structural and functional nephron damage [4]. In the late 1960s Brenner and coworkers described the pathophysiology of the adaptation processes to nephron loss in the rat remnant kidney model. They found that after removal of nephron mass, arteriolar resistance lowers and plasma flow increases in remnant glomeruli [5]. The tone of afferent arterioles was found to drop by a greater degree than those of the efferent arterioles, increasing glomerular capillary pressure and leading to increased filtration rate per nephron. Brenner also demonstrated that therapies attenuating these changes reduce decline of glomerular function and structural alterations.

Today, there is clear evidence from clinical studies that both hypertension and proteinuria are key players in the pathophysiology of CKD progression in humans [6–8]. The renin-angiotensin system is intrinsically involved in this process, and other potential contributors include genetic background, altered mineral homeostasis, dyslipidemia, renal anemia, inflammation and oxidative stress as well as general cardiovascular risk factors such as obesity, smoking and diabetes. The phenomenon of kidney failure progression following kidney injury or maldevelopment is a current focus of research in adults and children.

This chapter summarizes the current state of knowledge regarding the pathophysiology of kidney disease progression and discusses the evidence base of renoprotective strategies in pediatric CKD.

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Natural Course of Chronic Kidney Disease Progression in Children

Information on the natural course of CKD progression in children is still limited. The prospective, population-based ItalKid registry was started in 1990 and over the first 10 years 1197 children with various kidney disorders were registered, including 23% suffering from severe impairment of kidney function [9]. The incidence of kidney replacement therapy (KRT) was 7.3% per year with a 68% cumulative risk of developing CKD Stage 5 by age 20. The decline of kidney function was not linear, but rather characterized by a sharp decline during and after puberty. The probability of kidney survival decreased with lower glomerular filtration rate (GFR) at baseline. This finding supports the general clinical impression that in many children with hypodysplasia of the kidneys, the deterioration of kidney function accelerates around the time of puberty. However, it is still speculative whether this may be due to an increasing discrepancy between body size and functional nephron mass or whether the emerging production of sex steroid hormones at this age may influence kidney survival.

In a retrospective analysis of 176 patients with hypodysplasia of the kidneys, it was suggested that the natural course of kidney function in patients with congenitally reduced renal mass can be divided into three periods: an initial 'hypertrophy' phase characterized by improving kidney function (+6.3 mL/year on average during the first 3 years of life), a subsequent period of stable kidney function attained by 50% of the patients and lasting for a mean of 8 years, and a pattern of kidney function gradually deteriorating towards CKD Stage 5 (Fig. 55.1) [10]. The progression phase started just after infancy in 48% and around puberty in 23%. In 30% of patients kidney function remained stable even beyond puberty. Correlates of deteriorating kidney function were proteinuria, hypertension, past febrile urinary tract infections and lower GFR at onset [10].

An accelerated decline of kidney function was observed during the last 18 months before start of KRT in the Chronic Kidney Disease in Children (CKiD) cohort [11].

In an analysis of 4700 patients with congenital anomalies of the kidneys and the urinary tract followed in the ERA-EDTA Registry, more than two thirds of the patients attained ESKD at adult

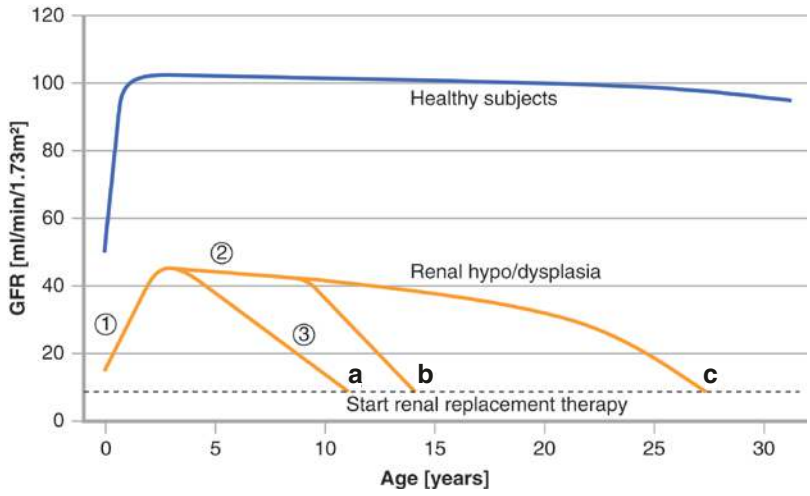

Fig. 55.1 Natural time course of kidney disease progression in children with renal hypodysplasia. ① denotes period of improving kidney function during infancy, ② period of stable kidney function, ③ period of kidney function deteriorating toward ESKD. Third phase is characterized either by rapid decline soon after infancy (**a**) or at early pubertal age (**b**), or by steady slow decline of renal function (**c**). Modified from Celedon et al. [10]

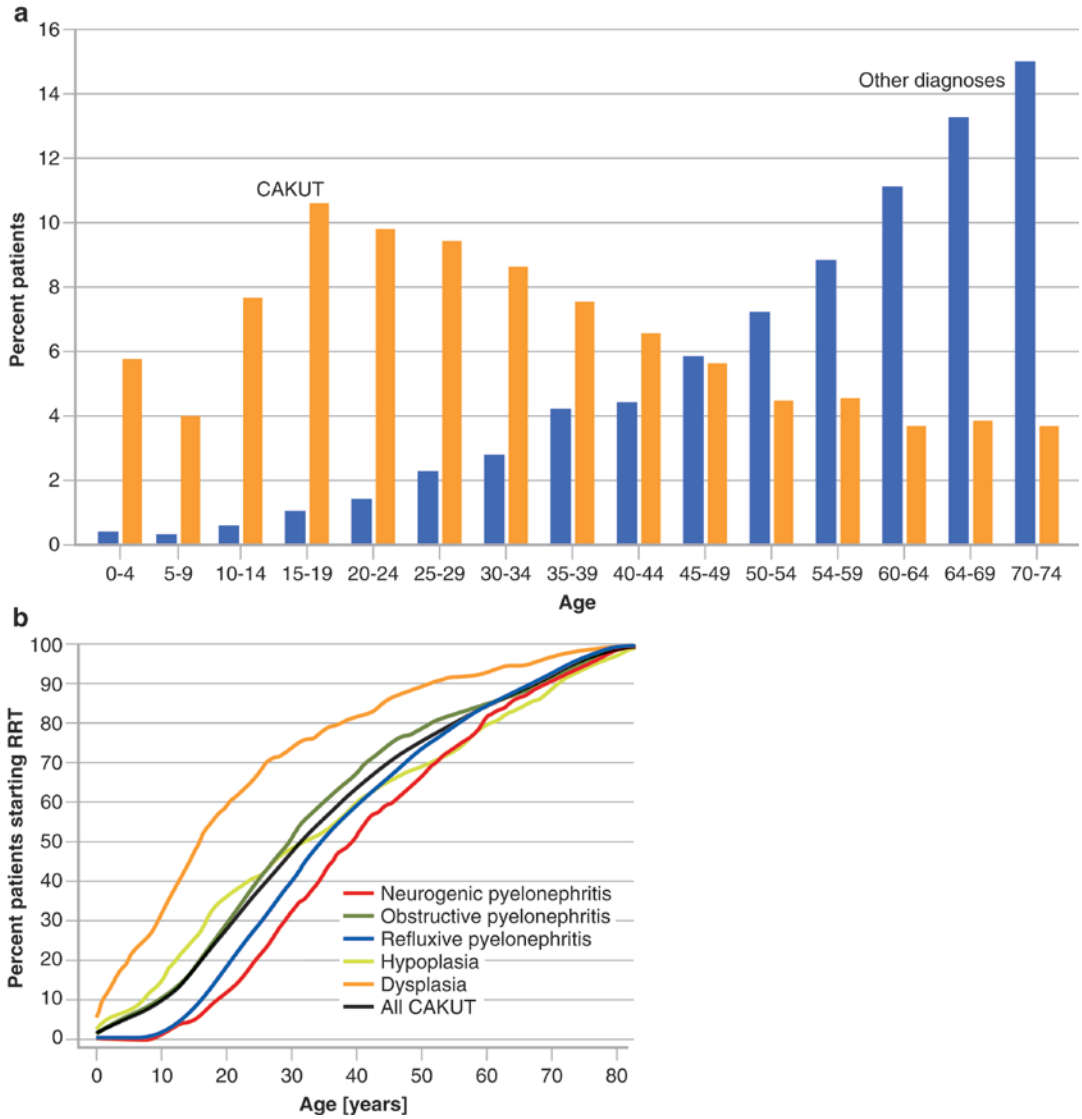

Fig. 55.2 Age distribution at onset of kidney replacement therapy in patients with congenital anomalies of the kidneys and the urinary tract (CAKUT) compared with non-CAKUT patients (**a**) and cumulative percentage of patients starting kidney replacement therapy by CAKUT subcategory (**b**). Data from the European Renal Association – European Dialysis and Transplant Association Registry. Used with permission of American Society of Nephrology from Wühl et al. [12]

age. The mean age at start of kidney replacement therapy was 34 years [12] (Fig. 55.2a, b). These findings support the concept that in patients with a congenital reduction in nephron mass, CKD progression due to ongoing loss of remnant nephrons is a lifelong process.

Mechanisms of Kidney Failure Progression

According to the Brenner hypothesis, any critical loss of functioning kidney mass, irrespective of the nature of the initial injury, leads to glomerular

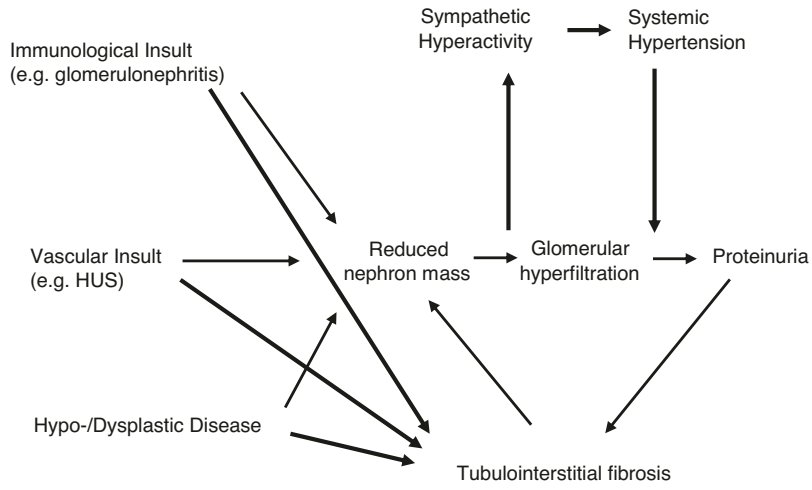

Fig. 55.3 Mechanisms of kidney disease progression in pediatric nephropathies

hyperfiltration with an increased single-nephron GFR (Fig. 55.3). The remaining nephrons lose their ability to autoregulate glomerular pressure, resulting in transmission of systemic hypertension to the glomerulus. Increased intraglomerular pressure induces proteinuria, which is the pathophysiological link between glomerular, interstitial [13] and tubular damage [14]. The degree of proteinuria in glomerular diseases correlates with the rate of CKD progression [15].

Recent research has attributed a central role to the podocytes, which are terminally differentiated and unable to respond to injury by proliferation and repair [16]. The key role of this glomerular cell type in the pathophysiology of CKD progression has been demonstrated by a growing number of specific genetic disorders specifically affecting the development, terminal differentiation and postnatal function of the podocytes, which result in congenital or infantile nephrotic syndrome inevitably leading to glomerulosclerosis and progressive kidney failure. The loss of lesioned podocytes may lead to focal and segmental adhesions of the denuded basement membrane to the Bowman capsule, with spreading of the ultrafiltrate into the tubulointerstitial compartment where it causes inflammatory injury ('misdirected filtration').

The formation and maintenance of the glomerular filtration barrier requires a complex interaction between podocytes and glomerular capillary endothelial cells. Vascular endothelial growth factor (VEGF) overexpression by podocytes has been noted in proteinuric states, and VEGF blocking antibodies prevent glomerular hyperfiltration, hypertrophy and proteinuria. Endothelial cell injury, resulting from disease-specific and/or from non-specific uremia-associated vasculotoxic and inflammatory insults, is frequently involved in progressive glomerular damage [17]. Vascular endothelial cells release endothelin, platelet derived growth factor (PDGF) and fibroblast growth factor (FGF) in response to fluid shear stress [18]. Moreover, injured endothelial cells express increased angiotensinogen and transforming growth factor β (TGFβ) [19], factors causing inflammation and fibrosis. In chronic glomerular injury, endothelial cells lose part of their anti-coagulant properties and intensify their pro-coagulant activity by increased expression of plasminogen activator inhibitor 1 (PAI-1). By release of adhesion molecules such as intracellular adhesion molecule 1 (ICAM-1), endothelial cells facilitate macrophage infiltration and attraction and proliferation of inflammatory cells. In addition, damage to endothelial cells may denude the glomerular basement membrane, all resulting in induction of local platelet aggregation and activation of coagulation with fibrin deposition and microthrombi formation.

Reabsorption of filtered proteins by the tubular epithelial cells can induce direct injury to intracellular lysosomal pathways, oxidative stress

and increased local expression of growth factors [20, 21] such as insulin-like growth factor 1 (IGF-1), TGFβ and hepatocyte growth factor (HGF). Moreover, stressed epithelial cells release an array of chemotactic factors including monocyte chemoattractant protein 1 (MCP-1), RANTES, connective tissue growth factor (CTGF), fibronectin and endothelin-1 (ET-1), which promote tubulointerstitial inflammation and fibrosis through recruitment and activation of macrophages [22–28]. Macrophages infiltrating the renal parenchyma in turn perpetuate the production of further cytokines and growth factors. The proteins of the complement system represent another component of proteinuria that may cause tubular damage, and once tubular cells are injured, they may vice versa activate the alternative complement pathway.

Both in the glomerulus and in the tubular apparatus, chronic inflammatory processes promote cell transdifferentiation towards a fibroblast phenotype, driven by a high tone of TGF-ß (epithelial mesenchymal transformation; EMT). Myofibroblastic transformation is characterized by the release of proteinases, cytokines and oxidants [29, 30]. Moreover, myofibroblasts and local fibroblasts begin to deposit fibronectin and laminin, the molecular framework for interstitial collagen deposition [31], and secrete extracellular matrix components including type IV collagen and collagens I, III, and V ('scar' collagens) resulting in scar formation [32]. In addition, the activity of matrix degrading enzymes is inhibited by overproduction of inhibitory peptides (PAI-1, tissue inhibitors of metalloproteinases (TIMPs)) [33, 34]. As a result of increased synthesis and reduced degradation, excessive tubulointerstitial collagen accumulation occurs. The fibrous masses in the tubulointerstitium are believed to compromise oxygen supply to the tubular cells, thereby further contributing to tubular atrophy and nephron loss.

Glomerular sclerosis, tubulointerstitial fibrosis and tubular atrophy result in a further loss of functioning renal mass, closing a vicious circle by further increasing intraglomerular pressure and hypertrophy of the remaining glomeruli (Figs. 55.3 and 55.4).

Angiotensin (Ang) II**,** the primary effector of the renin angiotensin system, is mechanistically involved in most of the mechanisms described above. Ang II is produced both systemically and locally in the kidney and exerts multiple endocrine, intercrine, autocrine and paracrine effects. Intrarenal Ang II concentrations are a thousandfold higher than in the circulation. The major source of intrarenal Ang II is auto/paracrine synthesis by tubular, juxtaglomerular and glomerular cells [35–37]. Renin release from the juxtaglomerular apparatus drives systemic Ang II generation and is involved in blood pressure upregulation, but has probably little relevance for intrarenal Ang II action. Most of the intrarenal effects of Ang II are mediated via the Angiotensin II type 1 (AT1) receptor [38]. Ang II is a potent vasoconstrictor that augments intraglomerular pressure by preferentially increasing the efferent arteriolar tone. Ang II also increases intracellular calcium in podocytes [39, 40], inducing cytoskeletal changes and altered podocyte function with resultant protein ultrafiltration even in the absence of structural glomerular damage [41, 42]. Moreover, Ang II increases the proliferation of smooth muscle cells and increases the glomerular and tubular expression of various growth factors, cytokines and chemokines, most importantly of the TGF-β/CTGF system, but also of tumor necrosis factor α (TNFα), PDGF, FGF and vascular cell adhesion molecule 1 (VCAM-1).

Ang II also stimulates oxidative stress, which perpetuates the up-regulation of cytokines, adhesion molecules, and chemoattractants [43, 44]. Finally, intrarenal Ang II stimulates afferent neurons, which are believed to activate central nervous structures regulating sympathetic tone. Hence, Ang II is pathophysiologically involved in the state of sympathetic hyperactivation which is characteristic of CKD and constitutes another important mechanism of renal disease progression and cardiovascular morbidity [45, 46].

In addition, uremia-induced deficiency of nuclear factor erythroid 2-related factor 2 (Nrf2), a transcription factor for the antioxidative stress response and activator of an array of cytoprotective genes, may enhance the susceptibility to kidney injury [47, 48].

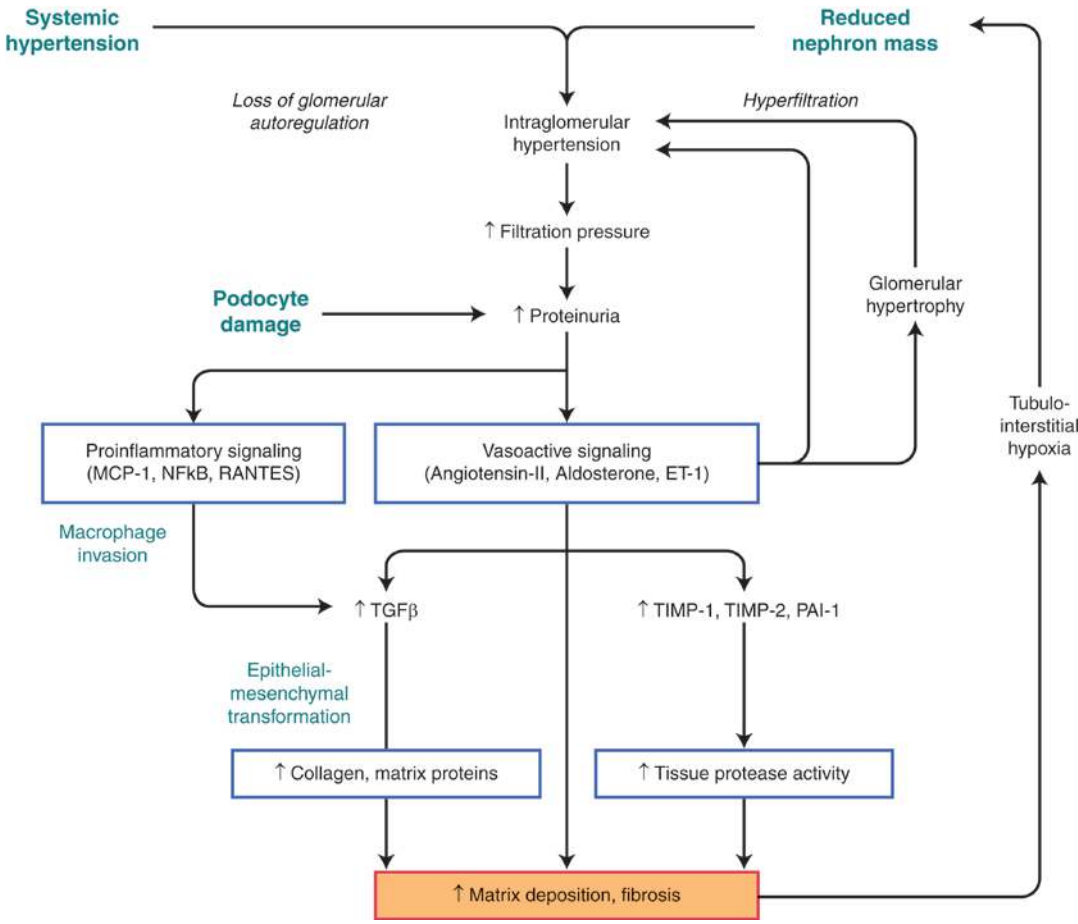

Fig. 55.4 Pathophysiological consequences of hypertension and proteinuria in chronic kidney disease

In patients with reduced nephron mass and anemia, hypoxia of tubular cells may occur due to increased oxygen consumption by tubular cells of the remaining nephrons, a decreased number of interstitial capillaries [49] and accumulation of extracellular matrix between interstitial capillaries and tubular cells, which hampers oxygen diffusion. In turn, hypoxia stimulates the production of profibrotic molecules such as TGFβ and ET-1 by tubular cells, further increasing the synthesis of extracellular matrix by fibroblastic cells and ultimately leads to tubular epithelial cell loss with formation of atubular glomeruli [50, 51].

In addition, hypoxia enhances the local production of reactive oxygen species (ROS). Oxidative stress also induces the release of pro-inflammatory and pro-fibrotic molecules, thereby enhancing the production of extracellular matrix by fibroblasts and favoring cell death.

Factors Modifying the Risk of Kidney Disease Progression

Age at Attainment of Kidney Mass Deficit

The age at which a significant loss of kidney mass occurs may influence the degree of glomerular hypertrophy and the long-term prognosis of kidney function. Patients born with unilateral kidney agenesis are at greater risk for proteinuria, hypertension and CKD than subjects undergoing unilateral nephrectomy by trauma or kidney

donation in later life, suggesting that the number of functional nephrons in congenital solitary kidneys might be decreased (possibly by hypogenesis) [52, 53].

Most patients with a solitary functioning kidney experience compensatory kidney growth of the remnant kidney. After unilateral nephrectomy for Wilms' tumor, compensatory renal growth was most marked in patients who underwent surgery at very young age. Marked kidney growth was associated with microalbuminuria in more than 30% of these patients [54]. The possibility that nephrons in infantile solitary kidneys are subject to greater hypertrophic stress is supported by quantitative morphometric studies showing glomerular volumes 5–6 times greater than normal [55].

However, most long-term follow-up studies of congenital solitary kidneys included heterogenous patient populations. Of note, patients with a congenital solitary kidney due to unilateral multicystic-dysplastic kidney disease (MCDKD) appear to have better functional outcomes over time than patients with unilateral kidney agenesis. In the long-term follow-up study by Sanna-Cherchi, 5% of MCDKD cases but 30% of unilateral kidney agenesis cases required dialysis after their second decade of life (HR 2.43) [53]. In addition, MCDKD patients had a lower prevalence of hypertension (3% vs. 19%) and proteinuria (3% vs. 12%) than patients with unilateral kidney agenesis [56]. It is tempting to speculate that the natural history differences observed with these single-kidney disorders may be related to different underlying pathogenic mechanisms variably affecting the long-term function of the remaining kidney.

Gender

Among adult patients with CKD, women have a higher overall prevalence of pre-dialysis CKD than men, possibly due to the longer life expectancy of women and to CKD overdiagnosis by use of estimated GFR equations. However, decline of kidney function in men is faster, maybe due to unhealthier lifestyles and hormonal differences, resulting in a higher rate of kidney replacement therapy in men [57].

A kidney survival advantage of females has been suggested by meta-analysis of patient cohorts with autosomal dominant polycystic kidney disease (ADPKD), IgA nephropathy and membranous nephropathy [58]. The advantage seems to be lost in post-menopausal women, suggesting a nephroprotective effect of female sex steroids; however, some confounding by lipid levels or the prevalence of smoking cannot be ruled out. In addition, at all levels of pre-dialysis CKD mortality is higher among men, whereas mortality on KRT is similar for men and women.

Data on a potential sexual dimorphism of kidney survival in children with CKD are scarce. A recent analysis of data from the CKiD Study was able to identify different trajectories of CKD progression in children. However, while females with glomerulopathies showed faster CKD progression than males, no significant sex differences were found in patients with non-glomerular disease. The differences in progression seem likely explained by sex differences in the underlying primary kidney disease and in baseline GFR rather than by a direct effect of sex on progression [59].

Underlying Kidney Disease and Genetic Pathology

Although the pathophysiological principles of CKD progression described above generally apply to all chronic kidney diseases, the time course of deterioration of kidney function for individual disease entities is highly variable. It is evident that patients with aggressive, incompletely controlled immunological nephropathies will have a more rapid progression of CKD than subjects with hypoplasia of the kidneys. However, even within groups of patients suffering from pathogenetically homogeneous hereditary kidney diseases, the rate of CKD progression can vary markedly between individuals. In a growing number of these entities, the progression phenotype can be linked to the underlying genetic defect. In disease entities caused by defects in

more than one gene, progression patterns may differ according to the gene involved. For instance, in adults with ADPKD, individuals with mutations in the PKD1 typically have a more severe disease course with earlier need for renal replacement therapy than those with mutations in the PKD2 gene. A gene-phenotype correlation is also obvious in children with the nephronophthisis complex. In children with mutations in the NPHP1 gene, CKD stage 5 is attained at a mean age of 13 years, compared to 8 months in NPHP2 and 19 years in NPHP3 mutation carriers [60]. Even within the same gene, the localization and type of the causative mutations is a key determinant of the renal prognosis. The highly variable times of disease onset and progression to ESKD in steroid resistant nephrotic syndrome caused by different mutations in the NPHS2 gene [61], and in Alport syndrome related to COL4A5 gene mutations [62] are classic examples for genotype-phenotype correlations in recessive and dominant forms of hereditary kidney disorders.

In addition to the causative role of genetic disorders in many if not most pediatric kidney diseases, common polymorphic variants in various genes may determine an individual's susceptibility for kidney injury, kidney disease progression and the response to renoprotective treatment. One of the first common variants studied in the context of CKD progression was the insertion/deletion polymorphism of the angiotensin converting enzyme (ACE) gene. The DD genotype has been shown to predispose for progressive renal failure in IgA nephropathy [63, 64]. Some, though not all studies, found an increased risk for parenchymal damage with the DD genotype in children with congenital urological abnormalities, particularly vesicoureteral reflux [65–67] and an increased risk for poor kidney survival in children with CKD due to kidney hypodysplasia [65].

In adults with non-diabetic proteinuric kidney diseases, the ACE polymorphism has been claimed to predict the nephroprotective efficacy of ACE inhibition. Proteinuria, the rate of GFR decline and progression to CKD stage 5 were lowered by ACE inhibitors in patients with the DD genotype, but not in those with the II or ID genotype [68, 69].

Also, common variants exist in various genes encoding cytokines, growth factors and regulatory peptides involved in CKD progression.

Recent and ongoing meta-analyses of genome-wide association studies (GWAS) are aiming to identify loci associated with incident CKD or the risk of KRT in general population based cohorts [70, 71], as well as in cohorts of adult and pediatric CKD patients [72]. An increasing number of common risk variants in genes related to nephrogenesis (e.g. *ALMS1*, *VEGFA*, *DACH*), glomerular filtration barrier formation and podocytes function (e.g. *DAB2*, *PARD3B*, *VEGFA*), angiogenesis (e.g. *VEGFA*), and renal function (e.g. *UMOD*, *SHROOM3*, *STC1*) have been identified [71]. For example, common variants in the region of the *UMOD* gene encoding uromodulin (Tamm-Horsfall protein) associate with CKD and GFR. Elevated urine uromodulin concentrations, which are associated with a common polymorphism in the *UMOD* region, precede the onset of CKD [73].

Genome-wide association studies may also allow identification of variants related to known CKD risk factors as hypertension or proteinuria in adults [74–76] as well as in children [77].

Epigenetics and Fetal Programming

Fetal programming describes the effect of environmental cues experienced during fetal development on an individual's susceptibility to diseases later in life through epigenetic changes that may be additionally modified postnatally.

Fetal programming may have direct consequences on kidney development and function. Barker and colleagues first demonstrated a correlation of low birth weight with the incidence of cardiovascular disease (CVD) in later adulthood [78]. Subsequent studies suggested that low birth weight is correlated with decreased kidney mass and nephron number, which in turn predisposes to adult hypertension [79]. In line with these findings, a recent study in more than 400 Japanese children born with very low birth weight showed a significant contribution of intrauterine malnutrition to chronic kidney damage [80]. Conversely,

higher estimated weights in utero, at birth and at 6 months post-gestational age were found to be associated with higher kidney volume and higher GFR at age 6–7 years [81].

Thus, in interaction with an existing congenital or acquired kidney disease, prenatal programming by epigenetic mechanisms may influence the course of CKD progression [82].

Hypertension

Hypertension is an independent risk factor for renal failure progression in adults [6–8]. While the degree of hypertension is a marker of the underlying renal disease severity, interventional studies have provided evidence that high blood pressure actively contributes to renal failure progression in human CKD. This has been confirmed by hypertension registry data of more than 40,000 adult patients demonstrating an association of systolic blood pressure with incident CKD. Each 10 mmHg increase of baseline or time-varying systolic blood pressure above 120 mmHg was associated with a 6% increase in the risk of developing CKD [83].

In pediatric nephropathies, renal hypertension is common, although typically less severe than in adult kidney disorders. Hypertension prevalence estimates in children with CKD range from 20% to 80%, depending on the degree of renal dysfunction [84]. However, even children with CKD stage 2 or renal hypodysplasia, conditions usually not strongly associated with hypertension, may present with elevated blood pressure [85]. In a large prospective study of children with CKD, systolic blood pressure greater than 120 mmHg has been associated with a faster GFR decline [86] (Fig. 55.5). Among CKD children included in the CKiD Study, time-varying blood pressure ≥ 90th percentile (compared to <50th percentile) was associated with an odds ratio of 3.75 in non-glomerular and of 5.96 in glomerular disease for the risk of losing 50% of GFR or progressing to ESKD [87].

Investigations on the physiological diurnal variation of blood pressure by ambulatory blood pressure monitoring have revealed that the integrity of the nocturnal fall of blood pressure ('dipping' pattern) plays a significant role in renal failure progression, in addition to and independent of the absolute blood pressure level.

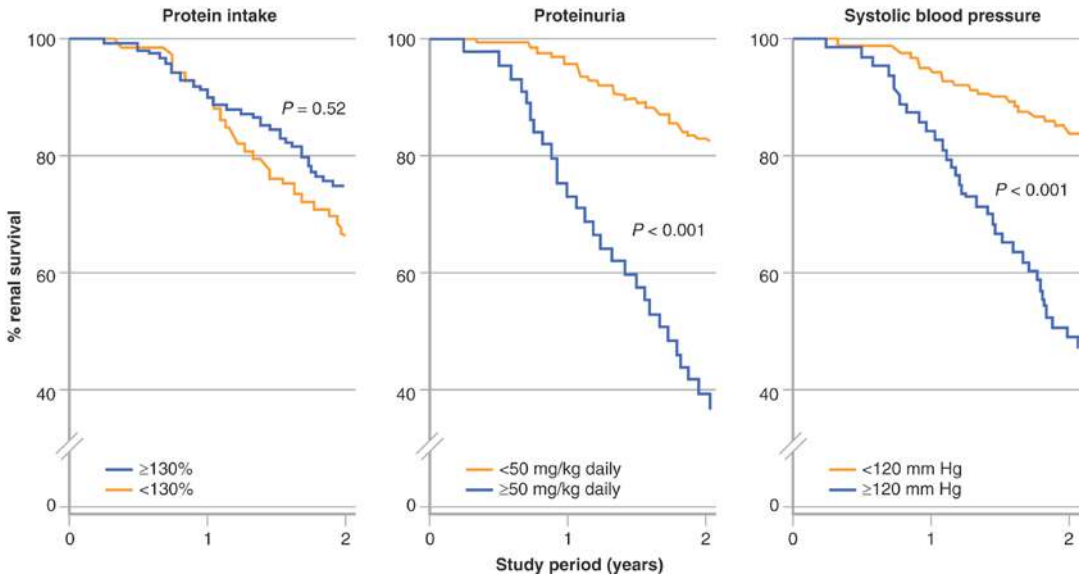

Fig. 55.5 Proteinuria and Hypertension predict the risk of disease progression in children with CKD. Used with permission of Elsevier from Wingen et al. [86]

Non-dipping, a well-known independent cardiovascular risk factor and common characteristic of renoparenchymal hypertension, is associated with more rapid progression towards kidney failure in adult CKD patients [88–91].

Similarly, elevated night-time blood pressure is correlated with an increased risk of CKD in the general population [92] and with a faster decline of GFR in CKD patients [93]. Overactivation of the sympathetic nervous system and endothelial dysfunction may contribute to the dysregulation of nocturnal blood pressure and circadian pattern [89].

Proteinuria

Population based studies in healthy individuals have demonstrated that proteinuria is an independent risk factor for CKD, KRT and overall mortality [94–96]. In adults with diabetic and non-diabetic kidney disorders, proteinuria is clearly predictive of the renal prognosis [15, 97, 98]. In the Ramipril Efficacy in Nephropathy (REIN) trial [99], urinary protein excretion was the only baseline variable correlated with GFR decline and progression to KRT.

In children, the spectrum of underlying kidney disorders markedly differs from adults. Congenital renal hypodysplasia with or without urinary tract abnormalities is the leading underlying pediatric kidney disorder, accounting for more than 60% of CKD cases. The European Study Group for Nutritional Treatment of Chronic Renal Failure in Childhood first demonstrated in 200 children with CKD stages 3–4 that proteinuria and hypertension are also major predictors of GFR decline in pediatric kidney disease [86] (Fig. 55.5). The ItalKid Project confirmed that proteinuria predicts renal disease progression in children with renal hypodysplasia [100]. In the ESCAPE (Effect of Strict Blood Pressure Control and ACE Inhibition on Progression of Chronic Renal Failure in Pediatric Patients) trial, baseline proteinuria and residual proteinuria during ACE inhibition were quantitatively associated with CKD progression [101, 102].

In a joint analysis of 1200 children with CKD stages 2–4 from the CKiD Study and the ESCAPE Trial, patients with glomerular disease showed a 43% shorter time to ESKD or 50% GFR loss than children with non-glomerular disease [103]. The average time to reach this endpoint was more than 10 years in non-proteinuric children with mild to moderate CKD as compared to 1.3 years in those with CKD stage 4 and nephrotic range proteinuria [103] (Fig. 55.6).

Metabolic Acidosis

In the Cardiovascular Comorbidity in Children with CKD Study (4C Study), pediatric patients with CKD stage 3–5 with time-averaged serum bicarbonate below 18 mmol/l had a significantly higher risk of CKD progression compared to those with a serum bicarbonate of 22 mmol/L or more [104]. These findings are in keeping with findings in interventional trials in adult CKD patients suggesting that *metabolic acidosis* is associated with faster decline in eGFR [105–107] (Table 55.1).

Dyslipidemia and Insulin Resistance

Abnormalities of lipid metabolism are a common complication in CKD. In the CKiD Study, 45% of children had *dyslipidemia*, with an increased risk in children with GFR < 30. Obese and overweight children in the cohort had significantly higher odds ratios of having dyslipidemia, with the highest risk in the most obese subjects. Dyslipidemia was also independently associated with nephrotic–range proteinuria. [108].

Epidemiological studies give some evidence that dyslipidemia is an independent risk factor not only for CVD but also for progressive chronic renal failure [109]. The dyslipidemic pattern differs between the major renal disease entities [110] and the degree of dyslipidemia parallels the degree of renal function impairment. Underlying mechanisms of uremic dyslipidemia include insulin resistance [111], obesity, hyperparathyroidism [112], malnutrition, acidosis [113] and impaired catabolism of triglyceride-rich lipoproteins by decreased activity of lipoprotein lipase and hepatic triglyceride lipase.

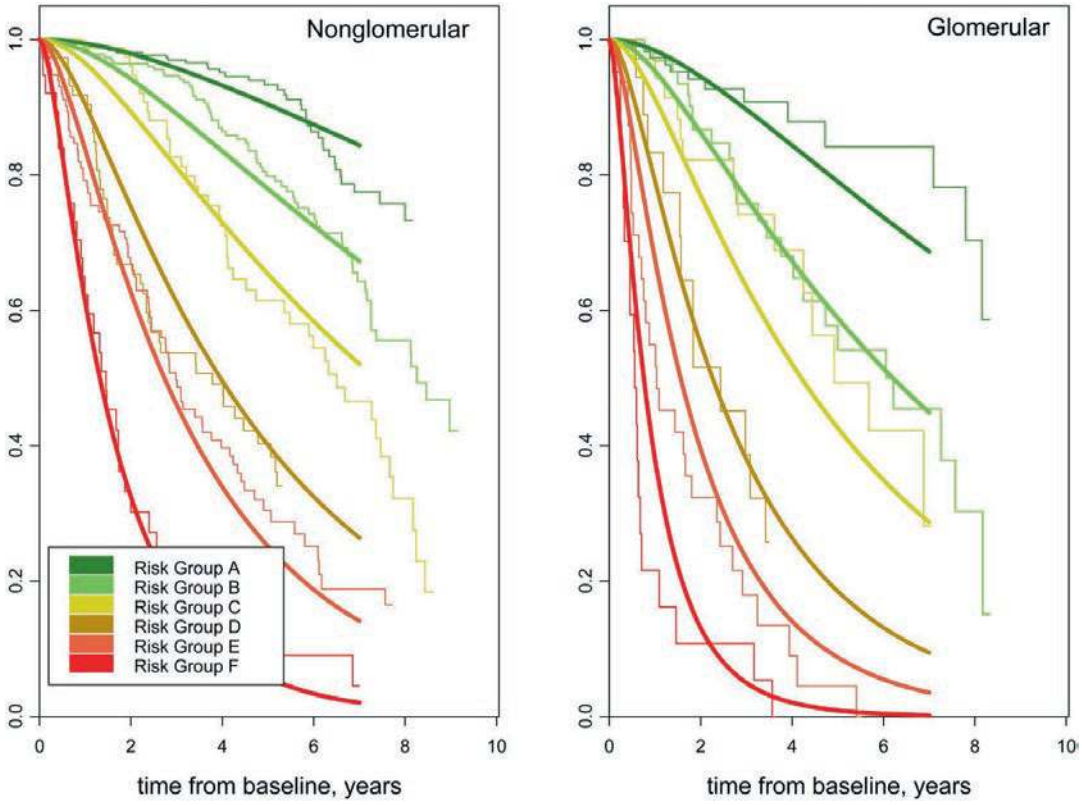

Fig. 55.6 Survival curves for the time from assessment of CKD (baseline) to a composite clinical event (50% GFR decline, kidney replacement therapy, or GFR less than 15 mL/min/1.73 m^2) stratified by CKD diagnosis and risk group. Used with permission of Elsevier from Furth [103])

In animal models, hypercholesterolemia accelerates the rate of progression of kidney disease [114]. A link between dyslipidemia and oxidative stress in the pathogenesis of renal damage was shown in unilaterally nephrectomized rats, in which hyperlipidemia increased glomerular and tubulointerstitial infiltration and aggravated glomerulosclerosis [115].

There are also observations in humans that the *insulin resistance syndrome* may underlie or mediate the association between lipids and loss of renal function. A strong relationship between the *metabolic syndrome* and the risk for CKD and microalbuminuria was found in a large, adult, non-diabetic general population [116]. A relationship between serum cholesterol and GFR decline was also shown in adult patients with type 1 diabetes and overt nephropathy [117]; patients with a total cholesterol level > 7 mmol/L showed an at least three times faster decline in GFR than subjects with lower cholesterol levels.

Table 55.1 Potential therapeutic strategies for prevention of kidney disease progression

Therapeutic target	Agents	Action	Treatment aim
Renin-angiotensin-aldosterone system	ACE inhibitors Angiotensin receptor blockers Aldosterone antagonists Renin inhibitors	RAAS blockade: antiproteinuric, antihypertensive, anti-fibrotic, and anti-inflammatory effects	Blood pressure control Reduction of proteinuria Attenuation of glomerular sclerosis and tubulointerstitial fibrosis
Hypertension	All antihypertensive drug classes	Antihypertensive Additional antiproteinuric effect by blood pressure control	Strict blood pressure control [101, 135, 136, 239, 240]: – BP target <75th Pct in non-proteinuric children – BP target <50th Pct in proteinuric children
Proteinuria	ACE inhibitors, some CCBs (non-dihydropyridines) and ß-blockers (e.g., carvedilol)	Antiproteinuric	Minimization of proteinuria: urinary protein excretion <300 mg/m^2/d [143, 144]
Metabolic acidosis	Bicarbonate	Renoprotective	Serum bicarbonate level > 22 mmol/L [107]
Inflammation	e.g., Endothelin A receptor antagonist, Pentoxifylline, Neprilysin, bardoxolone	Anti-inflammatory and antifibrotic action	Reduction of inflammation and fibrosis, attenuation of glomerular sclerosis and tubulointerstitial fibrosis
Dyslipidemia	Statins	Lipid lowering	Normalization of lipid profile
Anemia	Erythropoietin	Improved oxygen supply, reduced oxidative stress, direct protective effects	Normalization of hemoglobin levels
Mineral metabolism, Hyperparathyroidism	Phosphate binders (Calcium-free) Vitamin D Calcimimetics	Renoprotective Anti-fibrotic (vitamin D) Reduction of proteinuria, blood pressure, glomerular sclerosis (calcimimetics)	Calcium, phosphate, PTH and vitamin D levels within target range for CKD patients [241]
Hyperuricemia	Allopurinol	Renoprotective	Normalization of serum uric acid levels [229]
Renal disease progression	Low Protein Diet (0.8–1.1 g/kg/d)	Reduction of serum urea levels	Reduction of serum urea levels, delay of end-stage renal disease

RAAS renin-angiotensin-aldosterone-system, *ACE* angiotensin converting enzyme, *ACEi* ACE inhibitor, *ARBs* angiotensin receptor blockers, *CCBs* Calcium channel blockers, *BP* blood pressure, *Pct* percentile, *CKD* chronic kidney disease

Additionally, *obesity* appears to be a negative prognostic factor for both diabetic and non-diabetic nephropathies and an independent risk factor for accelerated progression [118, 119]. As adipose tissue generates high levels of circulating free fatty acids (FFA) and FFA uptake and oxidation in CKD are reduced by hypoadiponectinemia, leptin resistance, and increased cytokine release from adipose tissue and macrophages, intracellular FFA accumulate [120]. This may cause renal mesangial and epithelial cell injury and renal disease progression as a consequence of FFA-induced lipotoxicity.

However, the evidence for dyslipidemia as an independent risk factor for renal disease development and/or progression is not as strong in clinical studies as it is in experimental settings. It is unclear whether dyslipidemia independently increases the renal risk in those without other risk factors for kidney disease because most studies have been performed in patients with pre-existing renal disease or patients with associated renal risk factors such as hypertension or diabetes.

Hyperuricemia

Data regarding the role of hyperuricemia in the progression of CKD are conflicting. While in the Modification of Diet in Renal Disease (MDRD) study [121] a single elevated uric acid level was not predictive of future kidney failure in adults, a retrospective cohort study from Taiwan suggested that higher uric acid levels are associated with a more rapid eGFR decline and higher risk of kidney failure, particularly in patients without proteinuria [122]. In line with the latter findings, data from the CKiD Study indicated that hyperuricemia may also be an independent risk factor for faster CKD progression in children and adolescents [123].

Bone Metabolism

Disturbances of the *bone mineral metabolism* also may influence CKD progression. Phosphorus accumulation [124], elevated serum alkaline phosphatase [125] and low 25-hydroxyvitamin D levels are associated with progression, potentially via their relation to fibroblast growth factor 23 (FGF23), which has been identified as an independent risk factor for CKD progression in adults and children [126].

Environmental Factors

Additionally, environmental effects may affect progression of CKD, such as exposure to *smoking*. Smoking has been associated with an increased risk of renal disease progression in adults [127]. In the CKiD cohort, second hand smoking exposure was independently associated with nephrotic range proteinuria [128].

Treatment Strategies in Kidney Disease Progression

Based on the current understanding of the mechanisms of kidney disease progression, several principal nephroprotective strategies have emerged in recent years. These are based mainly on clinical evidence established in adult patients, but growing evidence also supports their efficacy in children. Efficient control of blood pressure and minimization of proteinuria are the two most important measures to preserve residual kidney function. Other approaches, such as the prevention and treatment of CKD-associated anemia, dyslipidemia and mineral metabolism disorder, have an experimental basis whereas their clinical importance is still less clear. The level of clinical evidence in support of the various nephroprotective strategies will be discussed below and is summarized in Table 55.2.

Blood Pressure Control

Numerous studies in adults have shown that antihypertensive therapy slows the rate of kidney failure progression [129]. There seems to be a linear relationship between the blood pressure level achieved by antihypertensive treatment and the rate of annualized GFR decline in CKD patients [129]. This relationship appears to persist well into the normal range of blood pressure [15, 130], although interventional trials comparing different blood pressure targets yielded somewhat conflicting results.

In children with CKD, the ESCAPE Trial provided evidence that intensified blood pressure control targeting for a 24-h mean arterial pressure below the 50th percentile provides superior long-term nephroprotection compared to a higher target range (50th to 95th percentile) [101]. In this randomized clinical trial, the ACE inhibitor ramipril was administered at a fixed dose in 385 children with CKD. Subsequently, the patients were randomized for conventional or intensified blood pressure control to be achieved by administration of additional antihypertensive drugs not affecting the renin-angiotensin-aldosterone system (RAAS). Within 5 years of observation, the risk of losing 50% of eGFR or progressing to KRT was reduced by 35% (from 42.7 to 29.9%) in the strict blood pressure control arm [101] (Fig. 55.7).

Intensified blood pressure control effectively improved kidney survival in children with an

Table 55.2 Evidence levels for efficacy of nephroprotective treatment strategies in adults and children with CKD

Renoprotective treatment	Evidence in adults	Evidence in children	Effect of Intervention		Drugs licensed for … in …
Antihypertensive treatment (RAAS blockade/CCBs/ß-Blockers)/**Strict BP control**	Multiple RCTS, meta-analyses	One RCT; few observational studies	+	BP control reduces risk of CKD; evidence for beneficial effect of strict BP control especially in proteinuric CKD	Hypertension in adults and children
RAAS blockade – antihypertensive + antiproteinuric treatment					
ACEi/ARBs	RCTs	RCTs	++	Antihypertensive + antiproteinuric	Hypertension in adults and children
Aldosterone antagonists	RCTs	RCT underway (finerenone)	+	Antihypertensive + antiproteinuric, reduced risk of CKD (finerenone)	Spironolactone: hyperaldosteronism in adults and children; eplerenone: left ventricular dysfunction in adults; Finerenone: diabetic and non-diabetic CKD in adults
Renin antagonists	RCT	None	+	Antihypertensive + antiproteinuric	Hypertension in adults
Combined RAAS blockade (ACEi + ARB or ACEi/ARB + aldosterone / renin antagonist)	RCTs	None	+	Antihypertensive + antiproteinuric; increased risk of side effects, combined RAAS blockade not recommended	NA
SGLT2 inhibitors	RCTs	None	++	Antihypertensive, antiproteinuric, anti-inflammatory, reduction of CKD progression rate	Diabetic / non-diabetic CKD in adults
Bicarbonate	RCTs	Observational studies	+	Reduced risk of CKD progression	Metabolic acidosis
Tolvaptan (in ADPKD)	RCTs	RCT	+	Retardation of cyst growth and CKD progression	Progressive ADPKD in adults
Antihypertensive chronotherapy	RCTs	None	+?	Nephroprotective effects reported, but quality of studies questioned	NA
Anti-inflammatory agents					
Endothelin receptor antagonists	RCTs	RCT underway (sparsentan)	+?	Antihypertensive + antiproteinuric, but unfavourable side effect profile for some compounds	Sparsentan: FSGS in adults

(continued)

Bardoxolone	RCTs	None	+?	Inconclusive findings	Not licensed
Pentoxifylline	RCTs	None	?	Inconclusive findings	Peripheral arterial disease in adults
Neprilysin	RCTs	None	?	Inconclusive findings	Heart failure in adults
Statins	RCTs	None	+?	Reduced risk of CKD progression in some studies	Dyslipidemia
Allopurinol, Febuxostat	RCTs	None	+?	Inconclusive findings	Hyperuricemia
Vitamin D	Observational studies	Observational studies	+		Vitamin D deficiency, rickets, CKD
Erythropoiesis stimulating agents	Observational studies	None	?	Inconclusive findings	Anemia
Low protein diet	RCTs	One RCT	+?	Inconclusive findings	NA

RCT randomized, controlled trial; + positive effect; ++ strong positive effect; +? Positive effects reported, but findings still inconclusive;? effect unknown; *NA* not applicable, *ADPKD* autosomal dominant polycystic kidney disease, *FSGS* focal segmental glomerulosclerosis, *BP* blood pressure, *ACEI* angiotensin converting enzyme inhibitor, *ARB* angiotensin receptor blocker, *CCB* calcium channel blocker

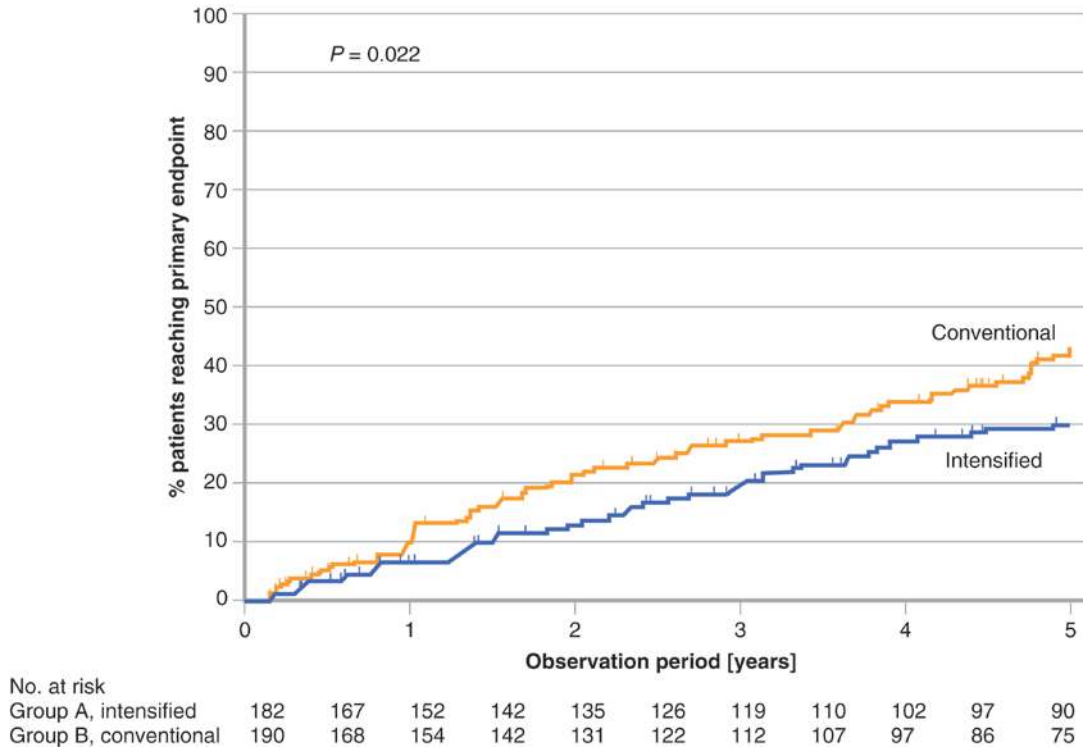

Fig. 55.7 Progression of chronic renal disease in children with CKD according to blood pressure control. The cumulative probability of reaching the primary composite end point of a 50% decline in the GFR or progression to end-stage renal disease is shown for patients randomized to either conventional or intensified blood pressure control in the ESCAPE Trial Used with permission of Massachusetts Medical Society from Wühl et al. [101]

underlying glomerulopathy as well as renal dysplasia or hypo-dysplasia, albeit not in other congenital or hereditary nephropathies [101].

The kidney survival benefit of intensified blood pressure control demonstrated by the ESCAPE Trial is in partial contrast to findings in other studies in adult CKD populations (MDRD Trial, ABCD Trial, AASK Trial, REIN-2 extension trial [131–133] in which no significant benefit was demonstrated for the cohorts as a whole. These partially discrepant findings may be explained by methodological and population differences. Patient age, ethnicity, pharmacological treatment protocols, duration of follow-up and dropout rates varied markedly between the mentioned studies. Furthermore, the use of ambulatory blood pressure monitoring may have enabled more sensitive monitoring of achieved blood pressure level in the pediatric ESCAPE Trial than in the adult trials, in which casual blood pressure readings were exclusively used. Remarkably, for all adult CKD studies mentioned above, patients with proteinuria were more likely to benefit from intensified blood pressure control. This is consistent with the results of a meta-analysis of nearly 10,000 CKD patients in which intensified blood pressure control in patients with proteinuria appeared to protect against renal failure [134].

In the ESCAPE trial [101], strict blood pressure control was tolerated very well in the vast majority of children. Since the absolute risk of cardiovascular events in children is very low, the "J curve" phenomenon, i.e., an increase of cardiovascular events in patients achieving a very low blood pressure level, seems to be largely confined to elderly patients with advanced atherosclerosis.

The findings of the ESCAPE Trial [101] have been adopted by the most recent pediatric guideline published by the European Society of

Hypertension, which recommends a blood pressure target (for casual blood pressure and for ambulatory blood pressure monitoring) below the 75th percentile in non-proteinuric children with CKD, and below the 50th percentile in those with proteinuria [135]. The AAP Clinical Practice Guideline for Screening and Management of High Blood Pressure in Children and Adolescents, published in 2017, recommends a blood pressure goal below the 50th percentile for all children with CKD [136].

For adults and adolescents above age 15, the latest European hypertension guideline recommends a systolic target blood pressure of 130–140 mmHg [137], while the US guideline recommends a target blood pressure of <130/80 mmHg for adults with CKD [138] and the most recent KDIGO guideline suggests a systolic target blood pressure of <120 mmHg by standardized office readings [139]. For further information, please see also Chap. 50.

Proteinuria Control

In line with the experimental evidence given above, multiple clinical studies have confirmed that proteinuria is not only a marker, but also an important mechanism of kidney disease progression. Its reduction is associated with a slowing of GFR loss in the long term [15, 140–142].

In the ESCAPE trial, the level of residual proteinuria during antiproteinuric treatment was negatively associated with kidney survival in children with CKD [101, 102]. In a post-hoc analysis of the trial data, the initial antiproteinuric effect of the pharmacological intervention predicted the long-term preservation of kidney function [102]. The maximal effect was observed in children who achieved a proteinuria reduction of more than 60%. In the MDRD and REIN trials in adults with CKD, each 1 g/d reduction in proteinuria achieved within 3–4 months of antiproteinuric therapy slowed the subsequent GFR decline by 1–2 mL/min per year [15, 143]. Hence, the goal of antiproteinuric treatment is to reduce proteinuria as much as possible, ideally to <300 mg/m^2/day [144, 145].

Pharmacological Treatment Options

Antihypertensive and Antiproteinuric Pharmacotherapy

While the different classes of *antihypertensive agents* are comparable with respect to their blood pressure lowering efficacy, they differ markedly regarding their effects on proteinuria and CKD progression [141, 142, 146, 147].

By virtue of their pharmacological properties, *ACE inhibitors* (ACEis) and *angiotensin II type I receptor blockers* (ARBs) are the first options in both adults and children with CKD. These RAAS antagonists have an excellent safety profile, which is almost indistinguishable from placebo. In adults with essential hypertension, treatment with RAAS antagonists has been associated with the best quality of life among all antihypertensive agents.

RAAS antagonists suppress the local angiotensin II tone (ACEis) or action (ARBs). This results in a reduction of intraglomerular pressure and proteinuria, diminished local release of TGF-ß and other growth factors, cytokines and chemokines, with consequently attenuated glomerular hypertrophy and sclerosis, tubulointerstitial inflammation and fibrosis [85]. They also normalize central nervous sympathetic tone by reduced renal afferent nerve stimulation.

Several randomized trials in adults with diabetic or non-diabetic kidney disease have demonstrated a more effective reduction of proteinuria, usually by 30–40%, by ACEis as compared to placebo and/or other antihypertensive agents [144]. In the long term, this is associated with a reduced rate of renal failure progression [140, 144, 148–156].

Very similar results were obtained in randomized comparisons of ARBs with placebo or conventional antihypertensive agents in diabetic nephropathy [147, 157, 158]. It has been reasoned that ACEis might have a specific renoprotective advantage by inducing accumulation of vasodilatory and antifibrotic bradykinins, but the course of GFR was similar in clinical trials comparing ACEi and ARB therapy [159, 160]. However, a recent network meta-analysis suggested that ACEis are superior to ARBs and other antihyper-

tensive agents in reducing adverse renal events in patients with non-dialysis CKD 3–5, but are not superior to ARBs in lowering odds of kidney events in patients with advanced CKD [161].

Prescription of RAAS antagonists typically reduces the risk of doubling serum creatinine or attaining ESRD by 30–40%, but the superiority of RAAS antagonists is related to the prevailing degree of proteinuria. In adults, the superior nephroprotection of ACEis over other antihypertensive agents has been most clearly shown in patients with proteinuria exceeding 500 mg/day.

While the maximal antiproteinuric effects of RAAS antagonists occur at doses exceeding those providing maximal antihypertensive action [162], regulatory authority approval is usually available only for the doses used to treat hypertension. Therefore, it is generally recommended to administer these drugs, after confirming tolerability in a short run-in period, at their highest approved doses [141, 163].

Early pediatric studies showed stable long-term renal function with ACE inhibition in children following hemolytic uremic syndrome [164], stable GFR during 2.5 years of losartan treatment in children with proteinuric CKD [165], and attenuated histopathological progression in children with IgA nephropathy receiving combined RAAS blockade [166]. The ESCAPE trial demonstrated efficient blood pressure control and proteinuria reduction by the ACE inhibitor ramipril [101, 167]. Subsequently, several randomized clinical trials demonstrated efficient, dose-dependent antihypertensive and antiproteinuric efficacy of ARBs in children [168–171]. One comparative trial suggested superior 24-h blood pressure lowering with the ARB valsartan relative to the ACEi enalapril, without any difference in antiproteinuric efficacy [168].

In the EARLY PRO-TECT Alport study, ramipril treatment significantly reduced the risk of disease progression in children and young adolescents with Alport syndrome by almost half and diminished the slope of albuminuria progression and the decline in GFR [172]. Early ACEi treatment is not curative, but may delay, or even prevent, the need for dialysis and kidney transplantation until relatively late in life for substantial numbers of patients with Alport syndrome due to hemizygous missense variants and even truncating variants [173].

The CKiD study has provided observational evidence that children with moderate to severe CKD benefit from ACEi and ARB use by a KRT risk reduction of 21–37% [174].

The antiproteinuric response within in the first 2–3 months of administration is predictive of the long term renoprotective effect [102, 143] and may be utilized to individualize the appropriate drug dose. The specific glomerulodynamic effects of RAAS antagonists induce an immediate drop of the GFR, usually by approximately 10–15%, when these drugs are first administered. In patients with CKD, this may cause a significant increase in serum creatinine. It is important to know that a marked initial GFR decline is actually a positive predictor of the long-term nephroprotective effect [175]. Rapid acute renal failure soon after administration of a RAAS antagonist is a very rare event and usually related to concomitant volume depletion or previously unidentified bilateral renal artery stenoses.

Notably, a gradual rebound of proteinuria after the second treatment year during ongoing ACE inhibition was observed in the ESCAPE trial [101]. This effect was dissociated from a persistently good blood pressure control (Fig. 55.8), and may limit the long-term renoprotective efficacy of ACEi monotherapy in pediatric CKD. In several adult studies, subsets of patients developed partial secondary resistance to ACE inhibitors (‘aldosterone escape’ by compensatory upregulation of ACE-independent angiotensin II production) [176–179]. Such patients might benefit from the use of ARBs, which should not be affected by aldosterone escape.

Combined RAAS blockade using ACEi and ARB concomitantly has only a minor effect on blood pressure (3–4 mmHg vs. monotherapy), but increases the antiproteinuric effect of ACEi or ARB monotherapy by 30–40% [180, 181]. However, the findings of the ONTARGET in adult populations with high cardiovascular risk (CKD and diabetes) do not support the concept of

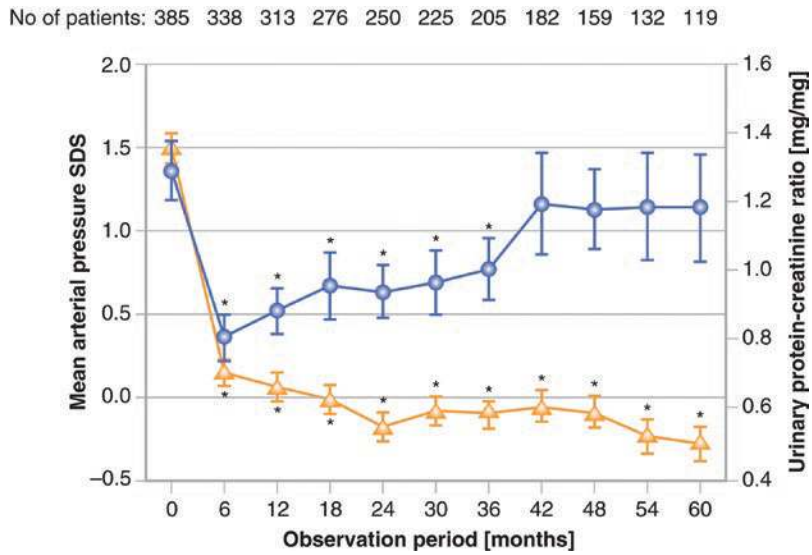

Fig. 55.8 Dissociation of antihypertensive and antiproteinuric effects of ACE-inhibition in children with CKD and fixed ramipril treatment (6 mg/m^2/day) participating in the ESCAPE Trial. Used with permission of Massachusetts Medical Society from Wühl et al. [101]

dual RAAS blockade in patients with low GFR or albuminuria [182, 183]. However, it is questionable whether these findings in a high-risk adult population with diabetes and established CVD can be extrapolated to pediatric CKD patients [184].

Aldosterone antagonists also act by RAAS suppression resulting in reduced blood pressure. While the use of spironolactone is limited by its endocrine side effects, the newer aldosterone antagonist eplerenone has minimal affinity for progesterone and androgen receptors; apart from the risk of hyperkalemia, reported side effects were similar to placebo [185]. Combined therapy of eplerenone and an ACEi increased patient survival in adults with congestive heart failure. However, the combination therapy of both compounds is limited in CKD patients by the potentiated risk of hyperkalemia. A Cochrane review analyzing the results of 44 studies on the use of aldosterone antagonists in combination with ACEi or ARB found uncertain effects of aldosterone antagonists when added to ACEi or ARB (or both) on the risk of death, major cardiovascular events, and kidney failure in patients with proteinuric CKD [186]. Aldosterone antagonists may reduce proteinuria, eGFR, and systolic blood pressure in patients who have mild to moderate CKD but may increase the risk of hyperkalemia, acute kidney injury and gynecomastia when added to ACEi and/or ARB.

In contrast, finerenone, a novel nonsteroidal selective mineralocorticoid receptor antagonist, has been shown in animal studies to block inflammation and fibrosis in both the heart and kidneys with only minor effects on serum potassium. In the placebo-controlled Fidelio-DKD trial involving >5700 adults with CKD and type 2 diabetes, finerenone significantly lowered the risk of CKD progression and cardiovascular events [187]. A clinical trial assessing the antiproteinuric efficacy of finerenone in pediatric CKD is currently underway.

The simultaneous use of novel, well-tolerated oral potassium binders may improve the tolerability of combined RAAS blockade, including aldosterone antagonists, in patients with increased risk for hyperkalemia [188].

The *renin-antagonist* aliskiren, which blocks the conversion from angiotensinogen to angiotensin I, has been shown to effectively reduce blood pressure. Preliminary data showed a blood pressure lowering effect comparable to that of ARBs and the combination therapy of aliskiren and valsartan at maximum recommended doses provided significantly higher reductions in blood pressure than did monotherapy [189]. However, due to the higher risk

of cardiovascular complications found in the ALTITUDE study, the combination therapy of aliskiren with ACEis or ARBs in patients with CKD is not recommended [190].

Calcium channel blockers (CCBs) are safe and can achieve blood pressure goals in patients with CKD. However, CCBs of the dihydropyridine type (amlodipine, nifedipine) fail to reduce progression of CKD. Although being excellent antihypertensive agents, dihydropyridine calcium channel blockers have no antiproteinuric effect and may actually promote proteinuria and more rapid CKD progression [142]. This is in contrast to non-dihydropyridine calcium channel blockers (diltiazem, verapamil), which have some antiproteinuric effect [142]. Therefore dihydropyridine CCBs should be avoided in proteinuric patients unless administered in combination with RAAS antagonists to improve blood pressure control [163], and may be acceptable as first-line antihypertensive monotherapy only in non-proteinuric patients.

CKD is often a state of overactivation of the sympathetic nervous system, and antiadrenergic drugs play an important role in its management. *β-blockers* are effective in lowering blood pressure in CKD patients; metoprolol and atenolol were the first antihypertensive agents for which beneficial effects on the decline of kidney function in CKD patients were demonstrated [149]. In the AASK trial, the beta-blocker metoprolol had an antiproteinuric effect almost comparable to ramipril, in marked contrast to amlodipine [141]. The antiproteinuric action may be due to sympatholytic effects. Newer β-blockers such as carvedilol have even better antiproteinuric effects compared to atenolol [191, 192].

Antihypertensive Combination Therapies

Because hypertension is a multifactorial disorder, monotherapy is often not effective in lowering blood pressure to the target range. Treatment with a single antihypertensive agent typically controls blood pressure in less than half of the patients.

Although most patients can be started on a single-drug regimen, consideration should be given to starting with two drugs of different classes for those with stage 2 hypertension. In addition, other patient-specific factors, such as age, concurrent medications, drug adherence, drug interactions, the overall treatment regimen, and comorbidities, should be considered. However, of those patients started on a single agent, many will subsequently require ≥2 drugs from different pharmacological classes to reach blood pressure goals. Drug regimens with complementary activity should be considered to maximize lowering of blood pressure. In addition, use of combination therapy may also improve adherence [137, 138].

In the ESCAPE trial [101], one third of patients required combination therapy to attain a blood pressure level in the normal range (<95th percentile), and 40% to reach a blood pressure in the lower normal range (<50th percentile). Likewise, 41% of the more than 700 patients participating in the European Cardiovascular Comorbidities in Children with CKD (4C) study were on two or more antihypertensive drugs [193], and 35% of the CKiD Study participants received combination therapy at baseline [194].

In CKD patients, RAAS antagonists are most commonly combined with a diuretic or with a calcium channel blocker, whereas their combination with a β-blocker usually does not exert an additive effect on blood pressure control. Fixed-dose combinations ('single pill') preparations are becoming increasingly popular in antihypertensive therapy, and may help maximize treatment adherence and efficacy [137, 138].

Antihypertensive Chronotherapy

In view of the fact that nocturnal blood pressure non-dipping is an independent risk factor for CKD progression, the timing of antihypertensive drugs may be relevant. Even with long-acting agents with recommended once daily administration, evening dosing lowers night-time blood pressure more efficiently, thereby partially restoring the physiologic nocturnal dipping pat-

tern. However, these effects seem to differ for individual antihypertensive drug classes. Whereas bedtime administration of calcium channel blockers and ACE inhibitors tends to restore the dipping pattern, evening dosing of β-blockers has no effect on the circadian blood pressure rhythm [195].

While antihypertensive 'chronotherapy' has not yet been demonstrated to affect CKD progression, in a study randomly assigning hypertensive CKD patients to either take all prescribed hypertension medications in the morning or at least one of them at bedtime, bedtime dosing was associated with improved blood pressure control and significant reduction of cardiovascular risk [196].

SGLT-2 Inhibition

Several drugs developed to enhance glucose control in patients with diabetes mellitus type 2, such as metformin, glucagon-like peptide 1 (GLP-1) agonists, dipeptidyl peptide 4 (DPP-4) antagonists, and specific *sodium-glucose cotransporter 2 (SGLT2) inhibitors***,** have recently been demonstrated to not only improve metabolic control but to exert additional beneficial effects on the cardiovascular system and the kidneys. Among these medications, SGLT2 inhibitors (gliflozins) have emerged as a particularly promising novel drug class. Apart from their glucose lowering effect, SGLT2 inhibitors decrease blood pressure, body weight, and albuminuria. The nephroprotective effects are based on a decrease of sodium delivery to the distal tubule and reduced glomerular blood flow leading to reversal of glomerular hypertension and hyperfiltration [197]. Large clinical outcome trials with the SGLT2 inhibitors dapagliflozin, canagliflozin, and empagliflozin in adult CKD patients with diabetic nephropathy have demonstrated a potent long-term nephroprotective effect of SGLT2 inhibition, with proteinuria reduction by 30–50% and impressive beneficial effects on hard renal outcomes such as doubling of serum creatinine, eGFR decline, need for KRT, and death from renal disease [198–200]. These nephroprotective effects have been confirmed in non-diabetic CKD patients [201]. To date, based on the results of the clinical trial programs, the SGLT2 inhibitors have been approved for the treatment of diabetic nephropathy. Unfortunately, pediatric trials with these novel compounds are currently not planned.

Due to the complementary effects of SGLT2 inhibitors and RAAS antagonists on glomerular perfusion and intraglomerular pressure (vasoconstriction of the afferent vessel by the SGLT2 inhibitor and vasodilatation of the efferent vessel by RAAS inhibition), combined therapy with these two drug classes might further reduce intraglomerular pressure and exert additive nephroprotective effects [202].

Anti-inflammatory Drugs

While RAAS antagonists exert indirect anti-inflammatory and antifibrotic properties via blockade of RAAS-dependent profibrotic and inflammatory pathways as outlined above, other non-specific *anti-inflammatory and antifibrotic drugs* may decrease fibrogenesis by reducing macrophage infiltration and subsequent recruitment of fibroblasts or generation of ROS. For example, pirfenidone, exerting anti-fibrotic, antioxidant, and anti-inflammatory properties, interfering with the expression, secretion and the effect of TGF-β, improved kidney function and proteinuria and reduced kidney scarring in animal models; however, in clinical trials in FSGS or diabetic nephropathy patients, pirfenidone showed conflicting results, failing to prevent proteinuria or GFR decline particularly in patients with renal dysfunction [203, 204].

Endothelin$_A$ (ET_A) receptor mediated inflammation, fibrosis and proteinuria can be reduced by *endothelin A receptor antagonists* (ERAs). ERAs have been shown to reduce albuminuria and blood pressure, but also cause sodium and fluid retention and anemia. A large randomized controlled trial in patients with diabetes mellitus type 2 and CKD using the relatively unselective ERA avosentan was terminated early because of an increased frequency of heart failure [205]. Atrasentan, a more selective endothelin receptor

antagonist, reduced the risk of renal events compared with placebo in pre-selected patients with diabetes and CKD [206]. Combined ET_A and angiotensin II blockade by sparsentan, a drug comprising moieties of irbesartan and atrasentan, achieved significantly greater reductions in proteinuria compared to irbesartan in patients with primary FSGS [207].

Despite their relevant side effect profile, the large body of pre-clinical data supporting a beneficial effect of ERAs, as well as encouraging results from clinical trials in IgA nephropathy, FSGS, Alport syndrome, sickle cell nephropathy, and resistant hypertension, hold promise that ET_A receptor antagonists may ultimately have a beneficial effect on a wide range of kidney disorders [208].

It has been shown that Nrf2 expression is consistently downregulated in CKD [209] and that activation of Nrf2 may prevent or attenuate fibrosis. Interventions that increase Nrf2 system components have been already described, but their effectiveness and clinical relevance require further clinical studies [209]. One pharmacological promotor of Nrf2 activity is *bardoxolone.* Initial phase 1 studies showed that bardoxolone increased GFR; however, subsequent phase 3 studies in patients with CKD and diabetes type 2 were prematurely terminated due to an increased risk of heart failure, elevation of transaminases, hypomagnesemia and muscle cramps. Worryingly, bardoxolone seems to increase urinary albumin excretion, indicating ongoing or even worsening glomerular damage. This might explain why after an initial increase GFR declined over 24 weeks in patients allocated to bardoxolone in the BEAM study [210]. Studies to further elaborate whether bardoxolone effectively retards kidney fibrosis and progression in specific kidney diseases such as Alport syndrome (CARDINAL trial) or ADPKD are underway.

Pentoxifylline (PTF), a non-specific phosphodiesterase inhibitor, has anti-inflammatory, antiproliferative, and anti-fibrotic properties. So far, the small clinical trials evaluating PTF in patients with CKD yielded conflicting data. A meta-analysis of 11 studies on PTF in combination with ACEi or ARB treatment including 705 adult patients suggested that combination therapy may lead to a greater reduction in proteinuria and attenuate the decline in eGFR in patients with CKD stages 3–5 [211].

Neprilysin (neutral endopeptidase or membrane metallo-endopeptidase) degrades numerous peptide hormones, including bradykinin, endothelin, and atrial and brain natriuretic peptides (ANP, BNP). Thus, *neprilysin inhibition* (NEPi) may be a therapeutic strategy enhancing the activity of the natriuretic peptide system, inducing natriuresis, diuresis and inhibition of the RAAS and the sympathetic nervous system. Sacubitril/valsartan, the first *angiotensin receptor-neprilysin inhibitor* (ARNI), has been shown to substantially improve not only cardiovascular outcomes in adults with heart failure, but also to delay CKD progression in this population. However, ARNIs have not shown similar effects on kidney function in the short-to-medium term in CKD patients [212].

In patients with ADPKD, *tolvaptan*, an oral selective antagonist of the vasopressin V2 receptor, has demonstrated beneficial disease-modifying properties in adults. Tolvaptan reduced total kidney volume and the decline of kidney function in adults with relatively early-stage ADPKD and high likelihood of rapid disease progression [213, 214] and also in those with more advanced disease [215]. Preliminary results of a placebo-controlled trial in pediatric ADPKD patients have shown efficient lowering of urine osmolality and a nominal reduction of total kidney volume after one-year of exposure to the drug [216].

Other Supportive Treatment Strategies

Metabolic acidosis is common in patients with CKD and may contribute to the development and worsening of proteinuria and tubulointerstitial fibrosis, accelerating the rate of decline in renal function. In a randomized controlled trial evaluating the renoprotective potential of oral bicarbonate supplementation in adult patients with CKD, only 4 of 67 patients receiving bicarbonate progressed to dialysis as compared to 22 of 67 patients in the untreated control group [107]. CKD progression

rate was reduced to 1 mL/min/year in patients with serum bicarbonate levels ≥22 mmol/L compared to >2.5 mL/min/year in patients with uncorrected low bicarbonate levels [107]. In adults with hypertensive nephropathy and relatively preserved GFR (mean GFR 75 mL/min/1.73 m^2), 5 years of oral sodium bicarbonate substitution (0.5 mEq/kg lean body weight daily) provided effective kidney protection when added to blood pressure reduction and ACE-inhibition [106]. Therefore, tight control of metabolic acidosis may become an important component of renoprotective therapy in patients with progressive CKD.

Treatment of uremic *dyslipidemia* is another component of renoprotective therapy. General measures to prevent dyslipidemia in CKD patients include prevention or treatment of malnutrition and correction of metabolic acidosis, hyperparathyroidism and anemia, all of which may contribute to dyslipidemia [112, 217, 218]. In addition, based on evidence from the general population, therapeutic life style modification (diet, exercise, weight reduction) is recommended for adults and children with CKD related dyslipidemia by the KDIGO Clinical Practice Guideline for Lipid Management in Chronic Kidney Disease [219]. Experimental evidence suggests that statins may retard kidney disease progression not only by their lipid lowering, but also by lipid-independent pleiotropic effects, inhibiting signalling molecules at several points in inflammatory pathways. Anti-inflammatory effects and improved endothelial function are thought to be partially responsible both for CVD risk reduction and improved kidney function [220]. Lipid lowering pharmacological treatment is common in adults with CKD, based on the benefit of this approach for primary and secondary prevention of CVD in the general adult population. However, in children with CKD, treatment of hypercholesterolemia is not recommended and treatment for hypertriglyceridemia should focus on severe cases due to lack of clear benefits coupled with safety concerns related to long-term use in children [219].

A meta-analysis including 10 studies on the impact of statin therapy on CKD progression suggested that high-dose, but not low-dose statin use, may be nephroprotective in CKD patients [221]. In addition, the degree of CKD and proteinuria may influence the effect of statin therapy on CKD progression. A study in almost 3500 adults with CKD demonstrated significant effects on GFR decline in patients with CKD stages 3b to 5 or proteinuria >1000 mg/d, whereas no significant effects were seen in mild to moderate CKD and non-proteinuric patients [222].

Several studies in adults with CKD suggested that dietary phosphorus restriction may stabilize kidney function [223]. However, no conclusions were possible from studies in children [224]. Experimental studies have suggested a PTH-independent beneficial effect of *phosphate restriction* on CKD progression. A high calcium phosphorus product may be detrimental to renal survival by aggravating intrarenal vasculopathy as well as by causing tubulointerstitial calcifications, which may stimulate tubulointerstitial inflammation and fibrosis. In view of these pathophysiological associations, it is currently discussed whether calcium-free phosphate binders may have some renoprotective potential in patients with CKD.

Sevelamer or colestilan may prove beneficial beyond phosphate lowering due to their pleiotropic effects, which include lipid lowering and anti-inflammatory properties. A lower all-cause mortality was found in patients with CKD stage 3–5D treated with sevelamer compared to patients on calcium-based phosphate binders [225].

Treatment with non-hypercalcemic doses of active *vitamin D* attenuates renal failure progression in uremic rats. This effect may be due to the immune modulatory and antifibrotic properties of vitamin D in combination with inhibitory actions on the RAAS [226]. In children with CKD, 25(OH)D levels >50 nmol/L were associated with better preservation of renal function, even in the presence of concomitant ACEi therapy [227].

In addition, there is increasing evidence for interactions between vitamin D, FGF23, and klotho regulating calcium phosphate homeostasis. Alterations of this endocrine axis may contribute to kidney disease progression by activation of the RAAS, vitamin D deficiency, reduced renal production of klotho and reduced FGF23

signaling [228]. FGF23 levels are independently associated with progression of CKD and may serve as a biomarker and mechanism of CVD [126]. These findings provide further arguments for close monitoring and early intervention to maintain mineral, vitamin D and PTH homeostasis in CKD.

Another biomarker of progressive CKD is serum uric acid. While allopurinol treatment improved renal outcome in CKD patients compared to placebo without a change in blood pressure level [229], and febuxostat treatment slowed down kidney disease progression [230], the CKD-FIX Trial could not demonstrate a beneficial effect of *uric acid lowering treatment* on kidney disease progression in stage 3–4 CKD patients [231].

Historically, *low protein diet* was prescribed for prevention of renal progression. However, the effects and consequences of this diet on CKD progression and delay of ESKD are inconclusive. A large randomized trial in adults failed to demonstrate efficacy of a low protein diet on the progression of non-diabetic kidney disease [232], whereas, in a recent review, protein restriction was associated with a risk reduction of kidney death [233]. Thus, though the progression rate was not significantly influenced by protein restriction, kidney replacement therapy could be postponed.

In children reduced protein intake to the minimal acceptable lower limit did not slow CKD progression [86, 234]. Further reductions may be effective but not acceptable for patients. Furthermore, a low protein diet may increase the risk of low-calorie intake, which may jeopardize the preservation of adequate statural growth. Therefore, it does not seem to be justified to prescribe a low protein diet in children with CKD.

Recently, studies in ADPKD patients have identified a chronic shift in energy production from mitochondrial oxidative phosphorylation to aerobic glycolysis as a contributor to cyst growth, making the cyst cells particularly sensitive to the availability of glucose. Therefore, low calorie or ketogenic diets may be a potential intervention to delay ADPKD progression [235]. A clinical trial evaluating the effect of a ketogenic diet on cyst growth and CKD progression in adult ADPKD patients is underway (NCT04680780).

Erythropoiesis stimulating agents (ESA) have emerged as a tissue-protective survival factor in various non-hematopoietic organs [236]. It has been suggested that normalizing hemoglobin in CKD patients might attenuate renal disease progression by increasing oxygen supply to the kidneys, preventing tubular atrophy and interstitial fibrosis. In addition, erythropoietin might counteract oxidative stress and apoptosis and may have direct protective effects on tubular cells [237] and podocytes [238]. However, this concept has not been substantiated in clinical trials on CKD progression to date.

Conclusions

Progression of childhood CKD towards kidney failure is common and once a significant impairment of kidney function has occurred it progresses irrespectively of the underlying kidney disease. Onset of CKD and progression rate in defined disease entities may be influenced by genetic factors. Hypertension and proteinuria are the most important independent risk factors for CKD progression. RAAS antagonists preserve kidney function, not only by lowering blood pressure, but also by their antiproteinuric and anti-inflammatory properties. Intensified blood pressure control exerts additional renoprotective effects in pediatric CKD; therefore, therapeutic strategies to prevent progression should comprise blood pressure control aiming for a target blood pressure below the 50th to 75th percentile. Furthermore, there is increasing evidence that treatment with SGLT2 inhibitors contributes to preservation of kidney function in adults with CKD. Other factors contributing in a multifactorial manner to CKD progression include anemia, metabolic acidosis, dyslipidemia and disorders of mineral metabolism. Measures to preserve renal function should therefore also comprise the maintenance of hemoglobin, serum bicarbonate, lipids and mineral metabolism in the normal range.

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56 Growth and Puberty in Chronic Kidney Disease

Dieter Haffner and Lesley Rees

Introduction

Maintaining optimum growth is one of the most challenging problems in the management of children with chronic kidney disease (CKD): reports published in 2013 showed that approximately 50% of children requiring kidney replacement therapy (KRT) before their 13th birthday had a final height below the normal range [1–4]. Short stature is a marker for increased mortality and hampers the psychosocial integration of pediatric CKD patients [5–7]. There is, however, evidence that, alongside advancements in the medical and technical management of CKD and kidney replacement therapy (KRT), height prognosis has substantially improved over the past decades [1, 2, 4, 8–11]. In 799 pre-dialysis children with mostly mild to moderate CKD (median age 11 years, estimated glomerular filtration rate (eGFR) 50 mL/min/1.73 m^2) followed in the Chronic Kidney Disease in Children (CKiD) Study, the median height standard deviation score (SDS) was −0.55. Among 594 patients from 12 European countries with CKD stage 3–5 (median eGFR 30 mL/min/1.73 m^2) who were followed prospectively in the 4C Study, mean height SDS was −1.57, with 36% of patients having a height below the third percentile. In 1001 children in the International Pediatric Peritoneal Dialysis Network (IPPN), the mean height SDS at initiation of dialysis was −1.97 [10]. Hence, these data from contemporaneous large cohort studies demonstrate that growth failure progressively occurs with decreasing eGFR. In the 2020 USRDS report the percentage of incident end-stage kidney disease (ESKD) patients with short stature was lowest in the oldest patients age groups, being 20.3% at 18–19.9 years, 25.8% at 14–17, 20.1% at 10–13, 38.8% at 5–9 and 51.9% at 0–4 years of age [11, 12].

There appears to be a positive trend over time: e.g. in Germany, the mean standardized height in children on KRT has increased over the past 20 years from −3.0 SDS to −1.8 SDS [1]. Yet this is not the case in all parts of the world, particularly in those with inadequate local resources, where height prognosis remains dismally low [2, 13]. Even across Europe, the 4C Study observed growth failure to vary widely between countries, from 7% to 44% [9].

There is no single cause of failing growth in children with CKD (Fig. 56.1). The two most important influences are the severity of CKD and young age at its onset, so that children with CKD due to congenital kidney abnormalities manifest-

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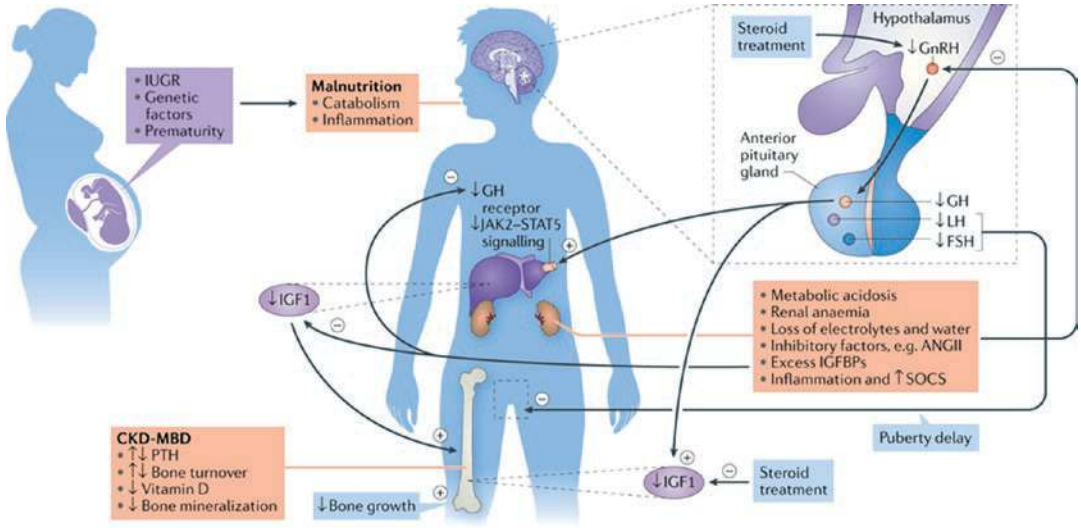


Fig. 56.1 The etiology of growth failure in CKD is multifactorial and includes intrauterine growth restriction (IUGR), genetic factors such as parental height and primary renal disease, prematurity, and malnutrition which especially limits growth in children with congenital CKD. Mineral and bone disorder (CKD-MBD), metabolic acidosis, anemia, loss of electrolytes and water, and disturbances of the somatotropic and gonadotropic hormone axes are additional factors. CKD is a state of growth hormone (GH) insensitivity, characterized by deficiency of functional insulin-like growth factor I (IGF-I), to reduced GH receptor expression in target organs like the liver, disturbed GH receptor signaling via the Janus kinase - signal transducers, activators of transcription (JAK2-STAT5) due to inflammation induced SOCS (suppressor of cytokine signalling), and increased IGF-binding capacity due to excess of IGFBPs. Finally, reduced release of hypothalamic gonadotropin-releasing hormone (GnRH), due to uremia-related inhibitory factors such as angiotensin II (AngII), and steroid treatment may result in decreased circulating levels of bioactive luteinizing hormone (LH), hypogonadism and reduced pubertal growth spurt. PTH, parathyroid hormone; FSH, follicle stimulating hormone; IGFBP, insulin-like growth factor binding proteins. [Reproduced with permission of [30]]

ing in infancy are usually more severely affected than those with acquired conditions developing in later childhood. This review summarizes the current knowledge of the phenotype, pathophysiology and therapeutic options for children with failing growth resulting from CKD.

Normal Growth

The physiological growth pattern can be divided into fetal, infantile, mid-childhood and pubertal phases (Fig. 56.2) [14]. During fetal life, nearly 30% of final height has already been achieved, so low birth weight and prematurity can substantially influence subsequent growth and final height attainment: although many otherwise normal infants born prematurely grow normally, and those who are small for gestational age (SGA) catch up in the first 6 months of life, around 10%, particularly if SGA, remain below the normal range for height into adulthood. A further one third to one half of total postnatal growth occurs during the first 2 years of life, and 20% during the pubertal phase. Throughout each postnatal phase the predominant influences on growth are different. Whereas in infancy, growth mainly depends on nutritional intake, growth in mid-childhood is driven by the somatotropic hormone axis. During puberty, the gonadotropic hormone axis stimulates growth via increased proliferation of growth plate chondrocytes and modulation of growth hormone (GH) secretion from the pituitary gland, resulting in the pubertal growth spurt (Fig. 56.2) [14].

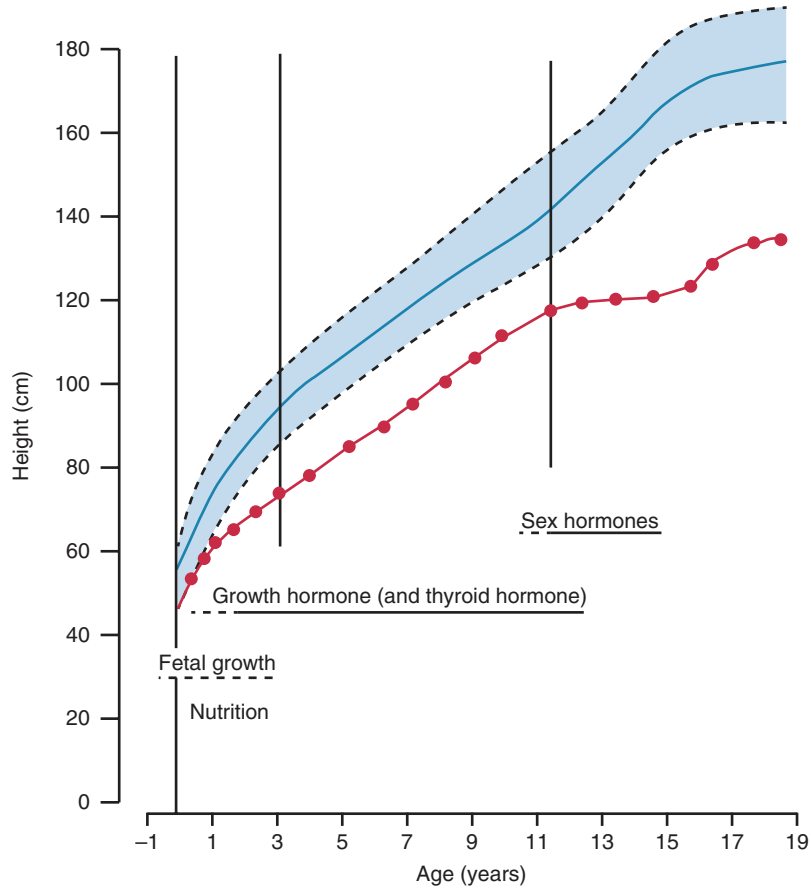


Fig. 56.2 Typical growth pattern in congenital chronic kidney disease. Relative loss in the nutrient-dependent infantile and gonadal hormone-dependent puberty phases, and percentile-parallel growth in the mainly GH-dependent growth period in mid-childhood are shown. The shaded area represents the normal range, third to 97th centiles. (Reproduced with permission of [14])

Effect of CKD on the Phases of Growth

The classical growth pattern for a child born with CKD was described in 1974 [15]. Length at birth is usually already below the mean (Fig. 56.2) [16]. As with any chronic disease, height velocity is most affected during periods of rapid growth. Marked growth retardation occurs during the first 2 years of life, followed by percentile parallel growth in mid-childhood, but catch-up growth is unusual. In the pre-pubertal years, the appearance of secondary sexual characteristics is delayed and the growth rate again decreases disproportionately [17, 18]. The pubertal growth spurt is later than normal and its magnitude impaired, resulting in further loss of growth potential and reduced final height (Fig. 56.2) [14]. Over the last 20 years, although these basic principles remain, this classic description has been reassessed as there have been new concepts and treatments for most patients in all postnatal phases of growth.

The Fetal Phase

Reduced fetal growth was described as a feature of the classic growth pattern of the child with CKD in 1994, and has been demonstrated in several studies since [16, 19–22]. Both prematurity and low birth weights are common. The incidence is particularly high in infants on dialysis but perhaps more surprisingly is high in children with less severe CKD as

well [23]. Registry data do not always distinguish between infants who do or do not have co morbidities (such infants often have below normal mean birth weight and length) but it has been shown that of just over 400 children with a mean eGFR of around 40 mL/min/1.73 m^2 in the US CKD registry, low birth weight (LBW, <2500 g) occurred in 17%, prematurity (gestational age < 36 weeks) in 12% and small for gestational age (SGA, birth weight < tenth percentile for gestational age) in 14%. It has been hypothesized that poor intrauterine growth conditions, e.g. maternal malnutrition and smoking, could cause both intrauterine growth retardation and kidney dysplasia [24, 25]. Likewise genetic abnormalities, e.g. dysregulation of Wilms' tumor suppressor gene, could cause both short stature and renal hypodysplasia [26]. Interestingly, in the US CKD registry 40% of patients had needed intensive care (ICU) at birth. The comparable overall incidence of abnormal birth history in the US population is 7–8%. Low birth weight, prematurity, SGA and requirement for ICU were all risk factors for poor growth outcomes, independent of renal function [21]. Likewise, intrauterine growth retardation, neonatal distress and parental height were shown to be important independent predictors of poor growth outcome in a cohort of 509 German children with CKD stage 3–5 [27].

The Infantile Phase

It is not surprising that adverse effects on growth are most intense during the infantile phase, and in particular the first 6 months of life, as the rate of growth is as high as 25 cm per year at birth, 18 cm per year at 6 months of age, and still 12 cm per year at 12 months of age. These figures are higher than at any other time during childhood and adolescence. Such growth challenges require nutrient intakes that are relatively higher than at any other age. As the infantile phase is predominantly dependent on nutrition, inadequate intake at this time can have a dramatic influence on growth. Indeed, any circumstances leading to decreased growth rates in this phase result in severe growth retardation and a potentially irreversible loss of growth potential [28, 29]. The decrease in mean standardized height can amount to 0.6 SD per month in infants with CKD stage 5. In recent years the increasing acceptance of KRT for all infants, including those with associated comorbidities, has increased the challenge to achieve normal growth in this age group [20]. Malnutrition in young children with CKD is due to inadequate nutritional intake and frequently recurrent vomiting as well. In addition, catabolic episodes due to infections, loss of water and electrolytes, and CKD-mineral and bone disorder (CKD-MBD) are major contributing factors to growth impairment in this period. If these disturbances are adequately controlled, severe stunting can be avoided in the majority of patients without untreatable comorbidities [19, 20, 30]. However, most infants suffering from severe CKD need supplementary feeding in order to provide adequate nutrient, water and electrolyte intake [31].

The Childhood Phase

During this phase, the somatotropic hormone axis becomes the most important influence on growth. Growth is closely correlated with the degree of kidney dysfunction in this period. Although there is no critical threshold, growth patterns are typically stable if the GFR remains above 25 mL/min/1.73 m^2 and tend to diverge from the percentiles below this level [23]. Sequels of CKD such as anemia, metabolic acidosis and malnutrition seem to be less important in mid-childhood. However, even a growth rate that parallels the centiles may not be 'normal', as children with good renal function and steroid-free immunosuppression following transplantation exhibit significant catch-up, compatible with the concept of continued suppression of an intrinsic catch-up growth potential in the uremic state [32, 33].

The Pubertal Phase

Pubertal Development

Delayed onset and progression of pubertal development was a common feature when KRT programs for children started [17]. Studies of the timing of pubertal onset have been hampered by the fact that bone age is only a crude marker for

assessment in CKD. Indeed, the distribution of bone age at pubertal onset varies at least as much as the distribution of chronological age in these patients. However, data from the late eighties demonstrated a delay of pubertal onset by 2–2.5 years in children with ESKD [17]. Menarche occurred after the upper limit of the normal age range (i.e. 15 years) in almost half of the girls treated by dialysis or transplantation [34]. Moreover, despite the achievement of pubertal stage IV or V, a substantial proportion of dialysis patients presented with permanently impaired reproductive function [35]. Fortunately, in the last twenty years most children requiring KRT before pubertal age present with normal or only slightly delayed pubertal onset. In two recent studies, mean age at pubertal onset as well as age at menarche did not differ between children on KRT and healthy children; and the serum levels of pubertal reproductive hormones were normal in the great majority of patients [1, 36]. Bone maturation in patients on KRT continues to be delayed by approximately 1.4 years compared to healthy children, although this does not negatively impact on pubertal development [1]. The age at onset of puberty has been positively correlated with the age at transplantation. Thus, early renal transplantation is a prerequisite for prevention of pubertal delay in children with stage 5 CKD [18, 36]. A recent analysis of the CKiD cohort including children with CKD stage 3–5 revealed delayed menarche in 10% of adolescent patients which was associated with African American race, lower eGFR, ever corticosteroid use, and longer duration of CKD [18, 37]. Delayed menarche was strongly associated with reduced height (<−2.0 SDS). Thus, delayed puberty is an important contributor to short stature in female patients even prior to dialysis. Patients who show delayed puberty—defined in boys by a testicular volume < 4 mL at the age of 14 years and in girls by a breast stage <B2 at age 13.5 years—should undergo work up by a pediatric endocrinologist including potential induction of puberty [30].

Pubertal Growth

During the last two decades in parallel with the improvement in sexual maturation has been an improvement in pubertal height gain [1, 2, 17, 18]. Longitudinal growth in 384 German children on KRT who were followed between 1998 and 2009 was compared with 732 children enrolled in the European Dialysis and Transplant Association (EDTA) Registry between 1985 and 1988 (Fig. 56.3) [1]. In line with previous

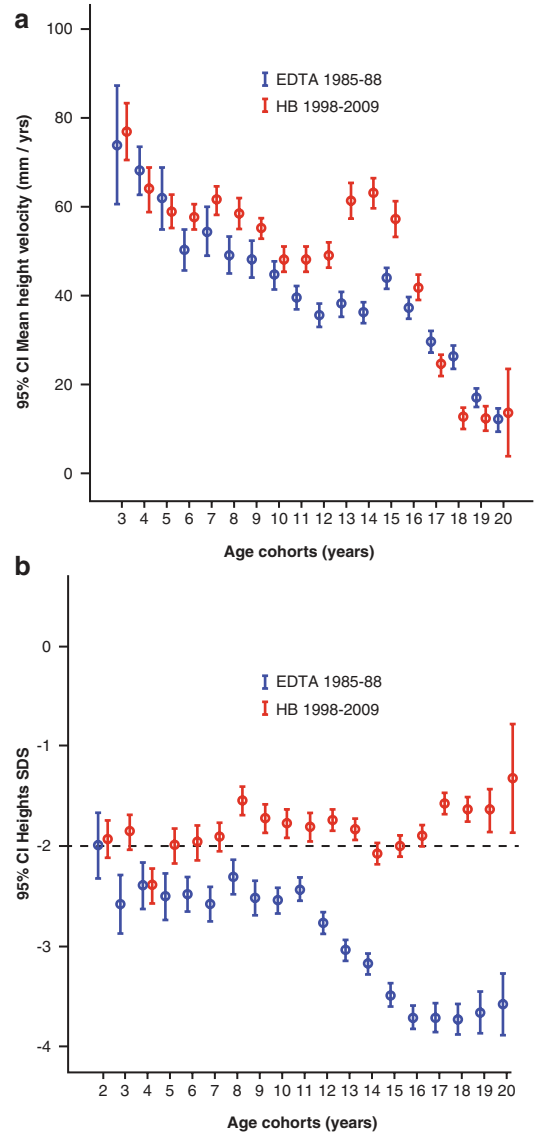


Fig. 56.3 (**a**) Mean height velocity of European children with renal replacement therapy in the EDTA study 1985–1988 (blue lines) versus the Hannover/Berlin (H/B) pediatric population cohort 1998–2009 (red lines) in different age cohorts. (**b**) Age-dependent height standard deviation score of European children on kidney replacement therapy 1985–1988 (EDTA study, n = 732, blue error bars) and in the HB group (n = 384, red error bars). EDTA European Dialysis and Transplant Association, CI Confidence interval. Reproduced with permission of [1]

studies, the pubertal growth spurt in the EDTA patients was delayed by approximately 2.5 years. In many patients no clear pubertal growth spurt was present, and consequently standardized height decreased during pubertal age. In contrast, a clear pubertal growth spurt was present and the onset of the pubertal growth spurt was within the normal range in the majority of the patients followed-up more recently. Consequently, standardized height even improved during puberty and until adult height. A strong negative correlation between total pubertal height gain and age at transplantation was reported in two studies a [18, 36]. Thus, whereas 20 years ago a loss of about 1.0 SD was expected during puberty, nowadays normal or only slightly reduced pubertal growth spurt can be expected if long-term dialysis is avoided.

Segmental Growth

It has been postulated that during malnutrition there is preferential preservation of growth of vital organs at the expense of less vital tissues such as the limbs, so that malnutrition during childhood results in disproportionate stunting with impairment of leg growth, and preserved trunk and head growth [38]. Consequently, relative leg length is increasingly used as a biomarker of childhood nutrition in epidemiological studies [39, 40]. Information pertaining to segmental growth has been collected in the CKD Growth and Development Study, in which more than 800 pediatric CKD patients before and after transplantation have been enrolled since 1998 [41–43]. Patients with a long-term history of CKD and KRT showed an age related disproportionate growth pattern [41–43]. Growth impairment and disproportionality was most obvious in early childhood. Sitting height was mostly preserved, whereas growth of the legs and arms was most severely affected. This resulted in a markedly elevated sitting height index (ratio of sitting height to total body height). Leg length was more affected in prepubertal patients. Consequently, body disproportion was less pronounced in pubertal patients. In addition to transplant function and steroid exposure, congenital CKD, smallness for gestational age, young age, and use of recombinant human growth hormone (rhGH) in the pre-transplant period were significantly associated with growth outcome (stature and degree of body disproportion. Catch-up growth after kidney transplantation is mainly related to improved trunk growth in children aged less than 4 years and stimulated leg growth in older children resulting in complete normalization of body proportions until attainment of adult height in the vast majority of patients [42, 44].

Adult Height and Height Prediction

When interpreting the adult heights of patients treated for CKD in childhood, it has to be remembered that the data obtained at any time will reflect treatment practices spanning the previous two decades. Furthermore, most reports of adult heights do not or incompletely discriminate according to patient characteristics (e.g. diagnoses, ages of onset of CKD, types and durations of KRT), and in particular registries do not separate out children with co morbidities that affect growth in their own right. With that in mind, reduced adult heights have been reported in up to 50% of CKD patients, although there has been a trend for improvement over the past decade [1–4, 17, 18, 45]. Mean adult heights vary from 148–158 cm for females and 162–168 cm for males (second centiles 151 and 163 cm respectively).

The ESPN/EDTA registry has shown that, after adjustment for age and period of start of KRT, final height increased significantly from −1.93 SDS in children who started KRT before 1990, to −1.78 in children in 1990–1999, and to −1.61 in those starting KRT after 1999 ($p < 0.001$). While 55% of patients attained an adult height within the normal range between 1990 and 19,995, this figure had risen to 62% between 2006–2011 [2]. The improvement in adult height over time was independent of age at the start of KRT (Fig. 56.4). Poorest growth outcomes were associated with earlier start and longer duration of dialysis or a diagnosis of a metabolic disorder such as cystinosis and hyper-

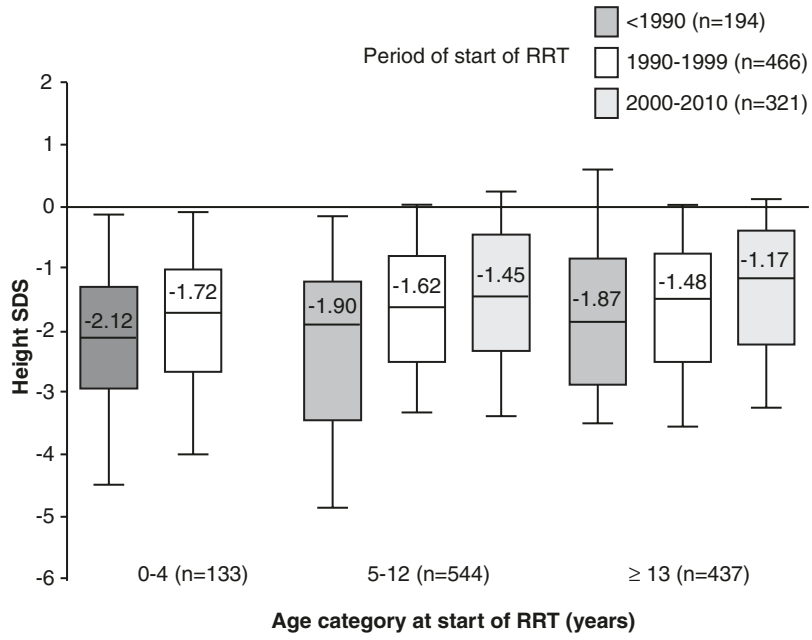


Fig. 56.4 Changes in final height SDS over time according to age and period of start of KRT (n = 981). The horizontal line in the middle of the box represents the median, the bottom and top of the box represent the lower and upper quartiles, respectively, and the ends of the whiskers represent the tenth and the 90th percentiles. (Reproduced with permission of [2])

oxaluria; whereas those with a longer time spent with a renal transplant and those treated with rhGH did the best [2–4, 46–49].

There is evidence that over the years, final height post transplantation is improving [3]. This is likely to be due to a combination of factors such as better growth attained pre transplant, e.g. by adequate nutrition and rhGH therapy, preemptive transplantation thus avoiding dialysis, and to the development of protocols that minimize the use of corticosteroids. European data show an improvement in final height from −2.06 SDS in children who reached adulthood in 1990–1995 to −1.33 SDS in 2006–2011[2]. In the 2014 NAPRTCS report, the mean height SDS of those >19 years of age was −1.37. Twenty-five percent of these patients had a height SDS of −2.2 or worse, and 10% were more than 3.2 SD below the mean. This has improved considerably as adult height SDS was −1.93 with the 1987–1991 cohort, −1.51 for 1992–1996 cohort; −1.06 for the 1997–2001 cohort, −0.98 for the 2002–2006 cohort and −0.89 for the most recent cohort (https://naprtcs.org/system/files/2014_Annual_Transplant_Report.pdf).

Older age at start of KRT, starting KRT more recently, cumulative time with a transplant, and greater height SDS at initiation of KRT were independently associated with a higher adult height SDS. Most impressively, recent results of the avoidance of steroids post transplant altogether are excellent, with mean final heights of 177 cm and 175 cm in males transplanted prepubertally and postpubertally respectively, with similar figures of 165 cm and 162 cm for females [4].

The application of adult height prediction methods in children suffering from CKD is not recommended. In several validation studies final height was overpredicted by 3–10 cm [17, 18, 46]. Most likely this reflects the complexity and thus unpredictability of growth and development under the conditions of chronic uremia, with highly variable and dynamic impacts of disease progression, medications, bone disease, KRT modalities, skeletal maturation, and pubertal timing [18, 46].

Causes of Growth Failure in CKD

Growth failure in CKD is due to a complex interplay of many different factors, with varying effects at different ages and stages of CKD (Fig. 56.1). While some factors, such as nutri-

tional and hormonal abnormalities, and hematological and metabolic derangements such as acidosis, electrolyte imbalance, and CKD-MBD are potentially correctable, the effects of others, such as birth parameters, associated syndromes, race and parental heights, are not [27]. There is a substantial global variation of the degree of growth failure in children on KRT which is at least partly explained by differences in economics. In a recent survey of the IPPN network gross national income was a strong independent predictor of standardized height, adding to the impact of other well-known factors, e.g. congenital CKD, anuria, and dialysis vintage as outlined below [50]. Likewise the country of residence was an independent predictor of growth outcome in a large European cohort of children with CKD stage 3–5, which may be related to differences in the timing of diagnosis and/or referral to a center specialized for children with CKD—which in turn may have economic causes [9].

Cause of Renal Disease

Congenital Abnormalities of the Kidneys and Urinary Tract (CAKUT)

Renal tubular sodium and bicarbonate losses are common in children with CAKUT, and can cause salt depletion and acidosis both of which can contribute to growth failure [51]. Supplementation with salt and bicarbonate may be necessary, along with free access to water.

Glomerulopathies

Growth may be affected in children with glomerulopathies, even in early CKD [52]. The nephrotic state per se and glucocorticoid treatment are known risk factors. Prolonged high corticosteroid doses lead to severe growth failure. Although partial catch-up growth can be seen after cessation of glucocorticoid treatment, this is usually restricted to young (prepubertal) patients [53]. Congenital nephrotic syndrome is often associated with severe growth retardation during the first months of life, even in patients with preserved kidney function, and seems to be secondary to persistent edema, recurrent infections, losses of peptide and protein-bound hormones in the urine, and/or protein-calorie malnutrition [54, 55]. In the Finnish-type nephrotic syndrome adequate nutritional support is vital and bilateral nephrectomies and initiation of dialysis may be necessary to stabilize growth. In less severe types of congenital nephrotic syndrome, unilateral nephrectomy and/or treatment with prostaglandin synthesis inhibitors and RAS antagonists can reduce proteinuria and thereby stabilize growth and the overall clinical condition [54–56].

Tubular and Interstitial Nephropathies

Tubular dysfunction characterized by losses of electrolytes, bicarbonate, and water can lead to severe growth failure even in the presence of normal glomerular function. The growth suppressive effects of isolated tubular defects are illustrated by the severe growth failure typically seen in patients suffering from renal tubular acidosis, Bartter syndrome, and nephrogenic diabetes insipidus [57]. Supplementation with electrolytes, water and bicarbonate may be able to prevent growth failure or even induce catch-up growth [58].

The most severe growth failure, which may be very difficult to treat, occurs in patients suffering from complex tubular disorders such as Fanconi syndrome [58–61]. In these patients, only partial catch-up growth can usually be achieved even with vigorous water and electrolyte supplementation. Systemic metabolic disorders (such as cystinosis, primary hyperoxaluria and mitochondrial cytopathies) resulting in complex tubular dysfunction, progressive loss of kidney function, and involvement of other vital organs (e.g. liver, bone, and brain) also lead to severe growth failure [61–63]. In children with nephropathic cystinosis, growth failure occurs already in infancy when glomerular function is typically not yet compromised. Progressive growth failure is further sustained by generalized deposition of cystine crystals altering the function of the growth plate, bone marrow, hypothalamus, and pituitary and thyroid glands. Early initiation of treatment with cystine depleting agents (cysteamine) results in an improvement of

growth rates and substantial delay in the development of ESKD [61, 64]. Nevertheless, in a recent European study mean standardized height in children suffering from nephropathic cystinosis with CKD 2–5 was 1.0 SDS lower compared to that in children with other causes of CKD at comparable age and degree of CKD, compatible with an additional impact of an underlying osteoblast defect in this disease [65, 66]. In patients with primary hyperoxaluria, supplementary treatment with citrate and pyridoxine can delay the progression of CKD in some patients, and possibly improve longitudinal growth [62]. However real catch-up growth after combined liver-kidney transplantation is rarely observed even in prepubertal oxalosis patients [48]. New ribonucleic acid interference (RNAi) therapies are expected to become the standard of treatment in these children, so that end-stage kidney disease, KRT and consecutive growth failure may be avoidable in the vast majority of cases in the future [67].

Stage of CKD and Dialysis

Even moderate reduction of GFR has been reported to result in impaired growth. The principal registry providing data on the epidemiology of growth in conservatively managed CKD is the North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS). The 2006 report covers the 10 years between 1994–2004 and includes a very large cohort of over 5000 children with GFRs of up to 75 mL/min/1.73 m^2. As expected, the most growth retarded were the youngest children, with a mean standardized height for under 2 years of age of −2.3 SD, but mean height SDS was reduced at all ages (−1.7, −1.4, −1.0 at 2–6, 6–12 and > 12 years respectively), with over one third overall being below the third centile for height. Standardized height worsened with progression of CKD, so that there was a strong correlation between creatinine clearance and height SDS (−3.2, −1.9, −1.5, and −0.9 for GFR <10, 10–25, 25–50 and > 50 mL/min/1.73 m^2 respectively) [68]. This means that many children, and particularly the very young, are already short at the time of entry to KRT programmes [69]. This has been confirmed more recently in a cohort of 42 children followed during the first 6 years of life, when the mean height SDS was normal at CKD stage 1–2, approximately −0.5 at CKD stage 3–5 and much less, at −1.5 SDS, for those on dialysis [70].

That short stature becomes more common in children on dialysis has been confirmed by the United States Renal Data System (USRDS), a registry collecting data on patients on KRT programs in the US. The 2007 report shows that the height and weight of approximately half of children on dialysis were below the 20th centile for the normal population [71]. Comparison of 2007 and 2016 data demonstrates that the prevalence of short stature in the incident pediatric ESKD population has not improved over the past 10 years [12]. The British Association for Paediatric Nephrology (BAPN) report for height SDS of prevalent patients on dialysis at the end of 2017 was −2 with interquartile range of −1 to −3, having been −1.3 (−0.3 to −2.4) SDS at the start of KRT (UK Renal Registry (2021) UK Renal Registry 23rd Annual Report – data to 31/12/2019, Bristol, UK. Available from renal.org/audit-research/annual-report). Children on dialysis were shorter than their transplanted peers whose mean Height SDS was −1.0 (−0.2 to −2), although both groups are below the heights of the normal age matched population. The International Pediatric Dialysis Registry collects data from more than 3000 children on peritoneal dialysis (PD) from around the world, and is, therefore, able to provide comparisons of all aspects of PD according to region in the largest cohort of children on PD to date. Currently, the mean standardized height on commencing PD is −2.35 SDS, and is below normal worldwide, but there is a large variation, ranging in 21 countries from −1.3 in the UK, to −3.5 in Brazil. Regional variations in resources are likely to contribute to these differences [31]. Given our knowledge that in the majority of reports standardized height declines with increasing time on dialysis, the obvious key to prevention of growth deterioration is pre-emptive transplantation [72].

Protein-Calorie Malnutrition

Anorexia is a common symptom of CKD, due to a combination of altered taste sensation, decreased clearance of cytokines that affect appetite and satiety, obligatory losses of salt and water leading to a preference for salty foods and large volumes of water, and the need for multiple medications. Vomiting is also common, particularly in infants, whose diet is liquid and therefore high volume, and because gastro-esophageal reflux is frequent and elevated polypeptide hormones result in abnormal gastrointestinal motility. PD results in raised intra-abdominal pressure, which may affect both appetite and cause vomiting and constipation, and dietary and fluid restrictions may result in an inadequate diet. Peritoneal dialysate losses of protein and sodium may be high. Finally, co-morbidities may cause poor feeding in their own right [31, 73]. Nutritional deficiency is, therefore, one of the most frequent and important factors contributing to growth failure. The Pediatric Renal Nutrition Taskforce has produced guidelines for the nutritional management of children with CKD stages 2–5 and on dialysis (*vide infra*) [74, 75].

Protein-Energy Wasting

Protein-energy wasting (PEW) is characterized by maladaptive responses including anorexia, elevated basic metabolic rate, wasting of lean body tissue, and under-utilization of fat tissue for energy [76]. It differs from malnutrition in which appetite is maintained, and weight loss is associated with protective metabolic responses such as a decreased basic metabolic rate and preservation of lean body mass at the expense of fat mass. Malnutrition can usually be overcome by nutritional supplementation or changes in the composition of the diet, whereas PEW can only be partially reversed by increased nutrition. Why some children develop PEW is unknown, but inflammation is likely to play a role [77]. However, growth failure is one of the main manifestations of PEW in children with CKD. New understandings of the pathophysiology of PEW in CKD have the potential for novel therapeutic strategies such as ghrelin agonists and melanocortin antagonists [31].

Obesity

At this stage it is important to mention obesity, which is emerging as a growing problem for children with CKD. In the ESPN/ERA-EDTA registry including 25 countries, of 5199 patients below the age of 18 years the prevalence of underweight was 4.3%, while 19.6% and 11.2% were overweight or obese respectively [78]. Receiving steroid therapy and living with a kidney transplant were independent risk factors for overweight. The incidence of obesity parallels that around the world in the normal population. The IPPN database demonstrates this regional variation, with BMI-SDS ranging from a mean of 0.8 in the US to −1.4 in India in children of all ages [31]. Twenty six percent of infants were obese in the US and 50% malnourished in Turkey [31, 58]. In North America, the frequency of obesity is increasing in the CKD population both before as well as at CKD stage 5 [79]. Obesity is a particularly a problem after kidney transplantation. This has been studied in the NAPRTCS database in a retrospective cohort study of 4326 children transplanted between 1995 and 2006, and followed up to 2007. Median BMI increased by 11% at 6 months but with no substantial changes thereafter [80]. In Europe, children with the lowest BMI and those over 5 years of age at transplant showed the greatest increases in BMI post-transplant [78]. UK 2017 data shows that the mean BMI of transplanted children was 1 SDS (0–1.8), whereas it was 0 (−0.8–1.1) for children on dialysis (UK Renal Registry (2021) UK Renal Registry 23rd Annual Report—data to 31/12/2019, Bristol, UK. Available from renal.org/audit-research/annual-report). The use of steroid sparing regimens may mitigate post transplant obesity [4]. Important to note, among transplanted recipients, a very short stature (OR: 1.64, 95% CI: 1.40–1.92) and glucocorticoid treatment (OR: 1.23, 95% CI: 1.03–1.47) were associated with a higher risk of being overweight/obese. Hence, at least in post-transplant patients, obesity is also a risk factor for poor growth.

Metabolic Acidosis

Metabolic acidosis (serum bicarbonate <22 mEq/L) usually begins when the GFR falls below 50% of normal, and is associated with decreased longitudinal growth and increased protein breakdown [81, 82]. Metabolic acidosis is also associated with endocrine consequences: in experimental uremia there is increased glucocorticoid production, increased protein degradation, and profound effects on the somatotropic hormone axis. The latter is characterized by down regulation of spontaneous GH secretion by the pituitary gland, decreased expression of the GH-receptor and insulin like growth factor I (IGF-I) receptor in target organs, and decreased IGF I serum concentrations [83]. Hence, metabolic acidosis induces a state of GH insensitivity, which is likely to contribute to impaired longitudinal growth in CKD patients.

Disturbances of Water and Electrolyte Balance

Although the relationship between salt loss and growth failure has not been formally proven in CKD, children with isolated tubular disorders resulting in urinary salt and water losses show severe growth retardation which can be at least partly resolved by adequate salt and water supplementation. The same applies to patients with a reduced chloride diet or with familial chloride diarrhea [84]. Growth impairment in diabetes insipidus supports the concept that polyuria may also contribute to growth failure in CKD patients [85].

CKD-Mineral and Bone Disorder (CKD-MBD)

It is widely accepted that skeletal deformities due to CKD-MBD contribute to uremic growth failure [86, 87]. Pronounced secondary hyperparathyroidism can interfere with longitudinal growth by destruction of the growth plate architecture, epiphyseal displacement and metaphyseal fractures. Severe destruction of the metaphyseal bone architecture may result in complete growth arrest. Treatment with 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) improves growth in uremic rats, but this not been demonstrated in children with CKD [88, 89].

PTH levels primarily reflect osteoblast activity. Therefore, it has been speculated that low PTH levels may be associated with poor bone and statural growth, and conversely high PTH levels might be expected in well growing children; and similarly, that poor growth might be expected with adynamic bone disease and better growth in its absence. Information on this issue is conflicting. Diminished growth rates have been shown in four dialyzed patients who had adynamic bone disease on bone biopsy, and PTH levels were positively correlated with the annual change in standardized height [90]. However, the proportion of patients showing adynamic bone disease in this population, where all subjects received high-dose intermittent calcitriol treatment, was rather high (25%). Therefore, this does not represent patients treated nowadays [91]. Indeed, low bone turnover was noted in only 4% of pediatric patients on dialysis in a recent study. In addition, there was no relationship between PTH levels and growth rates in 35 prepubertal children on dialysis for more than one year. Moreover, stable growth was seen with PTH levels only slightly above the normal range, and even catch-up growth occurred in children younger than 2 years [92]. In addition, one well-designed direct histomorphometric assessment in children on dialysis revealed no association between low bone turnover and body growth [93]. The IPPN offers the most up to date information from the largest cohort of PD patients: the annual prospective change in standardized height tended to correlate inversely with time-integrated mean PTH levels: patients with mean PTH levels >500 pg/mL (i.e. > 9 times upper limit of normal (ULN)) showed a significant loss in height SDS as compared to children with lower PTH levels (−0.28 versus −0.05 SDS per year; $p < 0.05$) [94]. Thus, dialyzed children with normal or only slightly elevated PTH levels have the potential for normal growth, whereas patients with high PTH levels (>500 pg/mL) are at increased risk of growth failure.

Anemia

Longstanding anemia in CKD patients has profound systemic consequences including anorexia and catabolism due to altered energy turnover, and multiple dysfunctions of organ systems. Retardation of growth and development is a hallmark of untreated chronic anemias of non-renal origin, such as thalassemia major. Theoretically, anemia may interfere with growth via mechanisms such as poor appetite, undercurrent infections, cardiac complications and reduced oxygen supply to cartilage. The advent of recombinant human erythropoietin in the late 1980s permitted study of the effects of anemia correction on longitudinal growth in CKD. Although short-term stimulatory effects of erythropoietin on longitudinal growth have been reported anecdotally, no persistent catch-up growth could be demonstrated in several multicenter clinical trials [95, 96] Partial correction of anemia has, though, improved exercise capacity and decreased heart rate and resting oxygen consumption [95, 97].

Endocrine Changes

Uremia interferes with the metabolism and regulation of various peptide hormones. This leads to inappropriate concentrations of circulating hormones and/or altered hormone action on target tissues (Fig. 56.1). Distinct alterations of the somatotropic and gonadotropic hormone axes have been identified, which are believed to play an important role in uremic growth failure [98].

Gonadotropic Hormone Axis

Gonadal Hormones

Low or low normal total and free testosterone (T) as well as dihydrotestosterone (DHT) plasma concentrations due to decreased synthesis and/or increased metabolic clearance have been reported in adolescents and adults with longstanding uremia [99]. The reduced conversion of T to DHT secondary to diminished 5α-reductase activity might contribute to the delayed pubertal development seen in some boys on dialysis [100]. Likewise, plasma estradiol levels in women tend to decrease in parallel with GFR reduction, and some adolescent girls show low-normal or decreased estradiol levels in relation to pubertal age [100, 101]. However, these observations were all made more than 20 years ago. At least in transplanted children this issue seems to be resolved nowadays. In a recent study, the majority of transplanted children without prior long-term dialysis had normal estradiol and testosterone levels [36]. This may at least partly explain the improvement of pubertal development in patients on KRT during the last decades [1].

Gonadotropins

Increased plasma concentrations of LH and FSH in combination with decreased or low-normal gonadal hormones suggest a state of compensated hypergonadotropic hypogonadism in patients with CKD stage 5 [102]. However, in patients on dialysis, gonadotrophin secretion may be inadequate relative to the degree of hypogonadism. This is compatible with an additional defect of pituitary gonadotropin release and the analysis of spontaneous pulsatile LH secretion has provided insights into the underlying pathophysiology [103, 104]. In dialyzed patients mean LH plasma levels are elevated despite significantly reduced pituitary LH secretion, due to the markedly impaired kidney metabolic clearance of LH. When kidney function is restored by kidney transplantation, pulsatile LH secretion normalizes and hypergonadotropic FSH and/or LH levels are only rarely observed [36, 103]. Animal studies suggest that a primary hypothalamic defect may contribute to the delayed onset of puberty in patients with uremia. The observed reduced release of hypothalamic gonadotropin-releasing hormone (GnRH) is due to uremia-related inhibitory factors and/or to an increased tone of the inhibitory neurotransmitter gamma-aminobutyric acid [105, 106].

Beyond the quantitative alterations of gonadotropin release, uremia also affects the biological quality of circulating gonadotropins. In pubertal and adult dialysis patients the proportion of bio-

active LH in relation to the total immunochemically measurable amount of LH is reduced. This might be due to altered glycosylation and/or accumulation of less active isoforms [105–107].

In summary, insufficient activation of the hypothalamic GnRH pulse generator, likely mediated via circulating inhibitors, appears to be the key abnormality underlying delayed puberty and altered sexual functions in patients with CKD stage 5. However, kidney transplantation is able to completely normalize all these alterations in the majority of patients if long periods on dialysis treatment are avoided.

Somatotropic Hormone Axis

Growth Hormone Secretion and Metabolism

In both pediatric and adult CKD patients fasting GH concentrations are normal or even increased, depending on the stage of CKD. GH, a 22-kilodalton protein, is almost freely filtered by the glomerulus (sieving coefficient ~0.82) and thereby ultimately cleared from the circulation [108]. Indeed, a linear relationship between GFR and the metabolic clearance rate of GH has been shown; GH clearance is reduced by approximately 50% in patients with CKD stage 5 [108, 109]. The prolonged plasma half-life of GH, rather than increased endogenous secretion, explains the increased circulating GH concentrations in uremia. Pituitary GH secretion is unaltered in prepubertal patients but decreased in adolescents with CKD, suggesting insufficient stimulation by gonadal steroids during puberty [110, 111]. In addition, malnutrition and metabolic acidosis negatively impact GH secretion rates in children with CKD [112].

Growth Hormone Receptor and GH Signaling

Experimental studies have advanced our understanding of uremic GH resistance. Both, the GH-induced hepatic as well as the growth plate cartilage IGF-I synthesis is diminished, due to either decreased expression of the GH receptor (GH-R) and/or a postreceptor signaling defect [113, 114]. Whereas reduced expression of the GH-R encoding mRNA in liver and growth plate chondrocytes was seen, hepatic and growth plate cartilage GH-R protein levels were comparable in uremic and non-uremic animals when corrected for uremia-associated anorexia by pair feeding [113–115]. Thus, whereas a decreased GH-R abundance in the liver and the growth plate cartilage is questionable, a postreceptor GH signaling defect was identified as cause of the diminished hepatic IGF-I secretion upon GH stimulation. In fact, aberrant GH-dependent JAK-STAT signaling has been noted in experimental animals [116]. Binding of GH to its receptor leads to activation of the JAK-STAT cascade by tyrosine phosphorylation, and transcriptional activation of IGF-I synthesis and proteins of the suppressor of cytokine signaling (SOCS) family. The latter are responsible for dephosphorylation of the GH-activated cascade and as such provide a GH-regulated negative feedback loop. However, under the conditions of chronic uremia the equilibrium between GH-induced transcriptional activation of IGF-I and SOCS is shifted towards SOCS overstimulation. Recent studies suggest that the chronic inflammatory state associated with CKD contributes to GH resistance, as SOCS are also induced by inflammatory cytokines [117, 118].

In humans, GH binding protein (GHBP), which enters the circulation by proteolytic cleavage of the extracellular receptor domain, is taken as a measure of GH receptor expression. In line with animal experiments, GHBP plasma levels in CKD patients are decreased and are related to residual kidney function [119].

Insulin-Like Growth Factor Plasma Binding and Tissue Action

Apart from GH resistance, insensitivity to IGF-I is also found in patients with advanced CKD [120–122]. While serum concentrations of IGF-I and IGF-II are usually within the normal range in children with CKD stage 1–4, IGF-I levels are slightly reduced and those of IGF-II mildly increased in dialyzed patients [123]. In contrast to the unchanged total amount of circulating immunoreactive IGF, somatomedin bioavailabil-

ity is reduced in advanced CKD pointing to the existence of circulating inhibitors [124]. A low-molecular weight somatomedin inhibitor (~1 kDa) was reported to circulate in uremic serum in an early study, but this has not been characterized further. Later studies focused on the accumulation of the specific high-affinity IGF-binding proteins (IGFBP1–6), which are normally cleared by the kidneys and are considered the main cause of diminished somatomedin bioactivity in uremia. In particular, the concentrations of IGFBP-1, −2, −3, −4 and − 6 increase as kidney function declines and IGFBP-1, -2 and -6 have been shown to inhibit IGF I bioactivity in-vitro [125, 126]. In contrast, the serum concentration of IGFBP-5 is normal and IGFBP-5 proteins undergo intense proteolytic cleavage in chronic uremia [126]. Likewise, the elevated level of IGFBP-3 is mostly due to the accumulation of proteolytic fragments whereas intact IGFBP-3 is markedly diminished [127]. The molar excess of IGFBPs over IGFs is approximately 150% in children with CKD and 200% in children on dialysis as compared to 25% in children without CKD. An inverse correlation between growth retardation and IGFBP-1, -2, and -4 serum concentrations has been described [128]. Reduced IGF bioactivity can be normalized by removing unsaturated IGFBP [124]. These data are in favor of the concept that serum IGFBPs increase with progression of CKD, and that the greater excess of IGFBPs by CKD stage 5 contributes to the more severe growth failure and reduced response to rhGH therapy in these children. In addition, cellular IGF signaling is impaired in the uremic state; it remains to be elucidated whether a postreceptor mechanism similar to the one observed for GH signaling is responsible for this phenomenon [118, 122, 124].

In summary, the marked deficiency of IGF-I synthesis, the modest elevation of GH levels due to decreased metabolic clearance, and increased IGF plasma binding capacity, strongly support the concept of a multilevel homeostatic failure of the GH-IGF-I system.

Corticosteroid Treatment

Long-term glucocorticoid treatment in patients after transplantation leads to diminished longitudinal growth by impairment of the somatotropic hormone axis. High-dose glucocorticoid treatment suppresses pulsatile GH release from the pituitary gland mainly by reduction of pulse amplitude [129]. The physiologic increase in GH secretion during puberty is reduced in allograft recipients receiving glucocorticoid treatment ($\geq$ 4 mg/m^2 per day) and the association between sex steroid plasma concentrations and GH release observed in healthy adolescents is blunted [129]. These changes are mainly due to increased hypothalamic somatostatin release.

In addition to reduced GH release, corticosteroids suppress GH-R mRNA and protein in animals and most likely also in humans [130]. Consequently, hepatic IGF-I mRNA levels are reduced in animals receiving glucocorticoids. However, plasma concentrations of IGF-I in patients treated by glucocorticoids are normal or only slightly reduced. In individual children on corticosteroid treatment impaired longitudinal growth occurs despite normal GH secretion and plasma IGF-I levels, suggesting insensitivity to GH and IGF-I at the level of the growth plate. Indeed, a direct growth inhibiting effect of dexamethasone on the growth plate was shown by local injection in rabbits [131]. In cultured growth plate chondrocytes glucocorticoids decreased DNA synthesis and cell proliferation in a dose-dependent fashion, associated with reduced expression of the GH receptor and diminished paracrine IGF-I synthesis [132]. In addition, pharmacological doses of glucocorticoids also impaired the proliferative response to the calciotropic hormones calcitriol and PTH [132]. This is at least partly related to a diminished release of paracrine IGF-I secretion by these hormones. IGF-I modulates its own activity in cultured rat growth plate chondrocytes by the synthesis of both inhibitory (IGFBP-3) and stimulatory (IGFBP-5) binding proteins. This is modified by

glucocorticoid treatment. Therefore, glucocorticoid treatment not only interferes with the somatotropic hormone axis with respect to GH secretion and GH / IGF-I receptor signaling, but also by modulation of paracrine IGF-I synthesis and binding by IGF binding proteins. Even more important, based on *in vivo* studies in rabbits catch-up growth after glucocorticoid exposure may remain incomplete in general [131]. Consequently, all efforts must be undertaken to reduce steroid exposure in children with CKD before and after kidney transplantation [133].

Treatment of Growth Failure in Chronic Kidney Disease

General Measures

The main measures for prevention and treatment of growth failure in children with CKD are summarized in Table 56.1. Close growth monitoring with intervals depending on previous growth, age and stage of CKD is essential. Early referral to a pediatric nephrology center followed by careful nutritional and metabolic management is vital in the prevention of growth retardation [134]. Growth retardation is clearly correlated to the degree of CKD. Therefore, adequate measures should be undertaken to preserve GFR, and to provide adequate dialysis in those children who require maintenance dialysis [72]. Preservation of kidney function requires treatment of elevated blood pressure, aiming for blood pressure values below the 50th and 75th percentile in proteinuric and non-proteinuric children, respectively [135]. Renin-angiotensin aldosterone system inhibitors, preferentially angiotensin-converting enzyme inhibitors or angiotensin receptor inhibitors, should be used to treat high blood pressure and ameliorate proteinuria in children with CKD [136]. Nephrotoxic medication should be avoided, and urinary tract infections in children with congenital abnormalities of the kidneys and urinary tract (CAKUT) should be treated. Finally, disease specific treatment, e.g. treatment with cysteamine in patients with nephropathic cystinosuis, may be required.

Table 56.1 Main measures for prevention and treatment of growth failure in pediatric CKD

Prevention:
• Close growth monitoring with intervals depending on previous growth, age and stage of CKD
• Preserve kidney function by:
– treating elevated blood pressure and reducing proteinuria, preferrably using RAAS inhibitors
– avoiding nephrotoxic medication
– prompt treatment of urinary tract infections
• Provide adequate energy and protein intake and consultation with a renal dietician.
– Consider enteral feeding by gastrostomy or nasogastric tube in cases of persistent insufficient oral intake
• Substitute water and electrolyte losses and correct metabolic acidosis
• Keep PTH levels in the recommended CKD stage-dependent target range and substitute native vitamin D in cases of low vitamin D levels
• Aim for early (preemptive) kidney transplantation with minimal steroid exposure in patients with end-stage CKD
• Provide adequate dialysis in patients requiring maintenance dialysis
Treatment:
• Consider use of growth hormone treatment in cases of persistent growth failure, i.e. height < third percentile and height velocity < 25th percentile, excluding patients who have received a transplant within the last 12 months
• Consider intensified dialysis or hemodiafiltration in in patients requiring maintenance dialysis presenting with persistent growth failure
• Consider use of rhGH therapy in pediatric kidney transplant recipients for whom expected catch-up growth cannot be achieved by steroid minimization, or for patients where steroid withdrawal is not feasible due to high immunological risks, particularly in children with suboptimal graft function (eGFR <50 mL/min/1.73 m^2)

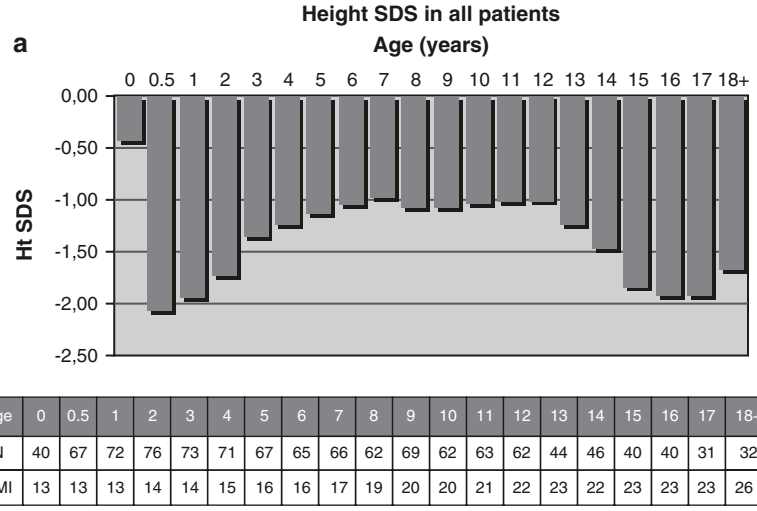
CKD chronic kidney disease, *RAAS* renin-angiotensin aldosterone system, *PTH* parathyroid hormone, *eGFR* estimated glomerular filtration rate

Adequate **nutritional management** is crucial in infants and young children, who frequently need supplementary feeding via nasogastric tube or, preferably, gastrostomy [75, 137]. In a retrospective analysis of growth in 101 infants and young children with severe CKD it could be demonstrated that persistent catch-up growth can be achieved in the majority of patients when there is good metabolic control and enteral feeding is commenced at the first sign of growth delay (Fig. 56.5) [20]. However, spontaneous growth as well as catch-up growth after initiation of enteral feeding is limited in patients with comorbidities [20, 138]. The use of enteral feeding varies around the world, as has been clearly demonstrated by the IPPN report of 153 infants on PD: gastrostomies are most commonly used in the US, where 80% of infants on PD are gastrostomy fed; 20% have gastrostomies in Europe, but there are very small numbers or none in the rest of the world [13]. Nasogastric feeding is commonest in Europe and Latin America. Gastrostomy feeding, rather than demand or nasogastric tube feeding, is associated with better preservation of linear growth in the infants in the IPPN database (Fig. 56.6)) [13]. This may be related to decreased vomiting with gastrostomies as compared to nasogastric tubes. Catch-up growth in children started on enteral feeding after the age of 2 years may be substantial but is markedly reduced in those on dialysis and strongly negatively correlated with age [139]. The assurance of adequate caloric and energy intake requires the patient and families to be advised by a kidney dietician, especially when supplementary feeding via nasogastric or gastrostomy tube is required [74, 137] In general, the initial prescription for energy intake in children with CKD should approximate that of healthy children of the same age (suggested dietary intake, SDI) [74]. To optimize growth in children with suboptimal weight gain and linear growth, energy intake should be adjusted towards the higher end of the SDI [74]. Caloric intake should account for growth failure and be related to "height age" rather than to chronological age if the child's height is below the normal range. Calorie intake in excess of 100% of SDI may not improve catch-up growth but rather results in obesity and may thereby negatively contribute to long-term cardiovascular morbidity in CKD patients [140, 141]. If there is excessive weight gain dietary energy intake should be reduced for children on PD to compensate for the energy derived from dialysate glucose, estimated at 8–12 kcal/kg per day. In addition; to promote optimal growth, target protein intake in children with CKD should be at the upper end of the SDI [74]. In patients on PD, a slightly higher intake (+0.2 g/kg/day) is recommended to compensate for dialytic protein losses. The aim is to maintain a normal serum albumin and a urea below 20 mmol/L as far as possible. High protein intakes should be avoided since, despite many attempts, anabolizing or growth promoting effects of high-protein diets have neither been demonstrated in animal models nor in children with CKD. On the contrary, high protein diets may be detrimental by aggravating metabolic acidosis and augmenting the dietary phosphorus load.

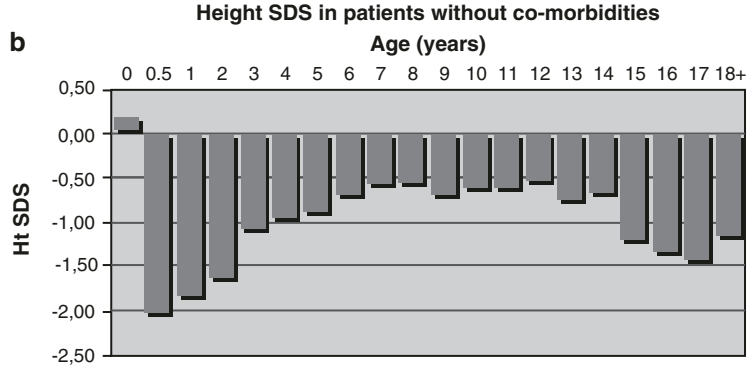
Metabolic acidosis should be vigorously treated by alkali supplementation aiming for serum bicarbonate levels equal or above 22 mEq/L. This can be assured by treatment with sodium bicarbonate and/or the use of HCO_3-based or lactate based dialysis solutions in patients on dialysis [11]. In addition, supplementation of water and electrolytes is essential in patients presenting with polyuria and/or salt losing nephropathies. Supplementation of sodium chloride is also important in young children on PD, since significant amounts of sodium chloride (i.e. 2–5 mmol/kg body weight) may be eliminated via ultrafiltration.

Dialysis and Intensified Dialysis

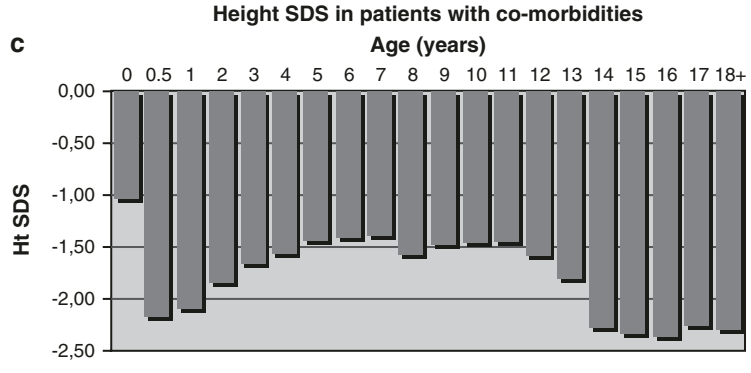
Although dialysis attenuates the uremic state, longitudinal growth is not usually improved and long-term PD or HD are associated with a gradual loss of standardized height in children and adolescents, and can be as high as 1 SD per year in infants [9, 142–144]. Children on dialysis who maintain some residual kidney function have the best growth; indeed residual kidney function may be a better predictor of longitudinal growth than dialytic clearance [145, 146]. However, a recent



Age	0	0.5	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18+
N	40	67	72	76	73	71	67	65	66	62	69	62	63	62	44	46	40	40	31	32
BMI	13	13	13	14	14	15	16	16	17	19	20	20	21	22	23	22	23	23	23	26



Age	0	0.5	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18+
N	20	33	38	39	38	37	35	33	33	30	29	31	27	28	23	23	17	17	12	17
BMI	14	12	12	14	14	15	16	16	17	18	20	21	20	22	23	23	23	23	22	25



Age	0	0.5	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18+
N	20	24	34	37	35	34	32	32	33	32	30	31	26	24	21	23	23	23	19	15
BMI	13	13	13	14	14	15	16	16	17	19	19	20	21	22	23	22	22	22	23	26

Fig. 56.5 Course of mean standardized height and body mass index (BMI) of children presenting within the first 6 months of life with a glomerular filtration rate less than 20 mL/min/1.73 m^2 receiving tube feeding in order to provide at least 100% of the recommended daily allowance (RDA) of healthy children. (**a**) Height SDS and BMI values for all patients. (**b**) Height SDS and BMI values for patients without comorbidites. (**c**) Height SDS and BMI values for patients with comorbidities. (Reproduced with permission of [20])

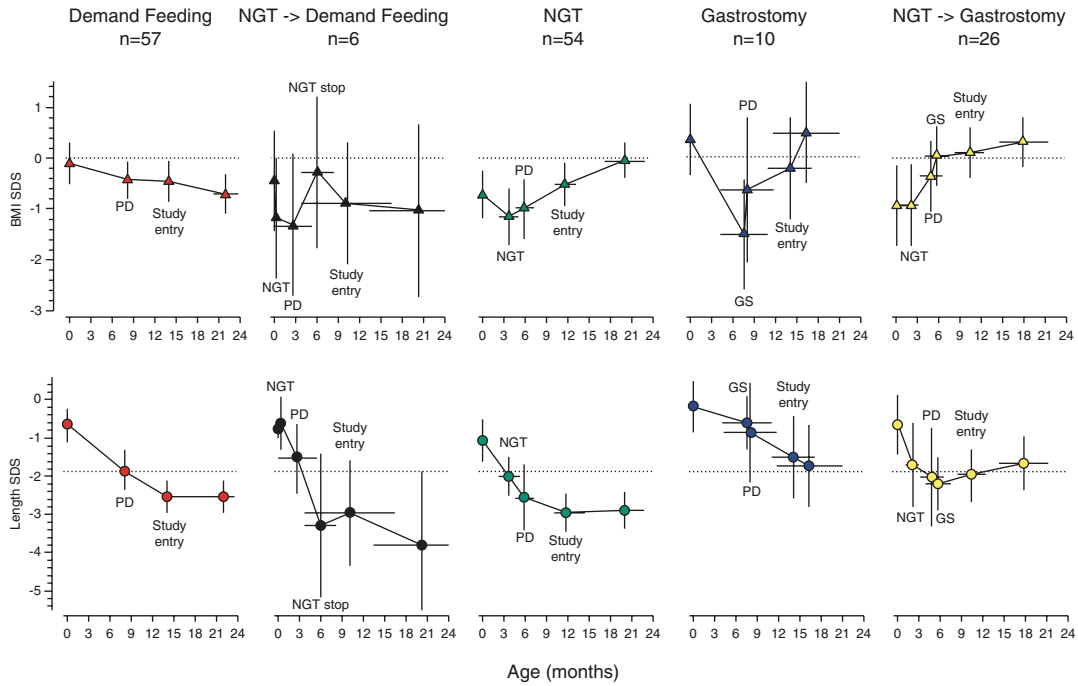
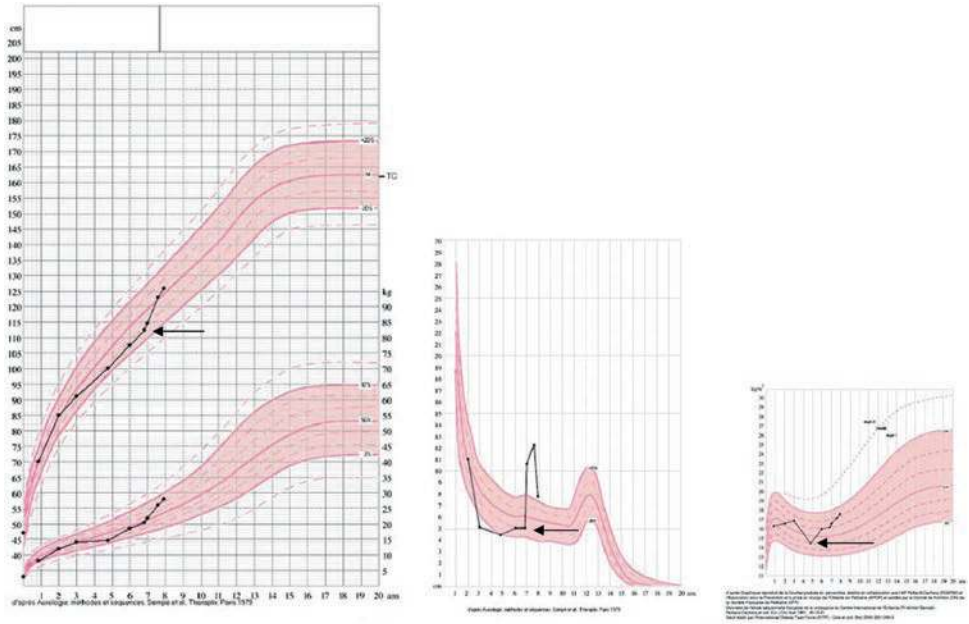


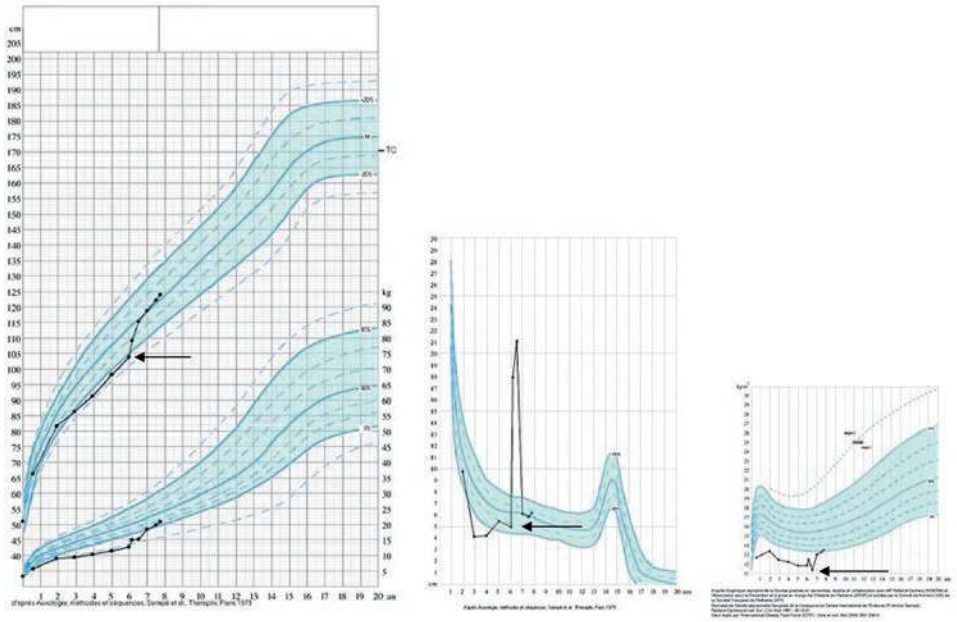
Fig. 56.6 Whereas both nasogastric tube (NGT) and gastrostomy (GS) feeding improve nutritional status, only GS feeding associates with stabilized linear growth in young infants undergoing CPD. The data points represent mean estimates at key time points of postnatal development, (i.e., birth, commencement of CPD, initiation and discontinuation of nasogastric tube or gastrostomy feeding, enrollment to IPPN [study entry], and last available observation). Two-dimensional error bars denote the 95% confidence intervals to mean age and SDS at the respective time point. [Reproduced with permission of [13]]

Italian study in infants on chronic PD reported catch-up growth in 50% of patients [147]. High peritoneal transporter status, a condition associated with increased morbidity and mortality in adults, is associated with poor longitudinal growth in children on chronic PD [148]. This might be due to the putative association of high peritoneal transport with inflammation, which can suppress statural growth by interference with GH signaling (*vide supra*), or excessive losses of proteins and amino acids important to growth.

It has been suggested that intensified dialysis, achieved by either extended thrice-weekly sessions, daily nocturnal or short daily sessions, might be able to induce catch-up growth [148–150]. According to a French study, catch-up growth can be maximized when intensified hemodiafiltration (3 h, 6 times a week) and rhGH therapy are combined [148, 149, 151–154]. Using this approach in 15 mainly prepubertal children for an average observation time of 21 months, there was an average increase in growth velocity from 3.8 cm/year at baseline to 8.9 cm/year (Fig. 56.7). This resulted in a mean of 1.7 SDS gain of standardized height, representing complete catch-up growth according to the attainment of the target height SDS [153]. A recent non-randomized study demonstrated superior growth in children treated with hemodiafiltration compared to those with conventional hemodialysis (HD) (Fig. 56.8) [155]. From a pathophysiological point of view, intensified hemodiafiltration (HDF) is a better substitute for physiological kidney function and may allow substantially better clearance of uremic toxins, e.g. middle-molecular weight compounds [155]. As a result, microinflammation and metabolic acidosis may be abolished, leading to improved appetite and tissue anabolism. The improved removal of inflammatory cytokines might reverse



Patient 14, a girl on daily on line hemodiafiltration
Protein diet intake(g/kg/d) : 2.4±0.4 ; protein nitrogen appearance(g/kg/d) : 1.3 ±0.14
Mean growth velocity (cm/year) : 9.6
Achieved height versus mid parental target height (SDS) : +0.3



Patient 1, a boy on daily on line hemodiafiltration.
Protein diet intake(g/kg/d) : 2.7±0.2 ; protein nitrogen appearance(g/kg/d) : 1.44 ±0.15
Mean growth velocity (cm/year) : 10.4
Achieved height versus mid parental target height (SDS) : +0.2

Fig. 56.7 Examples of growth charts (height and weight chart; growth velocity chart in centimeters per year; body mass under chart) of two patients on daily online hemodiafiltration (start indicated by bars) in addition to rhGH treatment . TC on height chart is the familial target height in (centimeters). [Reproduced with permission of [153]]

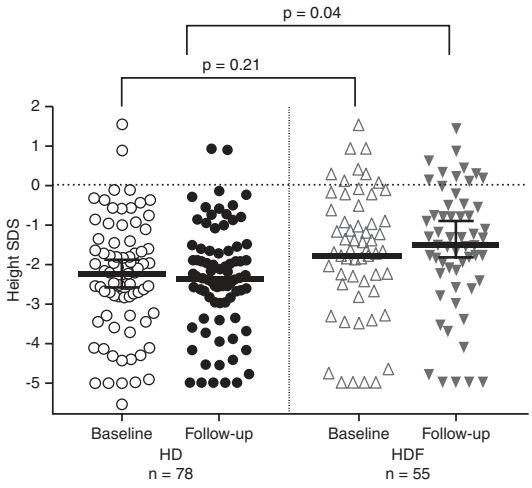


Fig. 56.8 Improved height SDS in children on hemodiafiltration (HDF) compared to hemodialysis (HD). The figure shows change in height SDS in the HD and HDF arms at baseline and 1-year follow-up. Data are shown as median and interquartile range. Within-group analyses performed by Wilcoxon matched-pairs signed-rank test and HD versus HDF cohorts compared by Mann–Whitney U test. At 12 months the height SDS in the HDF group was higher than in the HD group (P = 0.04). (Reproduced with permission of [155])

GH resistance enabling the full therapeutic potential of rhGH [152]. The positive effects of intensified dialysis, particularly home HD, usually outweigh the potential impact on psychosocial integration and treatment costs. Prospective randomized trials appear required to provide definite proof to this promising concept. In particular, the relative contribution of concomitant rhGH therapy to the growth improvement observed with intensified dialysis remains to be defined.

Transplantation

Although many of the metabolic and endocrine disorders contributing to uremic growth failure are resolved by kidney transplantation, post-transplant catch-up growth is usually restricted to young children and occurs far from regularly [3, 156]. As well as transplant function, age, severity of stunting at time of transplantation and glucocorticoid dosage are inversely associated with longitudinal growth. One retrospective study found that while standardized height was comparable at time of transplantation, it was significantly higher among living-related donor (LRD) than deceased donor transplant (DDT) recipients 5 years later [157]. This benefit of LRD grafts was independent of GFR arguing for preferential LRD in children with respect to post-transplant growth.

It must be stressed that even low-dose glucocorticoid treatment (<4 mg/m^2/day) results in growth suppression in children after transplantation. While complete steroid withdrawal has been associated with unacceptably high rejection rates in children with azathioprine and/ or cyclosporine A medication, withdrawal or even complete steroid avoidance appears much safer with the currently preferred immunosuppressants [158]. In a randomized trial of late steroid withdrawal in patients on treatment with cyclosporine A, mycophenolate mofetil steroid-free patients showed improved growth compared to controls (i.e. change in height SDS; 0.6 ± 0.1 versus −0.2 ± 0.1) within 27 months [159]. However, catch-up growth in pubertal patients was rather limited compared to that in prepubertal patients. It seems logical

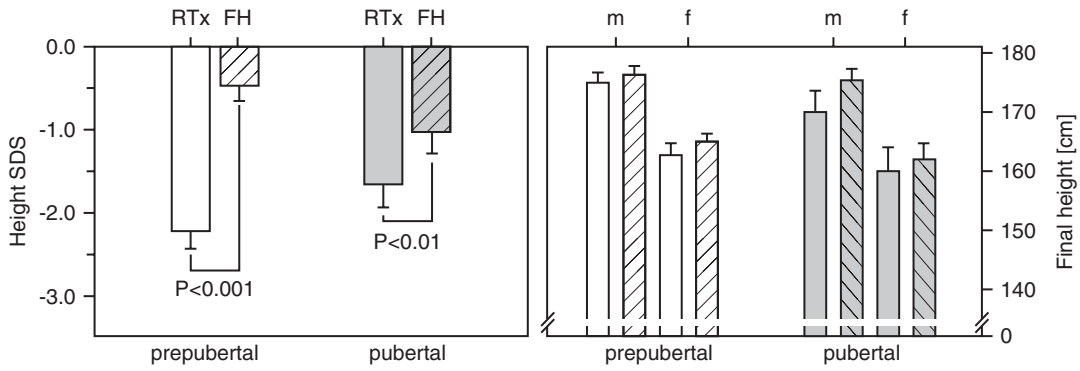


Fig. 56.9 Mean standardized height at time of renal transplantation compared to adult height and comparison of adult height with genetic target height in patients with steroid-withdrawal during month 4–6 after transplantation Mean standardized height at the time of transplantation and final height in prepubertal (n = 36) and pubertal (n = 24) patients. (d) Mean adult height (open bars) and genetic target height (hatched bars) in boys (n = 25) and girls (n = 17). Data in (c) and (d) are given as mean ± SEM. (Reproduced with permission of [4])

that if steroids are withdrawn at an early stage, or even completely avoided, a better growth outcome will be observed. Indeed, a retrospective analysis of longitudinal growth in a cohort of 74 children who had been weaned off steroids within 6 months of transplantation showed remarkable results [4, 159]. Mean adult height was −0.5 ± 1.1 SDS and −1.0 ± 1.3 SDS in prepubertal and pubertal patients and was within the normal range (>−2 SD) in 94% and 80% of them respectively (Fig. 56.9). Likewise, early steroid-withdrawal (< 6 weeks) and complete steroid avoidance improved standardized height by approximately 1.0 SDS within 3–5 years post-transplantation [32, 33, 160, 161]. Although experimental data indicate that mTOR inhibitors may interfere with chondrocyte proliferation and/or gonadal hormone synthesis, recent case control studies in transplanted children revealed similar growth rates in patients with and without mTOR inhibitor treatment [162].

In summary, efforts to avoid a height deficit before transplantation, early (preemptive) kidney transplantation, and immunosuppressive strategies characterized by the early withdrawal or even complete avoidance of steroids can improve adult height and normalize body proportions in children after successful transplantation.

Endocrine Therapies

Vitamin D

Calcitriol deficiency is a major cause of secondary hyperparathyroidism and CKD-MBD. Although calcitriol supplementation reverses the biochemical, radiographic, and histological signs of high turnover bone disease, neither experimental nor clinical studies demonstrate consistent improvement in longitudinal growth [89, 162–164]. These conflicting results might be due to differences in the mode of administration and to the pleiotropic calcitriol-specific effects on growth plate chondrocytes. In addition, only a week association between parathyroid hormone (PTH) levels and linear growth was reported in children with advanced CKD [94]. In general, minimal PTH suppressive active vitamin D analogues dosages should be used in order to keep PTH levels in the desired target range [165]. Finally, supplementation with cholecalciferol or ergocalciferol should be initiated in children with serum 25-hydroxyvitamin D concentrations below 75 nmol/L (<30 ng/mL) [166].

Calcimimetics

Uncontrolled and controlled studies provide evidence that calcimimetics are an effective therapy for secondary hyperparathyroidism in pediatric

dialysis patients [167]. Calcimimetics suppress PTH secretion by activating the calcium-sensing receptor (CaR). The CaR is expressed by epiphyseal chondrocytes; its stimulation stimulates chondrocytic proliferation and differentiation. Thus, calcimimetics may affect longitudinal growth in uremia as well. In fact, the calcimimetic cinacalcet improved food efficiency and body weight gain in uremic rats, but no effects on growth plate morphology and/or longitudinal growth were seen [168]. Likewise, no beneficial or adverse effect on longitudinal growth was noted during calcimimetic treatment periods of up-to three years in children on dialysis [167]. A comprehensive European guideline on the use of cinacalcet in children on dialysis was published recently [169].

Growth Hormone

Pharmacological treatment of children with CKD and growth delay with rhGH actually predated the elucidation of the pathomechanisms that underlie chronic uremic alterations of the GH-IGF-1 axis [114, 169–171]. Administration of rhGH markedly stimulates IGF-I synthesis with only a modest effect on IGFBPs, thereby normalizing somatomedin bioactivity and promoting longitudinal growth [172]. The efficacy and safety of long-term treatment with rhGH in children with CKD before and after kidney transplantation is well established and clinical practice recommendations on this topic were recently published in 2019 [30].

Efficacy of rhGH in Prepubertal Children

In prepubertal children with pre-dialysis CKD, rhGH therapy typically doubles height velocity during the first treatment year [173]. Catch-up growth continues asymptotically during extended treatment [174–176]. In dialyzed children, the treatment response is significantly attenuated compared to children with pre-dialysis CKD (0.8 SD vs. 1.3 SD) [177]. RhGH responsiveness is similarly poor in children on peritoneal dialysis and standard hemodialysis, but as noted previously, can be markedly improved when dialytic clearance is augmented by daily HDF [153].

Based on the current experience with rhGH in pediatric CKD patients, a model to predict growth response was developed [178]. The prediction model was developed using a cohort of 208 prepubertal children with CKD stage 3-5D followed in a pharmaco-epidemiological survey (KIGS), and validated in an independent group of 67 CKD patients registered at the Dutch Growth Research Foundation. The height velocity during the first rhGH treatment year (PHV) was predicted by the following equation: PHV (centimeters per year) = 13.3 – [age (years) × 0.38 + (weight SDS × 0.39)] – [hereditary renal disorder (0 when absent or 1 when present) × 1.16] + [Ln rhGH dose (milligrams per kilogram per week) × 1.04] + [GFR (milliliters per minute × 1.73 m^2) × 0.023]. This equation explains 37% of the overall variability of the growth response. The SE of the estimate or error SD of the prediction model was 1.6 cm and non-responders in the validation group were correctly identified. This model may help in predicting non-responders and in tailoring treatment strategies for growth retarded children with CKD.

Several RCTs have shown the benefit of rhGH therapy in short prepubertal renal transplant recipients. A meta-analysis of five prospective RCTs involving a total of 401 patients showed that patients receiving rhGH therapy had a significantly higher growth velocity 1 year after the initiation of therapy than the control group, with a mean height SDS difference of 0.68 (95% CI 0.25–1.11) [173].

Effects of rhGH on Pubertal Growth and Adult Height

In a study following patients with CKD and ESKD from late prepubertal age to final height, the average height increment in rhGH treated patients was twice that seen in a matched control group [46]. The main benefit for total growth and final height was achieved before the onset of the pubertal growth spurt, whereas no overall effect on pubertal height gain was observed (Fig. 56.10).

Data on adult height are available from 11 non-randomized trials in which rhGH was administered for at least 2 years, comprising a total of 836 patients on various modes of KRT [30]. In

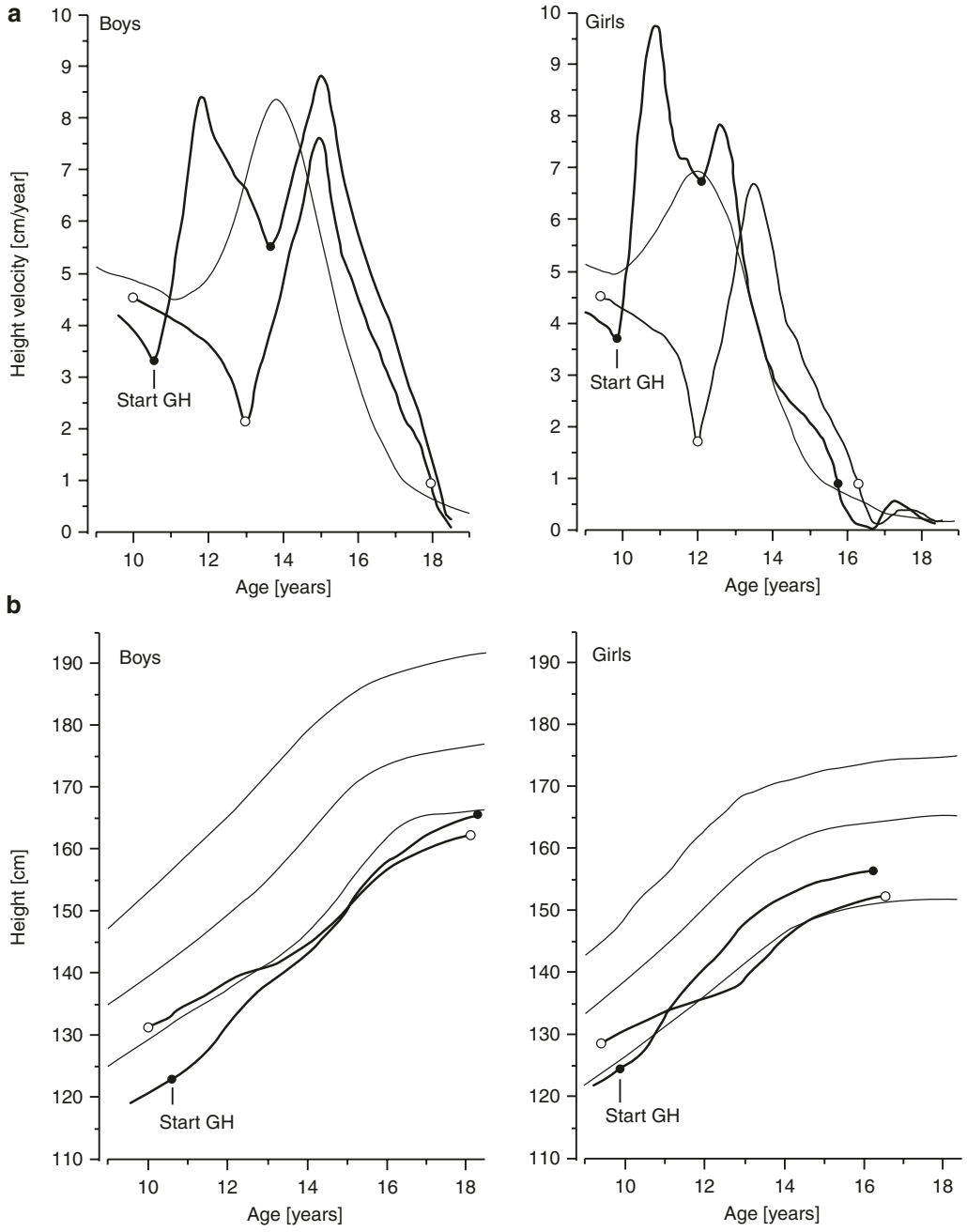


Fig. 56.10 (**a**) Synchronized mean height velocity curves of 32 boys (left panel) and 6 girls (right panel) with CKD during rhGH Treatment (closed circles), as compared with 50 children with CKD not treated with rhGH (open circles) and 232 normal children (thin lines). The dots indicate the time of the first observation, which corresponds to the start of rhGH treatment in the growth hormone-treated children, minimal prespurt height velocity, and the time of the end of the pubertal growth spurt. (Reproduced with permission of [46]). (**b**) Synchronized mean height curves of 32 boys (left panel) and 6 girls (right panel) with CKD during rhGH treatment (closed circles), as compared with 50 children with CKD not treated with rhGH (open circles). Normal values are indicated by the third, 50th, and 97th percentile. The dots indicate the time of the first observation, which corresponds to the start of rhGH treatment in the growth hormone-treated children, and the time of the end of the pubertal growth spurt. (Reproduced with permission of [46])

five studies a matched historical control group was included. The median change in standardized height until attainment of adult height amounted to 1.1 SDS (range 0.2–1.6 SDS) in rhGH treated patients ($p < 0.05$ for each final height measurement versus initial height measurement). This change corresponded to a median absolute increase in rhGH treated patients by 7.4 cm (range 1.4–10.8 cm) in boys and 7.0 cm (range 1.3–10.1 cm) in girls, based on European reference values. However, this calculation may underestimate the rhGH effect since in the non-rhGH treated controls adult height was significantly below the initial standardized height indices in all except one study. Height attained at the start of rhGH and throughout the duration of rhGH treatment were positively associated with final height, whereas time spent on dialysis, age at puberty onset, and age of start of rhGH were negatively associated with final height [46, 47]. Taken together, the available studies suggest that rhGH improves adult height in short prepubertal and pubertal CKD patients prior to and after kidney transplantation.

Efficacy of rhGH in Infants

According to standard concepts of the pathophysiology of uremic growth failure, malnutrition and fluid and electrolyte imbalances have a much greater impact on infant growth than alterations of somatotropic hormones. Consequently, correction of the nutritional status has been considered the primary measure to restore normal growth in growth retarded infants, postponing the option of endocrine therapeutic intervention to beyond the second year of life. This concept has been challenged by several reports of initiating rhGH in growth retarded infants with CKD [179–181]. A randomized controlled study involving 30 growth retarded infants (mean age: 16 months) with moderate CKD (mean eGFR: 25 mL/min/1.73 m^2) revealed excellent catch-up growth from from −3.0 to −1.1 SDS within 24 months of treatment, in contrast to no significant change in controls [179]. Another study reported an increase in height SDS from −3.3 to −2.2 within 12 months in 8 infants with a mean age of 22 months and CKD stage 3–4 [180]. In a randomized study of 16 infants with stage 3–4 CKD who were receiving at least 80% of the recommended daily allowence for calories and of whom seven were enterally fed, those randomized to rhGH showed significantly higher length gains (14.5 versus 9.5 cm/year; delta height SDS +1.43 versus −0.11) [181]. Hence, the results of these studies lend further support to the previous observation that the relative efficacy and cost efficiency of rhGH is actually best when initiated at young age, i.e. during infancy and early childhood [30]. While the provision of adequate nutrition is certainly vital to growth and development of infants with CKD, some children show growth failure despite adequate nutrition. In these patients, any further increases of energy intake typically lead to fat deposition, but not catch-up growth. Early rhGH therapy appears to be an attractive option to accelerate length and weight gain in such infants. The fact that the enhanced growth also helps the infant achieve the body size required for kidney transplantation more expeditiously is another substantial benefit [30].

General rhGH Treatment Strategies

Children from 6 months of age with stage 3–5 CKD or on dialysis should be candidates for rhGH therapy if they have persistent growth failure, defined as a height below the third percentile for age and sex and a height velocity below the 25th percentile, once other potentially treatable risk factors for growth failure have been adequately addressed, and provided the child has growth potential (open epiphysis on X-ray of the wrist) [30]. RhGH therapy should also be considered for children older than 6 months with stage 3–5 CKD or on dialysis who present with a height between the third and tenth percentile but persistent low height velocity (<25th percentile), once other potentially treatable risk factors for growth failure have been adequately addressed. Such early, preventive therapy is probably more cost-effective than starting at a more advanced age when growth retardation has become more evident and higher absolute rhGH doses are required.

Children suffering from nephropathic cystinosis often show severe growth retardation despite somewhat mild reductions in GFR, which is

thought to be related to the deleterious effects of Fanconi syndrome, resulting in hypophosphatemic rickets and malnutrition and/or an underlying obsteoblast/osteoclast defect [61]. Therefore, rhGH treatment is recommended in short children with nephropathic cystinosis at all stages of CKD [30].

The growth response to rhGH treatment is positively associated with residual kidney function, target height, initial target height deficit and duration of rhGH treatment, and inversely correlated with the age at start of treatment [46, 47, 174, 177, 178]. Daily dosing is more effective than three doses per week and the optimal dose is 0.045–0.05 mg/kg body weight per day by subcutaneous injections in the evening [30]. Parents and physicians should encourage children from about 8 to 10 years of age to do the rhGH injections on their own if adequate training and adherence can be assured, because this may ultimately improve patient adherence and self-esteem [30]. Whereas discontinuation of rhGH results in catch-down growth in approximately 75% of CKD patients this phenomenon is rarely observed when rhGH treatment is discontinued after kidney transplantation, highlighting the close relationship between kidney function and growth [182]. Furthermore, although the absolute height gain achieved by rhGH is independent of age, the reference range increases with age. Thus, rhGH treatment should be started as early as growth retardation becomes evident (i.e. height below third percentile) [30]. If height velocity in the first year of rhGH treatment is less than 2 cm per year over baseline, it is recommended to assess patient adherence to rhGH therapy through the measurement of serum IGF-I levels and/or the use of an injection pen with downloadable memory function, ensuring administration of the correct weight-adjusted GH dose, and addressing any, possibly subclinical, nutritional and metabolic issues [30].

The primary treatment target should be to return the child's height into her/his individual genetic percentile channel. Treatment may be suspended once this target is reached, but growth should be monitored closely as outlined above. In patients receiving rhGH while on conservative treatment rhGH should be continued after the initiation of dialysis, but stopped at the time of kidney transplantation. RhGH therapy should, however, subsequently also be considered for pediatric kidney transplant recipients for whom expected catch-up growth cannot be achieved by steroid minimization or for patients in whom steroid withdrawal is not feasible due to high immunological risk, particularly in children with suboptimal graft function (eGFR <50 mL/min/1.73 m^2). Growth should be monitored for at least 1 year post-transplantation before rhGH therapy is considered, in order to allow for spontaneous catch-up growth without need for rhGH therapy [30].

Potential Adverse Events Associated with rhGH Therapy

The safety of long-term rhGH treatment in CKD has been evaluated in several clinical studies and registries [30]. RhGH therapy in short children with CKD stage 3–5D and after kidney transplantation was not associated with an increased incidence of malignancy, slipped capital femoral epiphysis, avascular necrosis, glucose intolerance, pancreatitis, CKD progression, acute allograft rejection or fluid retention. Intracranial hypertension (ICH), manifesting in 3 out of 1376 CKD patients, was the only adverse event significantly associated with rhGH therapy [183]. However, in all three instances ICH occurred after discontinuation of rhGH. Due to the potentially increased risk of ICH in CKD, baseline fundoscopy is recommended prior to therapy initiation [30]. Furthermore, hydration should be carefully monitored in CKD patients receiving rhGH since overhydration may be a predisposing factor for ICH. In the presence of symptoms like headache or vomiting, an immediate workup for ICH including fundoscopy should be performed.

Although insulin secretion increases during the first year of rhGH treatment and hyperinsulinemia persists during long-term therapy, normal glucose tolerance is preserved during up to 5 years of rhGH administration in CKD patients on conservative treatment, dialysis, and after kidney tansplantation. Hyperinsulinemia is most pronounced in transplanted patients on con-

comitant glucocorticoid therapy. Hyperinsulinemia may, at least in theory, contribute to the development of atherosclerosis or induce diabetes mellitus by exhaustion of ß-cells. However, up to now this has not been observed in CKD patients receiving rhGH [183].

Aggravation of secondary hyperparathyroidism has rarely been reported in CKD patients on rhGH treatment, and the underlying pathomechanisms remain to be elucidated [184]. RhGH might have a direct stimulatory effect on the parathyroid gland and/or might have subtle effects on calcium homeostasis which in turn stimulate PTH secretion. Finally, increased longitudinal bone growth by rhGH treatment may unmask preexisting renal osteodystrophy. Therefore, bone metabolism should be evaluated carefully in candidates for rhGH therapy and severe hyperparathyroidism and renal osteodystrophy should be adequately treated before initiation of such therapy in CKD patients. Likewise, rhGH therapy should be stopped in patients with persistent severe secondary hyperparathyroidism (PTH > 500 pg/mL). RhGH may be reinstituted when PTH levels return to the desired PTH target range [30].

Future Perspectives

Despite attention to nutrition and the availability of rhGH therapy, the problem of CKD associated growth failure has not been resolved in the majority of dialysis patients. If early kidney transplantation is not possible, the concept of intensified hemodiafiltration combined with rhGH may be a promising option for patients suffering from growth retardation and GH insensitivity on conventional dialysis therapy. Independent of concomitant rhGH therapy, hemodiafiltration appears to allow improved growth rates than conventional hemodialysis and, if available, should be the preferred extracorporeal dialysis modality in all growing children.

Self-reported nonadherence to rhGH is associated with poorer growth velocity in children with CKD. Therefore, monitoring and optimizing adherence to rhGH therapy is key to satisfactory growth outcomes [185]. Another promising avenue of clinical research may be the provision of recombinant IGF-I administered as monotherapy or in combination with rhGH, as well as targeting of the SOCS2 signaling pathway [186].

A particular challenge is the management of severely diminished pubertal height gain seen in some adolescents with CKD. In such adolescents, pharmacological inhibition of epiphyseal closure may allow an extended duration of the remaining growth period. Since the closure of the epiphyseal growth plate is induced by local estrogen action, inhibition of estrogen synthesis is a principal therapeutic option. Whereas gonadotropin-releasing hormone analogues arrest pubertal progress, the potential growth benefit would come at the psychological disadvantage of delayed sexual maturation. In boys, aromatase inhibitors, which suppress local conversion of testosterone to estradiol, might extend the growth phase without affecting pubertal development and thereby increase the time window for the use of rhGH therapy. Indeed, the combined use of armomatase inhibitors and rhGH was associated with modest increases in adult height in boys with idiopathic short stature.

[187, 188]. However, some important questions about their long-term safety exist which at the moment prevent this from being generally recommended [189]. It would be fascinating to study its efficacy in adolescents on long-term dialysis. Nevertheless, successful early (preemptive) kidney transplantation with minimal steroid exposure is ultimately the best current measure to improve growth and final height in children with CKD stage 5.

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57 Neurodevelopment in Chronic Kidney Disease

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Introduction

Neurodevelopmental outcomes for young children with end-stage kidney disease (ESKD) historically were poor [1, 2]. As medical and surgical management of the disease have improved, cognitive and developmental outcomes have improved as well [3, 4]. However, our understanding of how the biochemical and hormonal milieu of kidney failure affects the growing brain remains incomplete. We also recognize that the treatments for kidney failure and their complications affect neurodevelopment, although the full effects may not be recognized for years.

Critical brain growth occurs *in utero* and throughout infancy [5], and it continues into young adulthood as well [6]. Early experiences and environment can affect vision, hearing, language, and responses to social cues [5, 7]. Structural maturation of specific neural pathways occurs throughout childhood and adolescence, which presumably corresponds to maturation of cognitive abilities and suggests that sensitive periods for neuronal architecture and function of discrete regions of the brain occur throughout these stages of life [8]. Thus, infancy, childhood, and adolescence are characterized by growth in cognition, memory and comprehension, and the long duration of childhood chronic kidney disease (CKD) has the potential to influence or interrupt brain development over an extended span of time.

The psychology literature is concordant with the observations of neuroscientists. Armstrong and Horn [9] presented a model of late effects that outlines how childhood chronic illness can impact neurodevelopment and neurocognitive function. They noted that most childhood chronic health conditions and their treatments affect the growth and development of children's brains. Brain structures, processes, and functions that develop prior to onset of disease are likely to be less affected, although exceptions such as toxic exposures or cerebral infarction may still have an impact. Secondly, the age of the child over the course of the disease determines the scope and severity of neurocognitive effects. Brain structures, functions, and processes that develop after the age of disease onset are likely to be most affected; thus, the younger the child is at onset of disease and treatment, the more global and severe the effects on development may be. We can synthesize this into a model for a given child who is treated for a chronic illness: the course will be an interaction between the age at diagnosis, the extent of disease

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and treatment intensity, whether the disease is successfully managed, the time since the treatment, and the age of the child when neurodevelopmental function is assessed [10–12].

This model of disrupted neurocognitive development is particularly relevant to childhood CKD and offers a useful paradigm for understanding the range of possible consequences on cognition [13]. CKD is a lifelong condition that differs from some insults originally envisioned by Armstrong and colleagues [11, 12]. Approximately 50% of all pediatric CKD diagnoses are due to congenital, non-glomerular anomalies [14] and *half* of all CKD patients with congenital disease will progress to kidney transplantation [15]. Some of these children will have a history of preterm birth and a complicated neonatal course with early hypoxia and mechanical ventilation, hypotension or hypertension, anemia, potential stroke, multiple surgical procedures with general anesthesia, and exposure to a wide range of drugs. Any of these exposures can adversely affect neurodevelopment.

Even children who develop kidney disease at a later stage of life may experience a prolonged course of disease with comorbidities. A large proportion of children with CKD will require chronic dialysis therapy prior to receiving a kidney transplant. While such treatment is lifesaving and more refined than decades ago, it provides far less clearance than normal kidney function and does not fully normalize the biochemical and hormonal milieu. In this chapter, we will review the effects of dialysis on brain structure and function. Patients, families, and the nephrology team often expect a great deal from kidney transplantation, including dramatic amelioration of the metabolic abnormalities associated with advanced CKD/dialysis. Yet transplantation requires exposure to a range of drugs known to have neurologic effects, including glucocorticoids, calcineurin inhibitors, cytotoxic agents, and monoclonal/polyclonal antibodies. These effects will be discussed at greater length later in the chapter.

Children with CKD experience frequent interruptions in schooling and may have a consequent lack of engagement in their education. School absences may have a higher than anticipated impact on future educational attainment, employment, and social adjustment. In fact, the social and emotional cost of chronic disease has a substantial impact on children with CKD, draining energy from the important work of cognitive and social growth and development. This is discussed in detail in Chap. 63.

This chapter will provide readers with both a summary of literature and an in-depth analysis of current knowledge. Tables 57.1, 57.2, and 57.3 provide a comprehensive listing of publications of cognitive and developmental testing in children with CKD: assessments of children diagnosed before 24 months of age (Table 57.1), assessments of children diagnosed after 24 months of age (Table 57.2), and comparisons of different treatment groups (e.g., pre- vs. post-transplant and dialysis vs. transplant) (Table 57.3). We have highlighted selected findings from these core studies in the sections below.

Table 57.1 Assessments of children diagnosed with CKD at less than 24 months of age

Study	CKD *n*	Treatment group[a]	Age at diagnosis[b]	Mean age at assessment (range)	Skills assessed	Tests	Control group
Popel et al. [24]	15	Transplant	<24 mos.	56 mos.	IQ/Cognitive Visual-Motor Integration	WPPSI-III VMI Fifth Edition	No
Hartmann et al. [25]	15	Transplant	<2.3 yrs.	8.3 yrs.	IQ/Cognitive Neuromotor	HAWK-III Zurcher Neuromotorik	No
Johnson & Warady [22]	12	Transplant	<16 mos.	11 yrs. (no range)	IQ/Cognitive Academic Memory/ Learning Executive Functions	WISC-IV WIAT-II-A WRAML2 BRIEF	Siblings

Table 57.1 (continued)

Study	CKD *n*	Treatment group[a]	Age at diagnosis[b]	Mean age at assessment (range)	Skills assessed	Tests	Control group
Laakkonen et al. [19]	21	PD	<24 mos.	16 mos. and 5+ yrs.	Infant (Broad Dev.) IQ/Cognitive Memory/ Learning Sensorimotor	AIMS MFED WPPSI NEPSY VMI DMA	No
Hijazi et al. [91]	31	Mixed	<18 mos.	8.4 years (no range)	Broad Development	Chart review	No
Madden et al. [21]	16	Mixed	<12 mos.	5.8 yrs. (1.6–12.1 yrs.)	Infant (Broad Dev.) IQ/Cognitive	Griffiths MDS WISC-III	No
Shroff et al. [18]	11	Mixed	<24 mos.	0–6 yrs.	Infant (Broad Dev.)	Griffiths MDS BSID	No
Ledermann et al. [92]	16	Mixed	<12 mos.	Half ≥5 yrs., half <5 yrs.	Infant (Broad Dev.) IQ/Cognitive	Griffiths MDS WISC	No
Warady, Belden, & Kohaut [4]	28	Mixed	<3 mos.	1 yr. and 4–7 yrs.	Infant (Broad Dev.) IQ/Cognitive	MDAT BSID WISC-III SB	No
Elzouki et al. [3]	15	Mixed	<16 mos.	14 mos. to 10.4 yrs.	Infant (Screen)	DDST	No
Honda et al. [93]	15	Mixed	<24 mos.	Every 6 mos., then 5–6 yrs.	Infant (Broad Dev.) IQ/Cognitive	Enjoji Method Tanaka-Binet	No
Bock et al. [94]	15	Mixed	<6 mos.	7.1 mos. (2–16 mos.); at Time 1; repeated	Infant (Broad Dev.)	BSID	No
McGraw & Haka-Ikse [1]	10	Transplant	<1 month	13 mos. to 4.5 yrs.	Infant (Broad Dev.)	Revised Yale Developmental Schedules	No
Rotundo et al. [2]	23	Mixed	<12 mos.	0.5–14 yrs. (n = 20)	Infant (Screen) Infant (Broad Dev.) IQ/Cognitive	DDST BSID SB	No

Att. attention; *CKD* chronic kidney disease; *Dev.* DEVELOPMENT; *ESKD* end stage kidney disease; *IQ* intelligence quotient; *Mod.* moderate; *PD* peritoneal dialysis

TESTS (alphabetical by abbreviation): *AIMS* Alberta Infant Motor Scale; *BRIEF* Behavior Rating Inventory of Executive Function; *BSID* Bayley Scales of Infant Development; *DDST* Denver Developmental Screening Test; *DMA* Direct Memory Access; *Griffiths MDS* Griffiths Mental Development Scales; *HAWK-III* Hamburg-Wechsler-Intelligence Scale for Children, Third Edition; *MDAT* Modified Developmental Assessment Test; *MFED* Munich Functional Developmental Diagnostic; *NEPSY* NEPSY: A Developmental Neuropsychological Assessment; *SB* Stanford-Binet Intelligence Scales; *WIAT-II-A* Wechsler Individual Achievement Test, Second Edition Abbreviated; *WRAML2* Wide Range Assessment of Memory and Learning, Second Edition; *VMI* Beery Buktenica Test of Visual Motor Integration; *WISC (III, IV)* Wechsler Intelligence Scale for Children, Third or Fourth Edition; *WPPSI (III)* Wechsler Preschool and Primary Scale of Intelligence

[a]Mixed: participant group that includes some combination of patients on conservative therapy, undergoing dialysis, or post-transplant

[b]Age at diagnosis presented as Mean (Range) unless otherwise indicated

Table 57.2 Assessments of children diagnosed with CKD at greater than 24 months of age

Study	CKD *n*	Treatment group[a]	Age at diagnosis[b]	Mean age at assessment (range)	Skills assessed	Tests	Control group
Hooper et al. [32]	368	Mild to mod. CKD	8 yrs. since diagnosis	6–16 yrs.	IQ/cognitive Academic Att./ Concentration Executive functions	WASI WIAT-II-A CPT-II BRIEF	No
Falger et al. [47]	27	Transplant	7.7 yrs. (0.25–15 yrs. dialysis onset)	14.1 yrs. (6.5–17 yrs.)	IQ/cognitive Sensorimotor	WISC-III KABC Zurich NMA	No
Slickers et al. [95]	29	Mixed	4.4 (0–16 yrs.)	12.5 (7–19 yrs.)	IQ/Cognitive Memory / Learning Att./ Concentration	WASI WRAML GDS	No
Duquette et al. [33]	30	Mixed	5.1 yrs.	12. 7 yrs. (6–18 yrs.)	IQ/Cognitive Academic	WASI WIAT-II	Healthy
Gipson et al. [35]	20	Mixed	7.2 yrs.	13.4 yrs. (17.5–19 yrs.)	IQ/Cognitive Memory / Learning Att./ Concentration Sensorimotor Language Executive functions	WASI WRAML GDS RFFT COWAT Tower of London WJ-III Numbers Reversed	Healthy
Bawden et al. [30]	22	Mixed	Unknown	11.8 yrs. (no range)	IQ/Cognitive Academic Memory / Learning Att./ Concentration Sensorimotor	WISC-III WRAT-3 WRMT-R Word Attack WIAT Reading Comprehension WRAML NVSRT EOWPVT VMI Grooved Pegboard Finger-Tapping Test	Siblings
Qvist et al. [31]	33	Transplant	<5 yrs.	8 yrs. (7–12 yrs.)	IQ/Cognitive	WISC-R NEPSY	No
Groothoff et al.	126	Mixed	0–14 yrs.	29.4 yrs. (21–42 yrs.)	IQ/Cognitive	WAIS (Dutch)	Normative comparison
Crocker et al. [96]	24	Mixed	Unknown	6–16 yrs.	IQ/Cognitive Academic Memory/ Learning Sensorimotor Language	WISC-III WRAT-3 WRAML WRMT Word Attack WIAT Reading Comprehension NVSRT EOWPVT VMI Grooved Pegboard Finger-Tapping Test	No

Table 57.2 (continued)

Study	CKD *n*	Treatment group[a]	Age at diagnosis[b]	Mean age at assessment (range)	Skills assessed	Tests	Control group
Hulstijn-Dirkmaat et al. [17]	31	Mixed	<5 yrs.	Repeated	Infant (Broad Dev.) IQ/Cognitive	BSID McCarthy Scales of Children's Abilities	No
Fennell et al. [26–28]	56	Mixed	6.1 yrs. (no range)	13.6 yrs. (6–18 yrs.)	IQ/Cognitive Memory/ Learning Att./ Concentration Sensorimotor Visuospatial	WISC-R WAIS-R RPM VMI SRT CPT Brown-Peterson Tasks Other	Healthy

Att. Attention; *CKD* chronic kidney disease; *Dev.* development; *ESKD* end stage kidney disease; *IQ* intelligence quotient; *Mod.* moderate; *PD* peritoneal dialysis

TESTS (alphabetical by abbreviation): *BRIEF* Behavior Rating Inventory of Executive Function; *BSID* Bayley Scales of Infant Development; *COWAT* Controlled Oral Word Association Test; *CPT, CPT-II* Conners' Continuous Performance Test, First or Second Edition; *EOWPVT* Expressive One-Word Picture Vocabulary Test; *GDS* Gordon Diagnostic System; *KABC* Kaufman Assessment Battery for Children; *NEPSY* NEPSY: A Developmental Neuropsychological Assessment; *NVSRT* Nonverbal Selective Reminding Test; *RFFT* Ruff Figural Fluency Test; *RPM* Raven's Progressive Matrices; *SRT* Buschke Selective Reminding Test; *WIAT, WIAT-II, WIAT-II-A* Wechsler Individual Achievement Test, First or Second Edition, Second Edition Abbreviated; *WJ-III* Woodcock-Johnson III, Tests of Cognitive Abilities; *WRAML* Wide Range Assessment of Memory and Learning; *WRAT-3* Wide Range Achievement Test, Third Edition; *WRMT, WRMT-R* Woodcock Reading Mastery Tests, First Edition or Revised; *VMI* Beery Buktenica Test of Visual Motor Integration; *WAIS, WAIS-R* Wechsler Adult Intelligence Scale, First Edition or Revised; *WASI* Wechsler Abbreviated Scale of Intelligence; *WISC (R, III)* Wechsler Intelligence Scale for Children, Revised or Third Edition; *Zurich NMA* Zurich Neuromotor Assessment

[a]Mixed: participant group that includes some combination of patients on conservative therapy, undergoing dialysis, or post-transplant

[b]Age at diagnosis presented as Mean (Range) unless otherwise indicated

Table 57.3 Comparisons of different treatment groups

Study	CKD *n*	Treatment group	Age at diagnosis[a]	Mean age at assessment (range)	Skills assessed	Tests	Control group
Icard et al. [44]	26	Pre-/Post Transplant CKD vs. Transplant	Unknown	Transplant group 10.7 yrs. (5.3–17.9) and 12.8 mos. later CKD Group: 7.8 yrs. (0.2–16.2) and 10.9 mos. later	IQ/Cognitive	Mullen Scales of Early Learning WASI	Healthy
Brouhard et al. [23]	62	Dialysis vs. Transplant	n = 34 < 10 yrs.; n = 28 > 10 yrs.	13.7 yrs.	IQ/Cognitive Academic	TONI-2 WRAT	Siblings
Lawry, Brouhard, & Cunningham [97]	24	Dialysis vs. Transplant	Duration CKD 2.8–4 yrs.	14.9 yrs. dialysis; 13.7 yrs. transplant; (6–18 yrs.)	IQ/Cognitive Academic	WISC-R WAIS-R WJ-R	No

(continued)

Table 57.3 (continued)

Study	CKD *n*	Treatment group	Age at diagnosis[a]	Mean age at assessment (range)	Skills assessed	Tests	Control group
Mendley & Zelko [45]	9	Pre-/Post Transplant	11.7 yrs.	14.2 yrs. and 15.8 yrs.	IQ/Cognitive Memory/ Learning Att./ Concentration Sensorimotor Visuospatial	WISC-III WAIS-R PASAT CHIPASAT SRT MVDT Grooved Pegboard Trail-Making Test CAT CPT	No
Davis et al. [16]	37	Pre-/Post Transplant	<30 mos.	14 months post-transplant	Infant (Broad Dev.) IQ/Cognitive	BSID SB	No
So et al. [46]	9	Pre-/Post Transplant	<7 mos.	<12 mos. and 3–22 mos. post-tansplant	Infant (Screen) Infant (Broad Dev.)	DDST BSID	No
Rasbury et al. [98]	18	Pre-/Post Dialysis	10.6 yrs. (ESKD)	12.8 yrs.	IQ/Cognitive Memory/ Learning	CFIT Learning Task	Healthy
Fennell et al. [99]	20	Pre-/Post Transplant	Unknown	11.7 yrs. and 1 mo. and 1 yr. post-transplant	IQ/Cognitive Academic Memory/ Learning Att./ Concentration Executive Functions	WISC PIAT HRCT Auditory Vigilance Task Free Recall Task	Healthy
Rasbury et al. [100]	14	Pre-/Post Transplant	Unknown	11.2 yrs. (no range) and 6 mos. post-transplant	IQ/Cognitive Academic Memory/ Learning Att./ Concentration	WISC-R PIAT CPT HRCT Auditory Vigilance Task Free Recall Task	Healthy

Att. Attention; *CKD* chronic kidney disease; *Dev.* development; *ESKD* end stage kidney disease; *IQ* intelligence quotient; *Mod.* moderate; *PD* peritoneal dialysis

TESTS (alphabetical by abbreviation): *BSID* Bayley Scales of Infant Development; *CAT* Cognitive Abilities Test; *CFIT* Culture Fair Intelligence Test; *CHIPASAT* Children's Paced Auditory Serial Addition Test; *CPT* Conners' Continuous Performance Test; *DDST* Denver Developmental Screening Test; *HRCT* Halstead-Reitan Category Test; *MVDT* Meier Visual Discrimination Test; *PASAT* Paced Auditory Serial Addition Test; *PIAT* Peabody Individual Achievement Test; *SB* Stanford-Binet Intelligence Scales; *SRT* Buschke Selective Reminding Test; *TONI-2* Test of Nonverbal Intelligence, Second Edition; *WJ-R* Woodcock-Johnson Revised, Tests of Achievement; *WRAT* Wide Range Achievement Test; *WAIS-R* Wechsler Adult Intelligence Scale, Revised; *WASI* Wechsler Abbreviated Scale of Intelligence; *WISC (R, III)* Wechsler Intelligence Scale for Children, Revised or Third Edition

[a]Age at diagnosis presented as mean (range) unless otherwise indicated

Early Development

Several studies have described developmental outcomes for children who were diagnosed with CKD during infancy. These studies have either assessed children shortly after their diagnosis or have reported developmental progress during the period of early childhood using broad measures (cognitive, motor, language) such as the Bayley Scales of Infant Development (BSID) or the Griffiths Mental Development Scale. Davis et al. [16] assessed 37 children younger than 30 months of age who were undergoing kidney transplant, and again an average of 14 months post-transplant, utilizing either the BSID or the Stanford-Binet Intelligence Scales (SB), depending on the child's age. Prior to transplant, most patients had significantly delayed psychomotor development and 18 patients had mental development scores classified as delayed. Post-transplant psychomotor performance improved, with the group mean reaching the low average range, and many of the patients with cognitive delay (12 of 18) improving their scores to the average range. Persistently abnormal cognitive function was associated with the earliest onset of kidney failure. In a similar study, 28 patients diagnosed with ESKD before three months of age [4] were assessed at a mean age of 5.9 years (the majority post-transplant), using a range of instruments, including the Modified Developmental Assessment Test (MDAT) and the Wechsler Intelligence Scale for Children, Third edition, (WISC-III) as well as the BSID and SB. Overall, 22 of the 28 children (79%) had general mental development that placed within the broad range of average.

Hulstijn-Dirkmaat et al. [17] prospectively evaluated 31 young children (mean age of 2.5 years) with a broad range of CKD, using the BSID and the McCarthy Scales of Children's Abilities. In this sample, 32% of patients had comorbid conditions or disease (cerebral complications or unexplained neurological impairment, visual/hearing disorder, congenital heart disease); children with comorbid conditions were evenly distributed between dialysis and non-dialysis groups. Overall, the patients had significantly delayed cognitive development, with a mean developmental index of 78.5 (SD 19.5), and a distribution of scores skewed below that of the normal population. Children *without* comorbidities who did not yet require dialysis had a group mean in the average range, compared to those on dialysis, whose group mean placed in the borderline range for cognitive functioning. Among children *with* comorbidities, those with CKD who did not yet require dialysis scored in the borderline range for cognitive development, while those on dialysis had a mean score in the impaired range. Thus, comorbid disease and more advanced CKD (i.e., need for dialysis) were independently associated with lower neurodevelopmental scores.

Other studies have found the developmental outcomes of very young children with CKD to be linked to comorbid medical conditions or risk factors. Shroff et al. [18] evaluated a group of 11 children who began kidney replacement therapy between 0 and 24 months of age; six achieved age-appropriate developmental milestones. The remaining five had significant special education needs, with developmental outcomes linked to medical co-morbidities including VACTERL association (e.g., vertebral defects, anal atresia, Cardiac defects, tracheoesophageal fistula, renal anomalies, and limb abnormalities), infantile hemiplegia, deafness, congenital hypothyroidism, transposition of the great vessels, and an undiagnosed syndrome that included dysmorphism, developmental delay, and non-functioning kidneys. Laakkonen et al. [19] examined neurodevelopmental outcomes for 21 patients who began kidney replacement therapy with peritoneal dialysis (PD) before 24 months of age and were at least 5 years of age. A comprehensive battery of tests was used (see Table 57.1) which targeted development, cognition, sensory and motor function, memory and learning. Six of these patients were considered to have normal neurodevelopment; the remaining 15 had some degree of neurodevelopmental impairment, ranging from minor (n = 9) to major (n = 6). Those

with major impairment all had documented predialysis risk factors (e.g., perinatal asphyxia, severe hypotension, alcohol exposure, severe neonatal respiratory distress).

In contrast to studies that have evaluated young children with more advanced stages of CKD, Hooper et al. [20] reported on preschool children with mild to moderate CKD. Measures of developmental level/IQ were administered to 124 children 12–68 months who had a mean estimate glomerular filtration rate (eGFR) of 50 ml/min per 1.73 m^2. While all mean scores placed in the average range in this group with mild disease, the authors noted that an unexpectedly high percentage (27%) had developmental/IQ scores that placed in the at-risk range, defined as scores more than 1 SD below the mean of a normal distribution.

To summarize, children diagnosed with CKD in infancy are at risk for delayed neurodevelopment; the magnitude of the delay is influenced by medical and neurological comorbidities, as well as the age of onset and severity of disease. There is some evidence that transplant early in life benefits neurodevelopmental catch-up.

Intellectual Function (IQ)

ESKD Onset in Infancy and Early Childhood

Many studies in pediatric CKD have specifically examined intellectual function (IQ). The most used measure to assess IQ is the Wechsler Intelligence Scale for Children (WISC); its most current version is the Fifth Edition (WISC-V). The WISC includes a Full-Scale IQ (FSIQ) and five index scores: Verbal Comprehension (VCI), Visual Spatial (VSI), Fluid Reasoning (FRI), Processing Speed (PSI), and Working Memory (WMI).

A few studies have looked at long-term intellectual functioning of children diagnosed with ESKD as infants or early in life. The previously noted study by Warady et al. [4] assessed IQ in 28 patients who were diagnosed with ESKD before 3 months of age. Using the WISC-III, they showed a divergence in verbal (acquired knowledge and verbal reasoning) and nonverbal (ability to understand and manipulate nonverbal stimuli such as colors, patterns, and shapes) performance at a mean age of 5.9 years: 72% of the children had average verbal scores, whereas only 56% reached average nonverbal scores. In a similar study, Madden et al. [21] assessed the IQ of 16 patients with ESKD who initiated dialysis during the first year of life. These subjects were also studied at a mean age of 5.8 years. The IQ range for the sample was 50–102 with an average IQ of 87 for the ESKD group (approximately 1 standard deviation below the mean). Ten children had IQ scores within one standard deviation of the mean. This average and distribution of scores were clearly abnormal (i.e., average IQ = 100) with a distribution that skewed lower.

Johnson and Warady [22] built on the above studies by adding a sibling control group. They assessed late neurocognitive outcomes for 12 patients diagnosed with ESKD as infants (mean age at testing: 10 years). All but one had functioning transplants, and for those with transplants mean eGFR was consistent with mild to moderate CKD. Mean WISC-IV index scores ranged from 80 to 82 (borderline to low average) and the mean FSIQ score was 76.8. The distribution of FSIQ scores ranged from 55–102 and are strikingly similar to those reported by Madden et al. [21] noted above. Nine of the patients were compared to their healthy siblings and these participants had FSIQ, Processing Speed Index (PSI), and Working Memory Index scores significantly lower than siblings. For example, only one-third of patients had an FSIQ in the average range or above, in contrast to 6 of 9 siblings. For the patient group, higher FSIQ and the VCI were associated with older age at start of dialysis; higher FSIQ and the PSI were associated with fewer months on dialysis, and higher PSI was associated with younger age at transplant. The Test of Nonverbal Intelligence (TONI-2), a test of intellectual functioning that does not require verbal responses, was utilized in another study of 62 children with CKD that included sibling controls [23]. While these subjects were diagnosed later in life, the pattern of intellectual functioning was

very similar: patients' mean percentile ranks for nonverbal intelligence ranged from the 27th to the 35th percentile, regardless of whether they were on dialysis or post-transplant, while siblings' mean percentile ranks ranged from the 32nd to the 56th percentile.

Popel et al. [24] found similar results using data from a longitudinal, prospective study to evaluate predictors of developmental outcomes for children who received a transplant before the age of 5 years. These patients started dialysis in the first 2 years of life (73% initiating dialysis in the first year of life), with a mean age of 30 months at transplant. At a mean age of 56 months (all post-transplant), mean scores on FSIQ, visual-motor integration, and adaptive skills were approximately one SD below the population normative mean. Hartmann et al. [25] assessed the neurocognitive and neuromotor outcomes of 15 patients with severe congenital CKD who received a kidney transplant at a mean age of 2.8 years and were evaluated a mean age of 8.3 years. Six of these patients received a pre-emptive kidney transplant and the other nine underwent dialysis for a mean of 11 months (±8.6 months). Neuromotor function was assessed as abnormal for eight patients. Performance IQ (PIQ) was lower (m = 89) than the normative mean of 100, and lower PIQ was associated with abnormal neuromotor function. Time on dialysis was inversely correlated with verbal IQ. Pre-emptive kidney transplant and duration of dialysis less than 3 months were protective; participants meeting these criteria demonstrated better neurocognitive outcomes.

Existing data indicate that children diagnosed with ESKD as infants or very early in childhood achieve intellectual outcomes which vary depending on comorbidities. Many achieve normal or near normal intellectual function, but with a distribution that is skewed below normal. Patients tend to perform less well than their unaffected siblings. Younger age at transplant and shorter duration of dialysis appear to benefit neurocognitive outcomes. These are impressive outcomes given the complexity of ESKD with onset in infancy, yet they indicate a substantial risk for learning impairment and academic difficulties.

Mixed Age Groups Including Mild to Moderate CKD

Children with onset of ESKD before school-age and those with mild-to-moderate CKD demonstrate IQ impairment that is similarly skewed below the population mean, although the effect is less striking than those diagnosed with ESKD as infants or in early childhood, and more children perform in the average to above average range. Fennell et al. [26–28] published some of the earliest data regarding associations between standardized neuropsychological tests and CKD. Compared to a healthy control group matched for age, sex, and ethnicity, a group of children with a range of eGFR, from moderate CKD to post-transplant, performed more poorly on selected indices of non-verbal IQ (Wechsler subtests Similarities, Digits Forward, and Digits Backward).

Gipson et al. [29] studied children and adolescents with advanced CKD (a mix of dialysis-dependent patients and patients being treated conservatively) and included a comparison group of healthy children. This sample of 20 children with CKD had an average age at diagnosis of 7 years and an age range of 7.5–19 years at the time of assessment. Patients with CKD scored significantly lower than the healthy controls on a measure of intelligence (Wechsler Abbreviated Scale of Intelligence [WASI] Full Scale IQ).

Using a sibling control group, Bawden et al. [30] evaluated 22 patients who were listed for transplant and either receiving dialysis or approaching dialysis. Intelligence was assessed via the WISC-III. Those with CKD/ESKD had a group mean score that was average to low average. Overall, the group scored significantly lower than the sibling group on measures of VIQ, PIQ, and FSIQ.

Qvist et al. [31] assessed children diagnosed in early childhood with ESKD secondary to congenital nephrotic syndrome. At a mean age of 8 years, this group had an average IQ of 87 (low average range) on the WISC-Revised, and 6–24% of patients were characterized as impaired based on the results of neuropsychological tests (NEPSY).

Hooper et al. [32] used a cross-sectional analysis of the Chronic Kidney Disease in Children (CKiD) study to describe cognitive function in 368 school-age children with mild to moderate CKD. While the group had mean IQ scores in the average range, the number of subjects whose scores placed one or more SDs below the mean on a measure of nonverbal ability (WASI block design) was more than twice that of the normative group. Several risk factors for low scores were identified in this study, including proteinuria, low birth weight (LBW), and history of seizures.

Duquette et al. [33] examined 30 patients (mean age = 12.7 years) with childhood-onset CKD, half on dialysis and half with pre-dialysis CKD, and compared them to a healthy control group. The two groups differed significantly on some key variables: the control group had higher maternal education and a larger proportion of children who identified as Caucasian. Nonetheless, after controlling for maternal education and race, the control group scored significantly higher on measures of VIQ, PIQ, and FSIQ than patients with CKD. Fifty-seven percent of the CKD group had FSIQ scores below the 25th percentile, compared to only 15% of the control group. Kidney function (eGFR) was a significant predictor of intellectual functioning, while age of CKD onset and duration of CKD were not associated with IQ in this study.

Groothoff et al. [34] obtained estimates of cognitive functioning in a group of 126 adults (mean age = 29.4 years) who began kidney replacement therapy between the ages of 1.9 and 14.9 years. The majority had a functioning transplant; 16 and 12 were on hemodialysis (HD) and PD, respectively. Groups mean scores for Verbal, Performance, and FSIQ placed in the average range. Compared to a matched control group, however, IQ scores and educational attainment were significantly lower for the CKD group.

Executive Function

Executive functioning (EF) is a term used in the field of psychology that refers to the management of complex cognitive processes, including planning, organization, problem solving, working memory, and attention. These skills are required for optimal performance on tests of intellectual functioning (IQ), but are assessed more directly with EF-specific cognitive tasks. A number of researchers have examined attention and memory abilities in children with CKD, believing these abilities may be particularly susceptible to effects of the disease. The early work of Fennell et al. [26–28] sought to examine the relationship between EF and CKD. Their subjects, a group of children receiving a range of kidney replacement therapies (HD, PD, transplant), scored worse than healthy controls on a variety of measures of EF (e.g., working memory, attention). The NEPSY was administered by Qvist et al. [31] to children with congenital nephrotic syndrome to assess attention, concentration, and memory skills. Results indicated that impairment was more frequent in children with a history of major central nervous system (CNS) infarcts, hemiplegia, and/or those with a seizure disorder or an abnormal EEG.

Gipson et al. [35] included measures of memory and executive function in their study of children and adolescents with CKD and compared them to healthy controls. Patients scored significantly lower on all of the memory indices and on the "initiation" and "sustaining" EF domains, which assess task initiation and ability to sustain attention. Memory and EF findings persisted even after the authors controlled for IQ and age. Of note, the severity of memory deficits was positively related to the age at onset of CKD.

In Johnson and Warady's (2013) long-term follow-up of patients who had been diagnosed with ESKD as infants, EF findings were similar to those for IQ: better verbal ability and memory were associated with older age at start of dialysis and fewer months on dialysis, and younger age at transplant was associated with higher overall memory scores. Shorter time on dialysis and younger age at transplant were also associated with better scores on the Metacognitive Index of the parent-report BRIEF. When compared to their healthy siblings, the patients scored significantly worse on the Wide Range Assessment of Memory and Learning, Second Edition Verbal Memory Index (but not the Visual Memory

Index), the BRIEF Metacognition Index, and the BRIEF Global Executive Composite score.

The CKiD study is the largest study to examine EF, but unlike many of the other studies discussed, included only children with mild to moderate CKD and without history of kidney transplant [32]. Mean scores were in the average range for a variety of attention-related measures (Conners' Continuous Performance Test, Second Edition) and parent ratings of executive functioning (BRIEF). However, on several of the BRIEF measures (Metacognition Index, Working Memory and Planning subscales, and the Global Executive Composite score) a larger than expected proportion of patients scored one or more SD below the mean ("at risk" for lower EF). If we consider these results broadly, 33–40% of children with early-stage CKD would be considered at risk for some impairment in EF. CKD-associated risk factors including low birth weight, proteinuria, hypertension, and history of seizures were associated with slightly worse scores on several measures. Conversely, higher eGFR appeared protective, as it was associated with a lesser chance of scoring in the "at risk" range. Older subjects had slightly worse scores, suggesting that EF growth was diverging from their peers. It is important to emphasize that overall mean scores were average and group findings were statistically, but not necessarily clinically, significant. In another study of CKiD participants, Johnson et al. [36]examined parent ratings of attention problems using the Behavior Assessment System for Children, Second Edition (BASC-2) Parent Rating Scales. Parents rated 28% of the sample as at risk for attention problems. Persistent hypertension and unresolved proteinuria predicted rating of attention problems. Taken together, these findings suggest that even mild to moderate CKD is associated with increased risk for sub-optimal neurocognitive functioning, which may be relevant for some patients. The CKiD study also collected parent ratings of attention and executive functioning for preschool participants with mild to moderate CKD. Thirty percent of the participants had parent ratings that placed in the "at-risk" range (ratings higher than 1 SD above the mean [worse]) for overall executive functioning [20].

Executive functioning was examined by the Neurocognitive Assessment and Magnetic Resonance Imaging Analysis of Children and Young Adults with Chronic Kidney Disease (NiCK) Study, a cross-sectional study of youth with CKD stages 2–5 [37]. This study compared 90 participants with CKD to 70 controls. Those with CKD scored significantly worse than controls in several areas of executive functioning, including attention, memory, and inhibitory control. In addition, performance was associated with eGFR: participants with CKD exhibited lower performance on tests of executive functioning as eGFR declined. Using ambulatory blood pressure monitoring, the authors also found that blood pressure abnormalities, including increased diastolic load and decreased diastolic dipping, were associated with neurocognitive impairment.

Lande et al. [38] also found an association between blood pressure variability (BPV) and executive functioning. They assessed 650 children 6+ years old with mild to moderate CKD enrolled in the CKiD study and had a mean follow-up period of 4 years. Children with systolic visit-to-visit BPV in the upper tertile obtained lower scores on a measure of executive functioning (D-KEFS Category Switching) than those with BPV in the lower tertile. This association remained significant in multivariate analyses that controlled for mean BP, demographic characteristics, and CKD-related variables.

Academic Achievement and Educational/Vocational Outcomes

There are limited data to describe academic achievement outcomes for children with mild to moderate pediatric CKD. An initial evaluation of academic achievement data collected as part of the Chronic Kidney Disease in Children (CKiD) multicenter prospective cohort study [39] suggested that general academic achievement scores were skewed toward the lower end of the normal range in CKD. The group's mean performance on the Wechsler Individual Achievement Test, Second Edition, Abbreviated (WIAT-II-A) was in

the average range, but across all academic domains more patients were "at risk" (scoring one or more SDs below the normative mean for age) than would be expected given a normal distribution. One third of subjects scored "at risk" on the WIAT-II-A Numerical Operations subtest, which is more than twice the expected rate. CKD-related characteristics including eGFR, proteinuria, hypertension, and anemia were not associated with low achievement. Among children evaluated in the CKiD cohort, ADD/ADHD was self-reported for 9% of the sample; however, this was not significantly associated with risk for academic underachievement. Children displaying lower total achievement scores had significantly more school absence (5.5 days versus 3 days). Use of an IEP or 504 plan was reported by 29% of the CKiD sample and children with lower total achievement scores had a higher rate of IEP or 504 plan usage (51% versus 17%).

When we consider the study by Duquette et al. [33], which compared 30 patients with CKD or ESKD to a group of 41 age and gender-matched healthy controls, we must keep in mind differences in key variables: the control group had higher maternal education and a larger proportion of children who identified as Caucasian. In addition, the CKD participants had more previous grade retentions, use of specialized educational plans, and more school absences. Allowing for these caveats, the patients scored significantly lower than the controls on the WIAT-II Word Reading and Math Reasoning domains. Higher eGFR was positively correlated with all academic scores, suggesting that less advanced disease causes fewer adverse educational consequences. The authors also examined whether children in either group met criteria for a learning disorder, using three different formulas: an ability-achievement discrepancy formula, which requires an academic standard score to be at least 15 points lower than FSIQ; a regression formula, which statistically adapts the discrepancy formula using achievement scores predicted by FSIQ; and a low achievement formula, which defines learning disability as a standard score below the 25th percentile for age. The two groups differed significantly only when the low achievement formula was used. Using this approach, 43%, 37%, and 40% of the CKD group met criteria for a learning disorder in reading, math, and spelling, respectively, compared to 7%, 2%, and 5% of the control group. Thus, for children with CKD, for whom the disease process may depress both cognitive performance and academic achievement, discrepancy formulas (which require a significant discrepancy between cognitive and achievement scores) may be of limited use. The authors also noted that children with CKD had a 40% rate of grade retention, compared to 2.4% of the control group, suggesting that current educational services are not meeting the needs of these patients.

Brouhard et al. [23] compared 62 dialysis and transplant patients (average age 13.7 years, roughly half with disease onset prior to 10 years) to a healthy sibling control group, using the Wide Range Achievement Test (WRAT), a brief measure of academic achievement that assesses three areas: spelling, arithmetic, and reading. There were no significant differences between the dialysis and transplant groups. When compared to siblings, the combined patient group scored significantly lower across all domains, with sibling mean scores placing in the average range and patient mean scores placing in the low average range. Using available data for approximately half the study sample, 85% percent of sibling participants were in regular education classes full-time while only 64% of patients were in a regular setting. Over half (56%) of the patients had missed 21 or more days of school in the last semester, compared to only one sibling (4%). The percentage of a patient's life with ESKD was negatively correlated with the WRAT Arithmetic score.

Early onset of disease, type of disease (e.g., congenital nephrotic syndrome), and associated comorbidities confer risk to academic achievement. Studies have historically varied in terms of whether those with significant CNS risk factors are included in analyses. When Johnson and Warady [22] compared the performance of nine children diagnosed with ESKD in infancy to that of their healthy siblings, patients with major neurological risk factors, including stroke or

asphyxia, Galloway Mowat syndrome, and Joubert syndrome, were excluded. Healthy siblings outperformed patients across all domains of the WIAT-II-A, including Word Reading, Spelling, and Numerical Operations, as well as the Total Achievement score. Mean scores for siblings placed in the average range; in contrast, all patient mean scores placed in the low average range. Based on parent report, only one of the siblings had ever been diagnosed with a learning or attention disorder, while three patients (33%) had been diagnosed with a learning disorder, and one with an attention disorder (11%). In another study that excluded patients with syndromes known to influence neuropsychological functioning, and that also included a sibling control group [30], 22 patient-sibling pairs participated in academic assessment and no significant differences were found on measures of achievement. These patients ranged in age from 6 to 16 years, were on a transplant waiting list, and were approaching or receiving dialysis. The average age of onset of ESKD was not reported. These results are more consistent with those from the CKiD study and suggest better academic achievement outcomes for children diagnosed later in childhood compared to those diagnosed in infancy.

In a more severely affected cohort, Qvist et al. [31] examined educational outcomes for children diagnosed with ESKD early in life. In this sample, a large proportion (88%) of children had a diagnosis of congenital nephrotic syndrome (severe Finnish type), many with comorbid events in early infancy including history of stroke. Thirty-three children who underwent transplant before 5 years of age were followed prospectively. The authors reported outcomes an average of 6 years post-transplant, when the patients were 7–12 years of age. Twenty-six patients (79%) attended a regular school, although six of these children required remedial education services. The remaining seven children attended a specialized school, two of them to accommodate hearing impairment rather than intellectual impairment. Risk factors for requiring a specialized educational setting included: longer time on dialysis, more hypertensive crises (the majority of which occurred during dialysis), more seizures (which occurred for some patients during dialysis and for others post-transplant), and major infarcts/hemiplegia. While major cerebral infarcts were more common in those requiring special education (3/7), watershed infarcts and abnormal EEGs were also seen among those in remedial and regular classes.

A small number of studies have examined much longer-term educational and vocational outcomes for patients who had ESKD as children. Having ESKD as a child appears to be associated with lower educational attainment, lower socioeconomic class, and higher rates of unemployment [34, 40], although at least one study reported more positive outcomes [40]. Offner et al. [41] described 124 adults with a mean age of 25 years who had received a kidney transplant between 1970 and 1993, when they were a mean age of 12.1 years. Mean eGFR had declined over the years, from 76 to 45 ml/min/1.73 m^2, and 80% of the sample had maintained or developed hypertension. Education and employment levels were roughly the same as the general German population at that time (9% unemployed in general population, versus 14% in the sample), although 45% lived with their parents. In contrast, a large study of 126 Dutch adults by Groothoff et al. [34] reported that only 42.2% of patients completed intermediate or advanced vocational training compared to 72.2% of the general population. Reynolds et al. [40] examined long-term outcomes for patients who underwent dialysis or transplant during adolescence (at a mean age of 14.4 years) and found that (1) the social distribution of kidney patients was lower than that of a healthy comparison group; (2) kidney patients had lower educational achievement; (3) significantly fewer were employed full-time, although two-thirds were employed in some capacity; and (4) that more kidney patients were receiving social security benefits compared to the control group (42% vs. 15%). Seventy-one percent of patients in this study reported that their disease and its treatment had significantly affected their education. The authors reported that onset of illness before 11 years of age and current self-reported low energy were associated with unemployment.

Qualitative research exploring educational and vocational outcomes have produced similar themes. Kerklaan et al. [42] interviewed 30 young adults 18–35 years of age who were diagnosed with CKD during childhood. These participants described long-term impact of school absences and reduced participation in social activities, including social anxiety, feelings of inferiority, and falling behind peers with activities and accomplishments. Longer duration of dialysis during childhood seemed to be related to more negative impact on friendships and participation in recreational activities. Participants described limitations to school, work, and social/leisure activities secondary to treatment burden; challenges with independence (e.g., moving out of parents' home); and difficulty forming adult relationships. Some described reorienting their plans and goals, being inspired to pursue certain professions (e.g., nursing, counseling), and unique, positive opportunities that arose secondary to the diagnosis of CKD.

The available data examining academic and vocational outcomes are consistent with what we know about the impact of CKD on cognitive and intellectual functioning. Children with CKD are at risk for lower academic achievement and poorer educational and vocational outcomes. The age of onset of CKD, disease severity, and comorbid conditions all influence the degree to which academic achievement is affected. The available evidence suggests that the academic achievement of children with CKD will be significantly below that of their siblings if kidney replacement therapy is required early in life. It is important to consider that in addition to the medical effects of CKD (such as uremia, acidosis, hypertension, and fatigue), children with CKD miss more academic instruction, and often have less time and energy to complete homework. The emotional and financial strain of kidney replacement therapy, as well as the time commitment, may also result in fewer opportunities for enrichment activities (music lessons, field trips) all of which also may impact a child's learning and achievement.

Pediatric nephrologists and allied health professionals have an opportunity to reduce the impact of CKD on academic achievement, particularly for children receiving chronic outpatient dialysis. This might include provision of school services in the dialysis unit, adjusting dialysis schedules to accommodate the school day, and working with families and schools to develop individualized education or remediation plans. Children who are struggling academically should be referred early for cognitive and psychoeducational evaluation.

Cognitive Outcomes and Kidney Transplantation

Kidney transplantation is a central goal in the management of children with advanced CKD, promising improved survival and quality of life. A substantial body of literature indicates that both cognitive performance and academic achievement are improved among children with kidney transplant when compared to those receiving dialysis. The available literature indicates that, in general, children with kidney transplant have improved performance on a range of tests of cognitive abilities and achievement post-transplant, although not full normalization. This is supported by more recent meta-analytic data from over thirty pediatric studies demonstrating that, in general, children with kidney transplant have intelligence equivalent to children with mild/moderate CKD and significantly better intelligence assessment than children receiving dialysis [43].

Icard et al. [44] reported results for six patients with advanced CKD who received a transplant. Compared to a control group of children with CKD being treated conservatively, children who received a transplant demonstrated a meaningful increase in their intellectual and developmental functioning. In fact, children receiving a transplant had, on average, a 12-point increase in their standard intelligence scores from pre- to post-transplant. Mendley and Zelko [45] obtained baseline intellectual assessment (WISC-III or WAIS-R) and performed within-subject comparisons of nine patients just prior to and 1 year after kidney transplant, using a wide range of neuropsychological tests. At baseline,

intelligence scores placed within the broad range of average, although skewed slightly lower than a normal distribution. All were attending regular education classes full-time with excellent attendance. After transplant, there was significant improvement in specific aspects of neurocognitive performance. Mental processing speed and decision-making speed (as assessed by the computerized Cognitive Abilities Test) improved from pre- to post transplant and subjects showed more consistent performance after transplant. Sustained attention (assessed by the Connors' Continuous Performance Test Signal Detection Index) and working memory (assessed by the Paced Auditory Serial Addition Test) also improved after transplant.

A study of an early cohort of infants (diagnosed and treated between 1978 and 1985) demonstrated how successful kidney transplantation has the potential to ameliorate developmental deficits [46]. All nine children had significant improvement in head circumference Z-score after transplant even as linear growth lagged. Performance on developmental assessment improved in most infants, with post-transplant Bayley cognitive and motor development scores placing within the broad range of average for most children. Seizures noted while children were on dialysis were not present after transplant.

Falger et al. [47] evaluated the intellectual functioning of 27 patients who had transplants in childhood (mean age at transplant was 9 years); evaluations took place an average of 6 years post-transplant (average age at assessment was 14 years). Median FSIQ was 97, but the range was wide (49–133). Five patients had neurological comorbidities, and their mean FSIQ score was 81, with a range of 49–101 (encompassing severe impairment to normal function). Twenty-one subjects had an FSIQ score > 85, and two were in the high average range (>115). The VIQ was significantly higher than the PIQ, which was significantly lower than a control population. The patients also scored significantly lower than a control group on 5 of 11 WISC-III subtests, even when excluding subjects with neurologic morbidity.

The assessment of long-term intellectual and metacognitive functioning and academic achievement for a group of 12 children transplanted in early life is described previously in this chapter [22]. Performance on some indices was related to younger age at transplant and fewer months on dialysis; however, post-transplant evaluation showed persistence of IQ and achievement gaps when a subgroup of patients was compared to their unaffected siblings. Molnar-Varga et al. [48] also examined how age and duration of dialysis impact later cognitive functioning. They compared 35 kidney transplant recipients who were a median of 28 months post-transplant to 35 healthy controls on a measure of intelligence (Woodcock-Johnson International Edition). The mean FSIQ score placed in the broad range of average, but greater than 1 SD lower than control participants (mean score 85 versus 107). Earlier age at onset of dialysis and longer time on dialysis were associated with lower scores in the transplant group, as was cumulative days hospitalized (standardized for age).

We must also consider how the introduction of a range of new medications at the time of transplantation has the potential to affect the CNS. While high dose glucocorticoid therapy is almost always of short duration, many children continue to receive lower dose glucocorticoids for years or even decades. The hippocampus has high concentrations of glucocorticoid receptors and neurochemical and electrophysiologic changes occur in the presence of these hormones [49]. Prolonged supraphysiologic exposure can accelerate neuronal loss in the hippocampus and increase the severity of other neurologic insults. Cellular toxicity, dendritic atrophy, and damage to hippocampal structure have been shown in a primate model of chronic exposure [50]. The effects of low doses over longer duration are unknown [51].

Calcineurin inhibitors are a mainstay of lifelong immunosuppression, but our knowledge of the neurocognitive effects of chronic use of this class of medications in children is limited. Acute neurotoxicity has been described in a subset of patients and can include headache, tremor, insomnia, paresthesia, mental status changes, visual and auditory hallucinations, cortical blindness, seizures and memory loss [52, 53]. These

side effects are more often observed in patients undergoing bone marrow or liver transplant, likely because of the greater intensity of immunosuppression required. In a study of 14 pediatric kidney recipients receiving calcineurin inhibitors, 86% reported myalgias, tremor, fatigue and headache, and half of the group had symptoms most of the time [54]. Acute, reversible encephalopathy with MRI findings was reported in two children, ages 7 and 17 years, after kidney transplant who were treated with tacrolimus at levels of 10–11 ng/ml [55]. Both subjects recovered completely and tolerated lower tacrolimus levels of 6–7 ng/ml.

Many potential mechanisms have been proposed to explain calcineurin inhibitor neurotoxicity, and these hypotheses are relevant to our concerns regarding brain development in young children with CKD. Calcineurin represents >1% of total brain protein, and intracellular binding proteins for cyclosporine and tacrolimus are found throughout the CNS. Both drugs may interfere with the activity of excitatory (N-methyl-D-aspartic acid [NMDA]) and inhibitory (γ-aminobutyric acid [GABA]) amino acid receptors through calcineurin, which may impact memory formation [52]. Intra-cranial injection of cyclosporine to day-old chicks disrupts memory formation [56]. Further, cyclosporine is toxic to glial cells in culture in a manner that appears to correlate with the white matter changes which are seen by CT and MRI in affected transplant recipients [52]. Cyclosporine has also been shown to induce apoptosis of oligodendrocytes and neurons in culture [57]. It is only possible to speculate to what degree these observed changes affect the normal dendritic pruning, myelination, and formation of complex connections which characterize infant and early childhood brain development.

Clinical observations suggest that sirolimus may not have independent neurologic toxicity [58]. However, it appeared to enhance the toxic effects of cyclosporine on brain mitochondrial glucose metabolism in a rat model [59]. In contrast, mycophenolate mofetil is not thought to have a neurotoxic profile.

Genetics of Neurodevelopment and Kidney Development

While only a fraction of adult CKD is caused by genetic disorders, genetic forms of kidney disease are common in pediatric CKD, representing approximately 40% of all cases. Single gene mutations explain an array of kidney diseases [60], yet there can be no doubt that there is much more to learn about genetic etiologies. A few genetic disorders are known to be associated with both CKD and mental retardation, but the full spectrum of neurodevelopmental abnormalities associated with genetic CKD has not been clearly defined. Causative genes have been identified in approximately half of kidney diseases with classic Mendelian inheritance [60], but we recognize other organ systems are affected in addition to the kidneys and urinary tract.

We now know that copy number variants (CNVs) are common in the human genome and can be identified and analyzed using array-based technologies [61]. Certain rare CNVs appear etiologic in developmental disorders, including schizophrenia, attention deficit disorder, developmental delay, behavior abnormalities and learning disability [62–64]. In a study of 522 patients with congenital kidney malformations, including kidney aplasia, agenesis, hypoplasia and dysplasia, rare CNVs were more likely in cases than controls, and large CNVs and gene-disrupting events were strikingly more frequent [65]. Among the genomic disorders associated with kidney developmental disturbance were those previously recognized to be associated with neuropsychiatric traits (e.g., DiGeorge, Wolf-Hirschhorn, Kallman, Potocki-Lupinski syndromes). Further, there were novel CNVs spanning genes having murine orthologs associated with kidney and neurodevelopmental defects.

These findings may change our understanding of the relationship between developmental and neurocognitive abnormalities and childhood CKD. Rather than attributing neurodevelopmental delay exclusively to uremic metabolic disturbance, we must consider that defects in pleiotropic genes involved in the morphology of brain and kidney may play a causative role in neurodevelopmental delays.

Neuroimaging

Neuroimaging provides a noninvasive opportunity to examine brain structure and function. Given the observed neurocognitive findings in the pediatric CKD population, research has more recently moved to defining the neural mechanisms for cognitive dysfunction. Neuroimaging studies in children with CKD and ESKD are few and are limited by broadly defined inclusion criteria including age at the time of evaluation, age at diagnosis, type of therapy, and underlying disease. In seeking a deeper understanding of the documented cognitive deficits in CKD, it is critical to ask: (1) is there a structural or functional brain basis for the observed neurocognitive deficits in this population; and (2) are there specific regions of the brain that are most associated with these deficits?

Imaging data can largely be reviewed by modality: computerized tomography (CT), structural magnetic resonance imaging (sMRI), and functional magnetic resonance imaging (fMRI). Twenty neuroimaging studies that include pediatric CKD patients have been published in the literature between 1977 and the present. Of these studies, 13 utilized CT-based neuroimaging and 7 utilized MRI. Most early studies on the topic of neuroimaging in pediatric CKD used CT. Within the more modern pediatric MRI subset, six have evaluated structural MRI and only one has examined brain function through use of regional cerebral blood flow. Study populations in the published literature vary widely in sample size, age, and primary disease etiology.

Contemporary neuroimaging data available from CT are available from 1997 to 2006. Nine of the thirteen published studies in the pediatric CKD literature using CT as a neuroimaging modality were case-control or prospective in nature. CT-based studies focused on pediatric populations with ESKD or post-transplantation without inclusion of less severe disease CKD phenotypes. In general, CT data from these studies demonstrate higher risk for global cerebral atrophy, silent white matter infarcts, and ventriculomegaly [3, 66, 67]. Cerebral atrophy is well-described in pediatric nephrology literature dating prior to 1990, with reports of up to 60% of patients having atrophy that was not associated with type of renal disease, hypertension severity, or corticosteroid therapy [68]. Cerebral atrophy in ESKD, however, has been correlated with age of onset of renal disease [66] and dialytic modality [69, 70]. Qualitative imaging also shows that lower cerebral density [69, 71] and ventriculomegaly secondary to brain atrophy [72] are more often associated with requirement for and duration of pediatric HD compared with receipt of PD [70].

The use of MRI can provide high-resolution qualitative and quantitative assessment of the brain. sMRI provides information related to volumes within and between regions of the brain. Additionally, if the MRI is performed in a research-based scan sequence, it is often possible to obtain sequences that evaluate white and gray matter as well as blood flow within the brain—the former representing sMRI and latter representing functional fMRI.

Data from the Neurocognitive Assessment and Magnetic Resonance Imaging Analysis of Children and Young Adults with Chronic Kidney Disease (NiCK) Study performed volumetric brain assessment using sMRI in youth with CKD compared to normal controls [73]. Although the sample size was adequately powered to detect a statistical difference between populations (N = 90), the CKD sample was very heterogenous with regard to chronological age, stage of disease, disease etiology, and inclusion of dialysis/transplant patients. Statistical analyses, including corrections for multiple comparisons and adjustments for age and sex, did not support any specific brain regional differences between CKD patients and controls. Furthermore, there were no CKD-related clinical predictors to link differences in brain regions of interest to neurocognitive performance. Single-center data by Solomon et al. [74] evaluated 18 males with mild to moderate CKD due to congenital anomalies of the kidney and urinary tract in comparison to matched, healthy peers. Cerebellar gray matter volume was significantly smaller in children with CKD compared to peers (Fig. 57.1). In contrast, cerebral gray matter volume was increased in CKD participants. Reduced

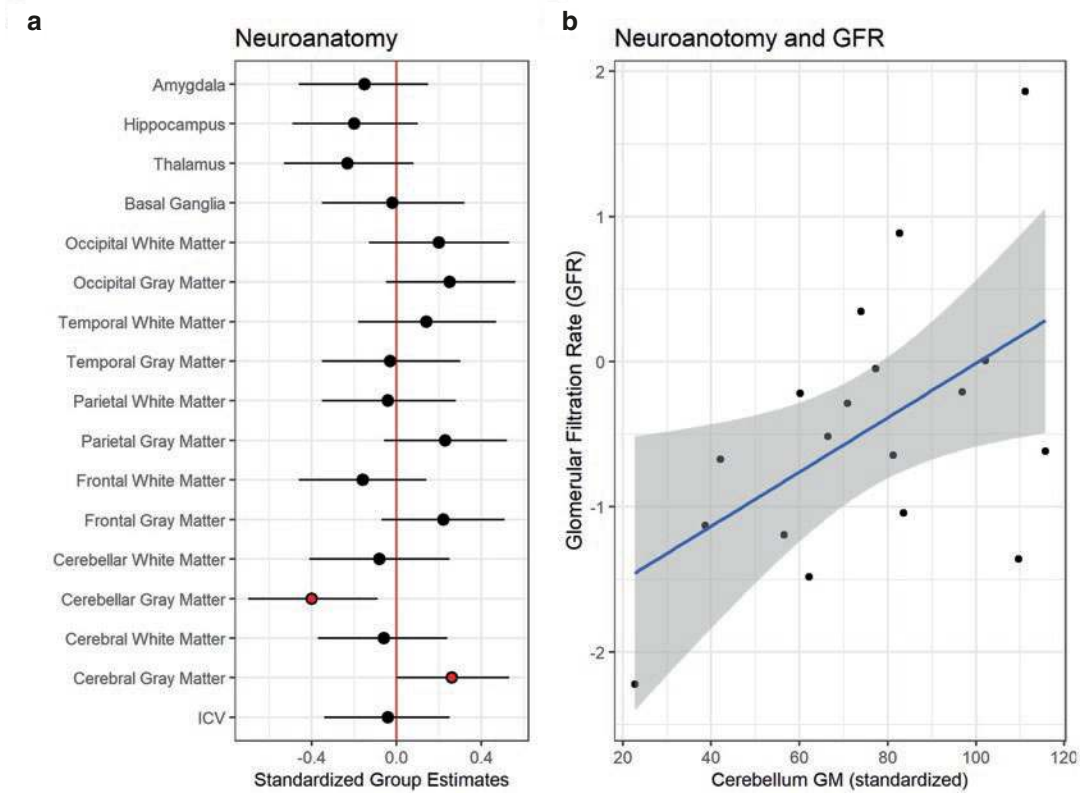

Fig. 57.1 Neuroanatomical differences between controls and pediatric chronic kidney disease [74]. Panel A shows the standardized group estimates (x-axis) and 95% confidence limits of the estimates for each of the regions of interest (ROI) included in the analysis (y-axis). Estimates are adjusted for age, socioeconomic status, and maternal education. The red (vertical) line marks 0, or no significant effect of group on ROI. Red circles mark significant group estimates. Panel B shows the relationship between estimated glomerular filtration rate, eGFR, (x-axis) and standardized cerebellum gray matter volume (y-axis) in the CKD group

cerebellum gray matter volume was associated with disease severity, operationalized as eGFR and predicted lower verbal fluency scores in the CKD sample. Enlarged cerebral gray matter in the CKD sample predicted lower scores on mathematics assessment.

sMRI also serves to inform the microstructural white matter integrity of the brain via diffusion tensor imaging (DTI). DTI is an MRI modality that allows for examination of white matter integrity—i.e., axonal or white matter microstructural changes that disrupt the diffusive property of water in the axon. These microstructural changes have been shown in diseases such as hypertension, diabetes mellitus, and atherosclerosis [75, 76]. The result of this microstructural disruption is a change in a physical diffusive property of the axon called decreased fractional anisotropy. Matsuda-Abedini et al. [77] conducted a quantitative white matter analysis utilizing a heterogenous sample of patients with varying CKD etiologies (including patients on PD and post-transplant) and control patients. This demonstrated the presence of decreased white matter integrity, specifically decreased fractional anisotropy, within the anterior limb of the internal capsule. It is possible that the entirety of the white matter integrity difference within the sample was not captured given the multisite nature of the study; specifically, MRI sequences are highly scanner dependent and statistically significant differences can emerge (or be lost) due to differences in magnet strength or brand of scanner utilized. Lastly, the sample did not have

parallel neurocognitive data to further inform the significance of white matter changes within the anterior limb of the internal capsule finding.

fMRI studies examine the use of oxygen within the brain or rate of arterial cerebral blood flow with specialized scan sequences called 'resting state' or 'arterial spin labeling,' respectively. Additional analysis of data from the NiCK Study lends evidence for regional cerebral blood flow abnormalities that may underlie cognitive changes in pediatric CKD [78]. In a study by Liu et al. [78], patients with CKD, including dialysis and transplant patients, showed higher global cerebral blood flow compared to control subjects. Perhaps of most interest, one area showed regional cerebral blood flow differences between patients and control subjects—including a region called the "default mode" network (Fig. 57.2). Neuroscience literature supports that the brain is organized into functional networks, and the

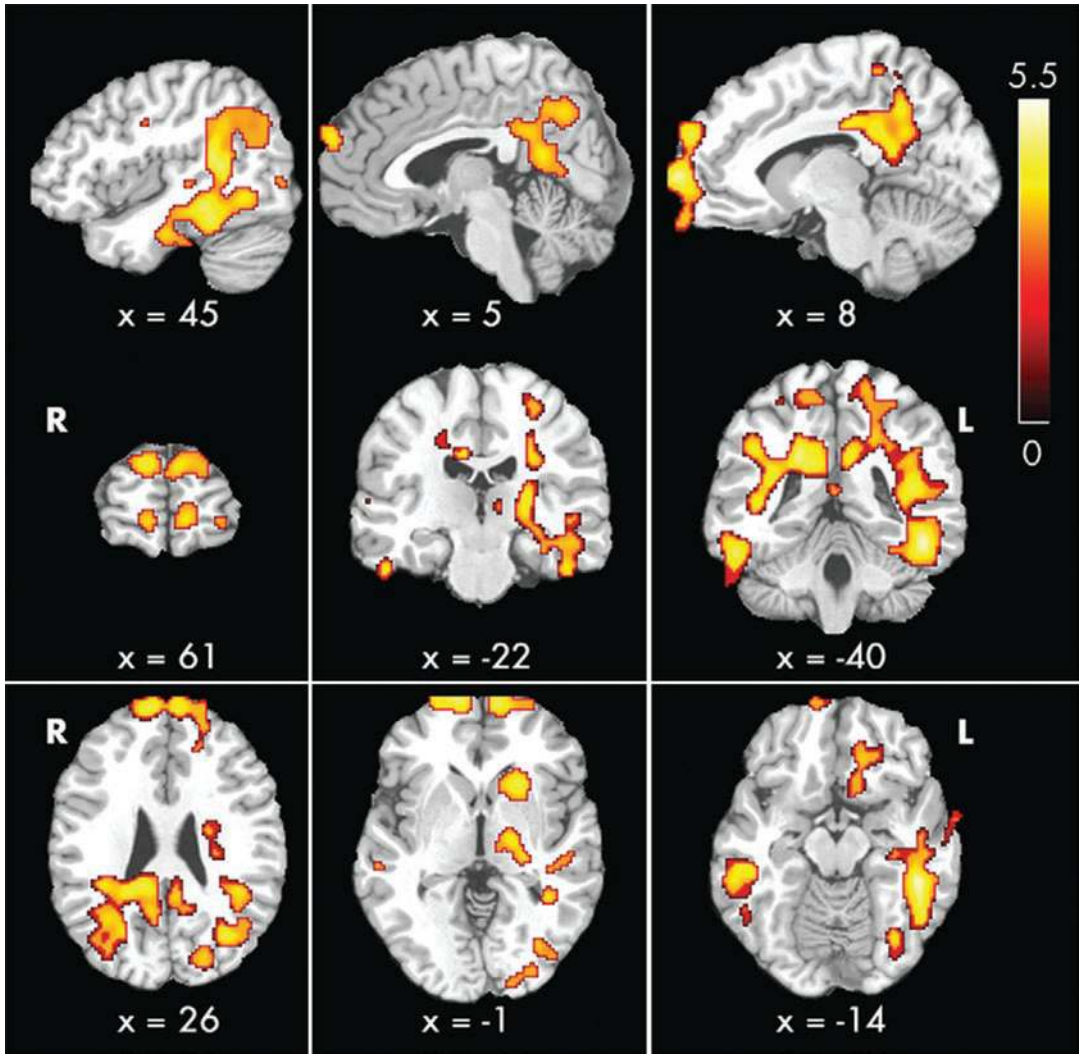

Fig. 57.2 From Liu et al. [78], reprinted with permission: demonstration of voxel-wise group comparison of cerebral blood flow after removal of effects of hematocrit level, age, and sex. Contrast shown here demonstrates the regions where those with CKD have greater cerebral blood flow than controls. Of note, there were no regions in this analysis where controls had greater regions than those with CKD. Color bar indicates *t* scores. *x, y, z* = coordinates in Montreal Neurological Institute (MNI) space

default mode network is a critical loop for attention regulation and, perhaps, EF processes [79–82]. Hematocrit-related effects explained most of the observed group differences in cerebral blood flow. Thus, Liu et al. hypothesized that chronic anemia experienced in pediatric CKD could be a potential cause of vascular endothelial damage due to increased compensatory blood flow to meet demands for deficits in tissue oxygen delivery.

More Frequent Hemodialysis and Neurocognitive Functioning

There were improvements in blood pressure, phosphate control and health-related quality of life in a prospective controlled trial of daily or nocturnal HD in adults [83, 84]. One cohort was studied with an expanded battery of cognitive tests; executive function and global cognition were not affected, but memory and verbal fluency were improved [85]. The subjects who received daily HD saw improvement on the Rey Auditory Verbal Learning Test Immediate Recall and the Controlled Oral Word Association Test after 12 months. The frequent nocturnal HD group did not see a benefit in cognition and had poorer performance on one test of attention. A smaller longitudinal study of 14 adults showed benefits to attention and working memory, psychomotor efficiency and processing speed, and learning efficiency when subjects were converted from thrice weekly HD to nocturnal treatments 5–7 times per week [86].

Reports of frequent HD and nocturnal dialysis in children have focused on improvements in growth and metabolic control, rather than on cognition. There remains interest in expanding these treatment choices to more children. Improvement in blood pressure control and middle molecule clearance coupled with reduction or removal of dietary restrictions could benefit both cognition and quality of life. Nonetheless, it is recognized that these treatment schedules are burdensome. In one of the largest series reporting patient outcomes for more frequent dialysis, daily in-center hemodiafiltration required 3-hour treatments six times per week [87]. Home nocturnal HD performed 6–7 nights per week provides improved metabolic control and elimination of dietary restrictions [88] but increased perceived treatment burden for the family. The NxStage System is most often used for home HD but is only appropriate for those over 30 kg; we do not have an alternative to PD for the smallest patients. These preliminary observations in selected dialysis programs have engendered enthusiasm for alternative regimens for HD in children and it has been recommended that pediatric nephrologists consider ways to implement more frequent HD and strategies for supporting families who wish to pursue it [89, 90].

Conclusion

The cognitive deficits observed in pediatric CKD patients represent a potentially under-recognized consequence of pediatric CKD in day-to-day clinical practice. The findings presented in this chapter demonstrate that our pediatric CKD patients are most at risk for cognitive dysfunction in the domain of executive functions; specifically, attention and working memory. Cognitive and academic performance may be associated with features on neuroimaging including differences in gray matter volume. These cognitive, academic, and neuroimaging differences likely emerge in early childhood and can be detected during the early stages of CKD. This signals a need for greater attention to the developing brain in the midst of a life-long, chronic disease process. Current data represent a limited understanding of the medical determinants of cognitive dysfunction in CKD and an even more minimal understanding of the genetic and epigenetic drivers of cognitive development and performance in CKD. Certainly, research efforts to understand cognition in CKD may be better served through parallel inclusion of a chronic disease control model that includes healthy controls. Nephrologists should remain aware of the burden of managing chronic disease at a young age and how that differs from the experience of adults who develop CKD later in life.

When treating children with CKD, it is important that nephrology care incorporate a psychologist as part of the treatment team to provide understanding for families and patients regarding the impact of CKD progression on neurodevelopment and neurocognitive performance and available academic accommodations and interventions. This will allow patients and caregivers to obtain more comprehensive information about the potential for cognitive rehabilitation and learn how best to support children and adolescents with CKD as they navigate educational and vocational endeavors.

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Nutritional Challenges in Pediatric Kidney Disease

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Rayna Levitt and Caitlin E. Carter

Introduction

Provision of adequate nutrition is a cornerstone of optimal management and improvement of outcomes for children with chronic kidney disease (CKD). Nutrition impacts short-term outcomes, including renal disease progression, growth, and development as well as long-term outcomes, including cardiovascular risk. Presently, the majority of guidelines for the nutritional management of children with CKD are based on consensus opinions and practice-based evidence.

Important considerations include the provision of adequate energy and macronutrients, the achievement of fluid and electrolyte balance, the provision of adequate micronutrients, and the prevention of metabolic bone disease. Extremes of body mass index, representing under-nutrition and over-nutrition are associated with poor outcomes and should be addressed when designing nutritional care plans for children with CKD. Promoting optimal nutrition for children with CKD necessitates multidisciplinary collaboration, including input from registered dietitians, physicians, nurses, and social workers.

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Nutrition Assessment

In 2009, in recognition of the growing population of children with CKD, the National Kidney Foundation published the Kidney Disease Outcomes Quality Initiative's (KDOQI) Clinical Practice Guidelines for Nutrition in Children with CKD [1]. These practice guidelines expanded upon guidelines that were published by KDOQI in 2000 by setting out evidenced based nutrition recommendations for pediatric patients with CKD, including patients receiving dialysis and those who have received kidney transplants. The guidelines were recommended as a starting point for those providing medical nutrition therapy to this complex patient population [2]. In 2019, the Pediatric Renal Nutrition Task Force, a team of pediatric nephrologists and pediatric renal dietitians from eight countries in Europe and North America, was established to develop clinical practice recommendations (CPRs) to update the 2009 KDOQI guidelines in areas where new evidence has become available [3]. This group conducted an in-depth review of evidence for current practices along with consensus opinion to establish updated practice guidelines.

The primary goal of evaluating nutrition status of children with CKD is to support growth and development, while preventing protein-energy wasting (PEW) and its well documented negative effects on patient outcomes [4, 5]. Provision of medical nutrition therapy in this population also

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aims to ensure that vitamin and mineral needs are met. There is a risk posed by over-nutrition as well, illustrated by obesity resulting from factors such as overzealous tube-feeding and the frequent use of corticosteroid therapy. Nutrition assessment in this patient population should involve the use of multiple measures of nutrition status as no single measure can provide an accurate picture. Children with CKD require more frequent monitoring of their nutrition status than their healthy counterparts, and the collection and comparison of measures over time can be used to form a more complete picture. The recommended components of assessment include anthropometric measures, evaluation of dietary intake, and review of biochemical data.

Anthropometric Measures

Height, weight and head circumference are the most easily attained measures used in nutrition assessment. They should be accurately measured using calibrated equipment and standardized techniques [1].

- Weight should be assessed at every clinic visit and weight gain or loss trends should be noted.
- Height should be evaluated in order to give context to the weight assessment. For children under 2 years of age, recumbent length should be measured. Standing height can be measured for those over 2 years who are able to stand. In order to determine if the height achieved is appropriate given a child's genetic potential, mid-parental height should be assessed. While anecdotal data can be used for this, it is best if the heights of both biological parents can be measured [6].

Mid-Parental Height Calculation:	
Boys:	[(mother's height + 13 cm) + father's height]/2
Girls:	[mother's height + (father's height-13 cm)]/2

(chart adapted from KDOQI 2009)

- Head circumference should be measured for children under 2 years of age.

These measures are plotted on percentile charts. In the U.S., the recommendations of the Centers for Disease Control and Prevention (CDC) are to use the World Health Organization growth charts to monitor growth for infants and children ages 0–<2 years of age and to use the CDC growth charts to monitor growth for children ages 2 years and older [7]. These values can also be expressed as standard deviation scores (number of standard deviations from the mean for a normal population of the same age). If an applicable underlying genetic disorder is present, it is appropriate to utilize a disease specific growth charts to assess growth parameters.

- Weight for length (for children <2 years) or BMI (for children ages 2–20 years) should be calculated every time weight and height are measured and plotted on percentile charts.

When evaluating BMI in children with CKD, it may be preferable to express their BMI relative to height age (the age at which the child's height would be at the 50th percentile). Children with CKD commonly experience poor linear growth and delayed skeletal maturation. Comparison of BMI to other children with the same chronological age may result in an underestimation of that child's BMI percentile. It is therefore more appropriate to compare BMI to those of children with similar height and maturation [1, 4, 5]. When evaluating weight or using weight to calculate BMI, it is important to first evaluate whether edema is present. For dialysis patients, estimated dry weight should be used when calculating weight for length or BMI.

In addition to anthropometric measurements, nutrition-focused physical exam (NFPE) can be included as part of the patient assessment as it can identify muscle wasting, subcutaneous fat loss, edema and macronutrient deficiencies [8]. The value of NFPE has been noted in the general pediatric population as it can identify malnutrition that has a negative impact on growth and

development. Since children with CKD are known to be at risk of poor growth, it may add value to the assessment of this patient population as well.

Dietary Assessment

It is recommended that dietary intakes of children with CKD be assessed with a minimum frequency of annually, or as often as every 2 weeks depending on the age of the child and stage of disease. More frequent assessments are recommended for younger children and children with advanced stages of CKD. Children who depend solely on enteral feeding should have more frequent monitoring. This is especially true of enterally fed infants who may benefit from feeding adjustments as often as weekly [6, 9]. The dietary intake assessment should either take the form of a prospective 3-day diet diary or a 24-h diet recall. A 3-day diary should include one weekend day to represent variation in the diet due to change in schedules on weekends. The 24-h diet recall may be better suited to adolescents who, may not be compliant with completion of a food diary. Either technique can be utilized by a registered dietitian to estimate daily intake of energy, macronutrients, vitamins and minerals. They can provide information about diet adequacy and, over time, can highlight changes in appetite that may lead to weight loss or increased risk of malnutrition. If dietary assessment reveals that the diet is inadequate, in addition to other considerations, the dietitian should evaluate whether food insecurity is a contributing factor. In 2018, one in seven U.S. households with children was affected by food insecurity, which can be exacerbated by families facing increased medical expenses [10].

Assessing Protein Status: Normalized Protein Catabolic Rate vs. Serum Albumin

Lower serum albumin is associated with increased morbidity and mortality [11]. The 2000 KDOQI Nutrition Guidelines recommended albumin be used as a marker of nutritional status in pediatric dialysis patients [2]. There are several shortfalls, however, in the use of albumin levels as a measure of nutritional status in children with CKD. Serum albumin concentrations decrease for various non-nutritional reasons, such as the presence of fluid overload, urinary and dialysate protein losses, inflammation, infection and liver disease [4]. In addition, it is possible for serum albumin to be normal in patients who are known to have a poor oral intake and who appear malnourished with a decrease in lean body or fat mass. Despite these limitations, hypoalbuminemia has been associated with mortality in children initiating dialysis therapy and albumin levels above 4 g/dL in adolescent patients on hemodialysis (HD) have been associated with a reduced risk of death and decreased hospitalizations [6]. For this reason, serum albumin levels continue to be monitored and are interpreted in the context of other assessment data.

Normalized protein catabolic rate (nPCR) has been validated as an objective tool that may assist in the nutritional assessment of adolescent HD patients. The calculation of nPCR is based on the increase in the level of blood urea nitrogen between the end of one HD treatment and the beginning of the subsequent treatment and it is theoretically more accurate than food diaries or diet recalls. Calculations using BUN values from the end of one dialysis treatment and BUN values at the start of a subsequent treatment may more accurately reflect protein intake than calculations using BUN values from the start and end of a same day HD treatment [12]. Higher nPCR in adolescents has been found to be a good predictor of weight gain, whereas lower nPCR has been shown to predict weight loss.

Normalized protein nitrogen appearance (nPNA) is a similar measure of estimated protein intake that can be calculated for patients on peritoneal dialysis (PD). It is calculated by determining the total daily excretion of urea in the dialysate and urine, as well as the daily dialysate protein losses. These values are entered into an estimating equation and are normalized for body size.

Equations for Calculation of Normalized Protein Catabolic Rate (nPCR) and Normalized Protein Equivalent of Nitrogen Appearance (nPNA)
nPCR
1. Calculate urea generation rate (G, mg/min):
G = [(C2 × V2) − (C1 × V1)/t
C1 = post-dialysis BUN (mg/dL)
C2 = pre-dialysis BUN (mg/dL)
V1 = post-dialysis total body water (dL; V1 = 5.8 dL/kg × post dialysis weight in kg)
V2 = pre-dialysis total body water (dL; V2 = 5.8 dL/kg × predialysis weight in kg)
t = time (minutes) from the end of one dialysis treatment to the start of the next dialysis treatment
2. Calculate nPCR:
5.43 × estG/V1 + 0.17
nPNA
1. **Calculate total nitrogen appearance (TNA, g/day):**
0.03 + 1.138 urea-Nurine +0.99 urea-Ndialysate +1.18 BSA + 0.965 protein-Ndialysate
2. Calculate protein equivalent of nitrogen appearance (PNA, g/day):
TNA × 6.25
3. Normalize PNA to body weight (nPNA):
PNA/weight (kg)

N = nitrogen, BSA = body surface area, BUN = blood urea nitrogen

Adapted from: National Kidney Foundation KDOQI Clinical Practice Guideline for Nutrition in Children with CKD: 2008 Update. Am J Kidney Dis. 2009; 53(3 Suppl 2):S1-S123 [1] and from Roman B. Nutrition Management of Pediatric Chronic Kidney Disease. Support Line. 2013 April;35(2):12–21 [4]

nPCR and nPNA are expressed in grams of protein per kilogram body weight per day (g/kg/day). The target nPCR or nPNA should be equivalent to the protein goal for age, which is based on the dietary reference intake (DRI) plus an allowance for dialytic protein and amino acid losses. Because nPCR fluctuates on a daily basis depending upon what is eaten, a single nPCR value does not give a good picture of protein intake; the observation of trends from month to month provides more information [1]. Among the shortfalls of nPCR are that it may overestimate protein intake in severely catabolic patients and underestimate protein intake in growing children, and has not been shown to have predictive value in infants and young children. For these reasons, like other assessment tools, it should be used in conjunction with other data to provide a complete picture of nutritional status.

Nutrition Management

Health care teams have moved away from prescribing "renal diets" as the standard diet for all children with CKD. Dietary modifications should be individualized based upon disease state and biochemical data as well as factors such as the child's age, development and food preferences. Children with CKD may require modifications to their intake of calories, protein, fat, phosphorus, calcium, sodium, potassium and fluid. In order to promote growth and development and facilitate adherence to diet recommendations, restrictions should be kept as liberal as possible. The diet can then be liberalized or tightened depending on the response in the relevant parameter. The nutrition care plan should be monitored frequently. Adjustments should be made over time in response to changes in the child's nutritional status, age, development, anthropometrics, food preferences, renal function, biochemistries, need for and mode of renal replacement therapy, medications and psychosocial status. Diet instructions should begin with a simple explanation of the role of the nutrient in the body, the rationale for the diet modification, the desired outcomes to be achieved (e.g., specific amount of weight gain, normalize serum phosphorus levels) and what happens if the modifications are not made. Guidelines for change should be practical, individualized to the patient's and the family's lifestyle, cultural food preferences and eating habits, and should be positive in that they emphasize the foods that the child can eat to replace those that need to be limited or avoided. Food models, pictures of foods from supermarket advertisements, and food containers with labels and ingredient lists can be used to make teaching sessions more interesting and relatable. During follow up, food records and diet histories can be used to assess the child's and family's understanding of and adherence to the diet to identify problems that may exist with modifying dietary intake. Nutritional counseling is recommended on an ongoing basis in order to address the dynamic nature of a child's development, food preferences, residual renal function and medical condition. Caregivers outside of the family (grandparents, school staff, daycare providers,

and babysitters) should be aware of diet modifications and should be provided with copies of teaching materials to use as reference guides to ensure consistent adherence to diet modifications. Adolescents should receive sufficient education to allow them to independently make appropriate choices when they are at restaurants, with friends, or at school.

Energy

Energy Requirements

Adequate energy intake can lead to increases in body weight and improvement in linear growth. Meeting caloric requirements is important to avoid using protein as an energy source through gluconeogenesis. There is no evidence that children with CKD have different energy requirements than their same-aged healthy counterparts. For this reason, the KDOQI 2009 guidelines and the Pediatric Renal Nutrition Task Force (PRNT) agree that initial energy goals for children with CKD 2–5D be the same as those for healthy children of the same chronological age [1, 3]. The PRNT utilizes the terminology suggested dietary intake (SDI) (see Table 58.1 from PRNT paper) as the guideline rather than estimated energy requirements used by KDOQI. The SDI provides a range of values. These SDI for energy can be adjusted for physical activity level and BMI [1, 3]. If weight gain and linear growth are poor, energy intake should be adjusted towards the higher end of the SDI. Weight and linear growth

Table 58.1 Energy and protein requirements for infants, children and adolescents with CKD2–5D aged 0–18 years

SDI for energy and protein: birth[a] to 18 years				
Month	SDI[b] energy (kcal/kg/day)	SDI protein (g/kg/day)	SDI protein (g/day)	
0	93–107	1.52–2.5	8–12	
1	93–120	1.52–1.8	8–12	
2	93–120	1.4–1.52	8–12	
3	82–98	1.4–1.52	8–12	
4	82–98	1.3–1.52	9–13	
5	72–82	1.3–1.52	9–13	
6–9	72–82	1.1–1.3	9–14	
10–11	72–82	1.1–1.3	9–15	
12	72–120	0.9–1.14	11–14	
Year	**SDI energy (kcal/kg/day)**		**SDI protein (g/kg/day)**	**SDI protein (g/day)**
–	Male	Female		
2	81–95[c]	79–92[c]	0.9–1.05	11–15
3	80–82	76–77	0.9–1.05	13–15
4–6	67–93	64–90	0.85–0.95	16–22
7–8	60–77	56–75	0.9–0.95	19–28
9–10	55–69	49–63	0.9–0.95	26–40
11–12	48–63	43–57	0.9–0.95	34–42
13–14	44–63	39–50	0.8–0.9	34–50
15–17	40–55	36–46	0.8–0.9	Male: 52–65 Female: 45–49

For children with poor growth, reference to the SDI for height age may be appropriate. Height age is the age that corresponds to an individual's height when plotted on the 50th centile on a growth chart

[a]Thirty-seven/40 weeks gestation. Premature infants have higher energy and protein requirements. The increased need for these and other nutrients (sodium, potassium, calcium, and phosphorus) must be balanced against the nutritional interventions to control the effects of CKD. This is outside the scope of this CPR

[b]Suggested Dietary Intake (SDI) is based on the Physical Activity Level (PAL) used by the international bodies: 1–3 year PAL 1.4; 4–9 year PAL 1.6; and 10–17 year PAL 1.8. Where guidelines have given a range of energy requirements for different levels of PAL, the lowest PAL has been taken for SDI energy in consideration that children with CKD are likely to have low activity levels

[c]Scientific Advisory Committee on Nutrition [13] reports energy requirements as kcal/day: male 1040 kcal/day; female 932 kcal/day

should be monitored over time and calorie goals should be adjusted according to the child's weight gain or loss and needs for catch-up growth.

There are many factors that can contribute to inadequate caloric intake in children with CKD. Decreased appetite is common. This may be related to alterations in taste perception that can decrease spontaneous intake of food [14]. High fluid intake requirements in polyuric patients or the requirement to take multiple medications can also diminish appetite. Other factors impacting intake include vomiting and gastroesophageal reflux, delayed gastric emptying and elevation of cytokine levels, including tumor necrosis factor [15]. Changes in hormones that regulate appetite such as leptin and ghrelin can also contribute to decreased intake. Frequent hospitalizations, multiple surgeries, nasogastric tube use and developmental delays impacting feeding with or without the factors previously listed can lead to oral aversion or delayed development of oral feeding skills. Nutrition supplementation should be initiated promptly if a downward trend in weight percentile is noted [3].

Obesity is increasing both in the population of healthy children around the world as well as in the population of children with CKD. In 2015–2016, the prevalence of obesity in the U.S. was 13.9% among preschool-aged children (2–5 years of age), 18.4% among school-aged children (6–11 years of age) and 20.6% among adolescents (12–19 years of age) [3, 16].

The International Pediatric PD Network (IPPN) registry found that children starting chronic peritoneal dialysis (PD) therapy had an overweight/obesity prevalence as high as 19.7% [3, 16, 17]. Nasogastric and gastric tube feeds have been found to be an independent predictor of overweight/obesity, which suggests that there may be over-utilization of supplemental feeds through NG/GT tubes in some patients, leading to an imbalance between caloric intake and energy expenditure [18]. The Chronic Kidney Disease in Children (CKiD) study found the median energy consumption of children with CKD to be higher than recommended in all age groups [19, 20]. With recognition that the epidemic of obesity in the general pediatric population has been linked to increased rates of hypertension, hypercholesterolemia, impaired glucose tolerance, insulin resistance, type 2 diabetes, sleep apnea and asthma, the potential adverse impact of these co-morbidities on children with CKD should be investigated. Future prospective studies should examine any potential link between obesity and poor outcomes, especially with respect to cardiovascular events, so that appropriate dietary and lifestyle modifications can be defined.

Appetite Stimulants

When patients are not meeting nutritional needs, nutrition counseling and calorie supplementation provide the first line of defense; however, complementary strategies for improving appetite and treating or preventing malnutrition have been examined. Megestrol acetate (MA), a synthetic progesterone derivative with appetite stimulating properties, has been studied in the pediatric CKD population. In a retrospective cohort study, Hobbs et al. followed 25 patients with a mean age of 8.9 ± 5.4 years who had demonstrated decreases in BMI and poor weight gain [21]. The mean duration of therapy was 5.4 ± 6.3 months. During treatment, there was a significant increases in BMI ($P < 0.0001$) and weight ($P < 0.0001$). Linear growth continued to improve. MA was well tolerated with the exception of one patient who experienced a side effect of cushingoid features. The authors conclude that MA may provide a safe short-term strategy to improve nutritional status in children with CKD. Possible side effects of MA such as diarrhea, headaches, dizziness, hyperglycemia, hypertension, adrenal suppression, adrenal insufficiency and thromboembolic events have been cited as causes for taking caution in its use in infants with CKD [22]. Cyproheptadine is an antihistamine that increases appetite by exerting an anti-serotoninergic effect on the brain. It has been studied as a potential appetite stimulant in underweight children who are otherwise healthy, children with cystic fibrosis and those with cancer-related cachexia, and has been found to significantly increase caloric intake and height velocity [23, 24]. The appetite stimulating effects of the gut hormone ghrelin suggest that it could be

an effective treatment for anorexia in adult CKD patients. This hormone is felt to potentially play a key role in the pathogenesis of protein-energy wasting, inflammation and cardiovascular complications in CKD [25–27]. Ghrelin has been found to acutely induce lipolysis and insulin resistance and could potentially increase the risk of diabetes [28]. Though further studies demonstrating the long-term efficacy of ghrelin are needed, along with evaluation of its use in children, it appears possible that administration of long-acting ghrelin mimetics holds the promise of improving appetite and nutrition in patients with CKD.

Nutritional Supplementation

When voluntary oral intake is low, calories can be maximized by adding concentrated sources of carbohydrate and fat to the diet, choosing calorie dense foods, and limiting calorie-free foods and fluids such as water. Calories can be added to foods using heart-healthy margarines or oils, cream and other fats, sugars, syrups, or commercial carbohydrate modules. Commercial calorie supplements such as milkshakes or energy bars may be useful; however, their phosphorus and potassium content should be taken into consideration. Standard pediatric enteral supplements have fairly high calcium and phosphorus content to support bone growth. These products are contraindicated in children with hyperphosphatemia and/or hyperkalemia. Standard products may be used in combination with low mineral and electrolyte pediatric renal products for these patients. Adult renal products can be used for older children and teens.

For infants with CKD, breastfeeding is the preferred method of feeding. If breastfeeding is not possible or breastmilk is not available, whey-based infant formulas are recommended. If a low potassium, low phosphorus formula option is needed, a low renal solute load infant formula such as Similac PM 60/40®(Abbott Laboratories, Chicago, IL, USA) can be used either to fortify or supplement breastfeeding or on its own. Breastmilk and infant formula should be fortified for infants who require fluid restriction or for those who require more energy or nutrient dense feeds.

To meet requirements, commercial glucose polymer powders, liquid fat products or powdered fat and carbohydrate combination products can be added to infant feedings to increase their standard energy density (20 kcal/oz., 0.67 kcal/mL) as high as 60 kcal/oz. (2 kcal/mL) without significantly increasing electrolyte and mineral content. The choice to add a carbohydrate module, fat, or a combination of both should be made after considering serum glucose and lipid profiles, the presence of malabsorption or chronic respiratory disease (carbohydrate metabolism increases carbon dioxide production), and the cost to the caregivers. When making more than two or three increases in energy density, the distribution of calories from carbohydrate and fat should be kept similar to the base feeding. Unless fat malabsorption is present, "heart-healthy" oils such as corn or canola oil can provide a low-cost option, especially for infants on PD who have hypertriglyceridemia as a consequence of absorbing excess glucose from the dialysate. Powdered products may be preferred over oils, however, due to the difficulty of blending oil with formula and the tendency of the oil to adhere to feeding bottles and feeding tubes. Increasing the caloric density of powdered formulas by concentration (i.e., adding more formula powder or liquid concentrate and less water) is typically not recommended because of the accompanying increase in sodium, potassium, and phosphorus concentrations. If concentration of feeds or if dietary supplements are required, they should be introduced in a gradual manner to optimize acceptance and tolerance. Increases in energy density of 2–4 kcal/oz. are generally better tolerated than larger increases.

If using adult formulas, clinicians should evaluate the protein and electrolyte content of the formula to ensure its safety and appropriateness based on the child's age and weight. Serum magnesium levels require monitoring when using adult renal formulas because their magnesium content is significantly higher than breast milk, infant and pediatric formulas. The acceptance of these products can be improved by mixing them with fruit, and/or ice to make shakes or slush-type drinks.

For children undergoing PD, energy contribution resulting from glucose absorption from peritoneal dialysate solutions may provide an additional 8–26 kcal/kg [2]. The amount of glu-

cose absorbed can vary depending on the mode of dialysis (time on dialysis, cycles and dwell times), the glucose concentration of the dialysate and the characteristics of the peritoneal membrane [3, 5] For underweight children on PD therapy, clinicians need not calculate the calories absorbed from dialysate as this might compromise the nutritional quality of the diet. These calories should be considered as "bonus" calories to help promote weight gain. However, for children on PD who are exceeding weight gain goals, it may be important to take the estimated calorie contribution from PD fluid into account.

Infant, Pediatric and Adult Renal Formula Options

Formula	Distributed by	Used for
Similac PM 60/40®	Abbott Laboratories, Chicago, IL, USA	Infant formula for infants requiring low renal solute load, low electrolyte formula Can be used alone or to supplement breast milk Can be used in combination with other formulas to achieve desired mineral and electrolyte balance
RenaStart®	Vitaflo, USA	Intended for children ages 1 and up For children requiring formula low in protein, calcium, chloride, potassium, phosphorus and vitamin A Can be used in combination with other formulas to achieve desired mineral and electrolyte balance Not intended for use as a sole source of nutrition
Suplena®	Abbott Nutrition	Calorie dense adult nutrition supplement for patients with potassium and phosphorus restrictions
Nepro®	Abbott nutrition	Calorie dense adult nutrition supplement for patients with potassium and phosphorus restrictions who require additional protein
NovaSource Renal®	Nestle nutrition	Calorie dense adult nutrition supplement for patients with potassium and phosphorus restrictions
Renalcal®	Nestle nutrition	Calorie dense adult nutrition supplement for patients requiring minimal additional electrolytes and minerals. Can be used in combination with other formulas to achieve desired mineral and electrolyte balance Not intended for use as a soul source of nutrition

Despite efforts to provide adequate calories through oral nutrition supplementation and formula concentration, many factors such as gastroesophageal reflux, vomiting, medication taste, uremia and thirst for large volumes of water due to polyuria can contribute to inadequate oral intake and poor weight gain. For children who are not able to meet their nutrition requirements orally, enteral feeding is recommended.

Enteral Feeding

Nasogastric, gastrostomy, gastrojejunostomy, and jejunostomy tubes have all been used successfully to provide additional nutrition, fluids and/or medications by intermittent bolus or continuous infusion. Nasogastric tubes are a good option for short-term enteral feeding or may be used as a bridge until a long-term tube can be placed, but they are not recommended for long-term use as they are associated with an increase in emesis. Although oral feeding is preferred when possible, tube feeding should be considered for children who are unable to meet their energy needs despite dietary intervention and who are underweight and/or growth delayed. In addition to the benefits of promoting weight gain and growth, enteral feeding can help relieve the stress that many caregivers experience when efforts to provide adequate oral nutrition are failing. If oral intake is limited or absent and enteral feeding is provided, oral stimulation is encouraged in order to promote

development of feeding skills and prevent the development of food aversion [1]. Oral stimulation and nonnutritive sucking opportunities should be provided to infants who are completely dependent on tube feeding to help smooth their transition to oral feeding after successful transplantation. Limiting practice with oral feeding can have a significant negative impact on oral motor skill development. Occupational therapists or speech therapists are central in facilitating the development and strengthening of oral feeding skills and preventing or extinguishing oral aversion. Using a multidisciplinary approach, the prospects for a transition to oral feeding post-transplant are good. Even if oral intake improves after transplant, some children will benefit from retaining the G-tube to help ensure adequate hydration and adherence to recommended medications.

The choices of formula and feeding plan are guided by age, serum chemistries, gastrointestinal function, and fluid allowance. In addition, caregiver related factors, including the ability of caregivers to mix and measure formula recipes and financial barriers to accessing ingredients, must be considered. Feedings are initiated and advanced according to pediatric guidelines and tolerance [29]. Volumes and rates that are based on body weight help to avoid intolerance in patients who are underweight or small for their age**.** Whenever possible, the volume of feeds should be minimized to optimize tolerance and keep the hours of feeding manageable within the child's daily schedule. Infants should be given intermittent bolus feeds to maintain normal blood sugars. Continuous overnight feeds are generally avoided for infants due to an increased risk of aspiration resulting from vomiting and gastroesophageal reflux associated with uremia. Continuous feedings may be required if the patient's tolerance of bolus feedings is poor. Continuous overnight feeds are generally preferred for children and adolescents to facilitate daytime hunger and optimize oral intake. Reported complications of enteral feeding include tube blockage, tube displacement emesis, leakage around the gastrostomy exit site, skin irritation and itching, exit site infection, hemorrhage and peritonitis [30], When vomiting and gastroesophageal reflux are not responsive to medical therapy, jejunal feeding or a fundoplication may be warranted. To decrease the risk of peritonitis, the placement of gastrostomy, gastrojejunostomy, and jejunostomy tubes should occur before or concomitant with insertion of a PD catheter, whenever possible [31–33]. In particular, percutaneous endoscopic gastrostomy insertion after PD initiation carries a high risk for fungal peritonitis and potential PD failure. Suggested precautions for lowering the risk of peritonitis include antibiotic and antifungal prophylaxis, withholding PD for 2–3 days, and gastrostomy placement by an experienced endoscopy team [9, 33].

Age	Initial hourly infusion	Daily increases	Goal
Continuous feedings			
0–1 year	10–20 mL/h or 1–2 mL/kg/h	5–10 mL/8 h or 1 mL/kg/h	21–54 mL/h or 6 mL/kg/h
1–6 years	20–30 mL/h or 2–3 mL/kg/h	10–15 mL/8 h or 1 mL/kg/h	71–92 mL/h or 4–5 mL/kg/h
6–14 years	30–40 mL/h or 1 mL/kg/h	15–20 mL/8 h or 0.5 mL/kg/h	108–130 mL/h or 3–4 mL/kg/h
>14 years	50 mL/h or 0.5–1 mL/kg/h	25 mL/8 h or 0.4–0.5 mL/kg/h	125 mL/h
Bolus feedings			
0–1 year	60–80 mL every 4 h or 10–15 mL/kg/feed	20–40 mL every 4 h	80–240 mL every 4 h or 20–30 mL/kg/feed
1–6 years	80–120 mL every 4 h or 5–10 mL/kg/feed	40–60 mL every 4 h	280–375 mL every 4 h or 15–20 mL/kg/feed
6–14 years	120–160 mL every 4 h or 3–5 mL/kg/feed	60–80 mL every 4 h	430–520 mL every 4 h or 10–20 mL/kg/feed
>14 years	200 mL every 4 h or 3 mL/kg/feed	100 mL every 4 h	500 mL every 4 h or 10 mL/kg/feed

Parenteral Nutrition

When oral and/or enteral nutrition intake is not sufficient or not tolerated, parenteral nutrition (PN) may be necessary to provide adequate nutrition. When delivering PN in fluid restricted patients, concentrated solutions of amino acids, dextrose, and lipids are required. Energy requirements during PN are 10% lower than enteral requirements because there is no thermal effect of feeding. Standard amino acid solutions (i.e., both essential and nonessential amino acids) are generally used and provided according to daily enteral protein recommendations specific to the child's age and renal replacement therapy. Amino acids, dextrose, and lipids can be advanced according to normal pediatric PN guidelines and serum urea, glucose, and triglyceride concentrations should be monitored. Mineral and electrolyte content should be adjusted to maintain acceptable serum concentrations, and acetate and chloride content should be adjusted to maintain normal acid-base balance. Standard pediatric dosages of parenteral multivitamins and trace elements can be used; the risk of toxicity, especially for vitamin A, is minimal with a daily injectable multivitamin provided that the child has no other exogenous source of vitamin A (i.e., oral diet is minimal).

Intradialytic Parenteral Nutrition (IDPN)

Intradialytic PN (IDPN) is a non-invasive method of delivering supplemental nutrition to malnourished patients on HD. It can be delivered using the HD access during treatments, allowing the volume of fluid to be removed through ultrafiltration. The KDOQI guidelines recommend a trial of IDPN for children receiving maintenance HD who are unable to meet their nutritional requirements through oral or enteral feeding [1]. IDPN is only supplemental and cannot be used as a sole source of nutrition, but it can be used to augment inadequate oral and/or enteral intake in malnourished children. It can provide a significant amount of protein, but will only meet a small percentage of overall caloric needs [34].

There have been several studies of IDPN use in children. Goldstein et al. demonstrated that IDPN could reverse weight loss and promote weight gain within 6 weeks of its initiation in three teenage patients who had experienced a $\geq$ 10% weight loss over a 3 month period [35]. In this study, IDPN provided 40% of the weekly prescribed protein intake. Orellana et al. examined IDPN in teenaged patients who had lost 10% of their body weight in a 3 month period and were below 90% of their ideal body weight [36]. Of the nine patients studied, seven patients who had organic illness demonstrated an improvement of weight or BMI during the first 5 months of IDPN therapy. The other two patients had psychosocial associated malnutrition and did not demonstrate similar improvement of weight or BMI. The lack of weight gain in these patients was postulated to be due to issues such as depression or inadequate access to food after initiation of IDPN, which may have limited their ability to improve their oral or enteral intake [37].

The optimal composition of IDPN has not been defined, but it is typically designed to provide amino acids in amounts to meet the estimated daily protein needs along with dextrose and 20% or 30% lipid components to increase calorie provision [1]. The goal infusion rate provides optimal caloric intake while avoiding hyperglycemia and hyperlipidemia. It is important to monitor for hyperglycemia, hypokalemia, hypophosphatemia and hyperlipidemia. It has been suggested to limit the glucose infusion rate to $\leq$9 mg/kg/min. This can help minimize hyperglycemia and prevent refeeding syndrome. Lipid infusions should be limited to no more than 1–2 g/kg/day, starting as low as 0.5 g/kg/day. It is recommended to hold or reduce intralipid (IL) infusions if triglycerides exceed 250 mg/dL [34]. It is also important to be aware of the additional fluid contribution in patients who routinely present with fluid overload between dialysis treatments. As critics of PN, Dudley et al. point to potential problems such as disorders of glucose homeostasis, acid-base, fluid and electrolyte disturbances, impaired renal function, metabolic bone disease and nephrolithiasis. They state that IDPN is not more beneficial than enteral supplements in patients who are compliant with supplementation and have adequate intestinal function.

They do, however, recommend a minimum 3 month trial of IDPN for malnourished children on HD in whom enteral support has not demonstrated benefits or is not viable [38]. Although IDPN is costly, it is recommended for patients who have experienced a weight loss of >10% for three consecutive months and who are below 90% of their ideal body weight (or who have a BMI for height age that is below the fifth percentile) and who have not responded to oral and/or enteral supplementation [1, 34, 37].

The use of IL infusion alone has been studied in children receiving HD as a method of sparing protein degradation and supporting positive nitrogen balance. Hasken et al. found that the provision of 0.5–1 gram/kg IL therapy during each dialysis session could contribute to improvements in serum albumin, predialysis BUN, and nPCR in malnourished pediatric HD patients [39]. The cohort exhibited a 5% weight gain without any changes in cholesterol or triglyceride levels. One benefit from this approach is a reduction in cost compared to delivering full IDPN.

Limiting Energy Intake

Increased appetite and excessive energy intake are common in children treated with high-dose corticosteroid therapy for conditions such as nephrotic syndrome, vasculitis, and renal transplantation. Children and caregivers should receive early education about the potential for overweight and obesity and be provided with strategies for controlling caloric intake, optimizing dietary balance and increasing physical activity to maintain a healthy weight. Overweight is sometimes seen in infants and children on PD as a result of significant dialysate glucose absorption, which is usually greater in young infants because of the enhanced permeability of their peritoneal membrane for small molecules. To help control weight gain in these patients, dialysate calories should be considered when estimating energy intake. Icodextrin dialysate, which contains a poorly absorbed, high-molecular-weight, starch-derived glucose polymer to provide the osmotic force for ultrafiltration, can be used to lower the caloric load without sacrificing clearance of metabolic waste or fluid removal.

Protein

Protein Requirements

Children need to be in positive nitrogen balance to support growth. Along with providing adequate protein, the diet must also provide adequate non-protein calories from fats and carbohydrates in order to prevent protein from being utilized to meet energy needs. It is also critical that the correct balance of amino acids be provided. The PRNT suggests a range for the SDI for protein that is designed to represent the daily amount of protein considered to meet the needs for 97.5% of the population (see Table 58.1) [3]. They suggest that the target for protein intake should be at the upper end of the SDI in order to support optimal growth. The lower end of the SDI is considered to be the minimum safe intake. Children treated with HD or PD may need to have protein intakes above the SDI in order to compensate for protein losses into the dialysate. During periods of peritonitis, there may be an increase in peritoneal protein losses that necessitates a further increase in protein intake. Protein needs may increase due to proteinuria, glucocorticoids, acidosis, other infections and catabolism. In contrast, children who have a persistently high BUN level that is believed to be associated with dietary intake may need their protein intake adjusted towards the lower end of the SDI. If protein intake is excessive, patients may experience increased accumulation of nitrogenous waste products and increased symptoms of uremia. For children following a lacto-ovo vegetarian or vegan diet, it is recommended that the SDI be increased by a factor of 1.2 (lacto-ovo vegetarian) to 1.3 (vegan) to compensate for the lower bioavailability of non-animal protein. The restriction of dietary protein in the early stages of CKD is not recommended as it may increase the risk of malnutrition, protein-energy wasting and poor growth. A high intake of protein may also be detrimental to health as it may negatively impact acid-base balance and urea levels as well as increase phosphorus intake [3]. During the immediate post-transplant period, protein needs are increased by approximately 50% in association with surgical stress and the catabolic effects of

steroids. Needs are decreased back to normal recommendations approximately 3 months after transplantation [1].

Modifying Dietary Protein Intake

Despite anorexia and decreased appetite, voluntary protein intake usually exceeds recommendations, which can be acceptable as long as serum urea and phosphate levels are within acceptable limits. Occasionally, protein intake may be inadequate as a result of anorexia, oral-motor problems, low meat intake, or a low phosphorus diet that limits protein-rich dairy foods. Persistently low urea levels (i.e., <50 mg/dL or <18 mmol/L) may be a sign of overall inadequate protein and caloric intake in children receiving dialysis. KDOQI guidelines recommend the use of protein supplements to augment poor oral and/or enteral protein intake [1]. Powdered protein modules (see Table 58.2) can be added to expressed breast milk, infant formula, beverages, pureed foods, cereals, or other moist foods to boost their protein content. Minced or chopped meat, chicken, fish, egg, tofu, or skim milk powder can be added to soups, pasta, or casseroles. High levels of phosphorus often accompany high protein diets and this should be considered when making efforts to increase dietary protein content. Renal nutrition supplements that are rich in protein can also be given orally or enterally to increase protein intake.

Carbohydrates

Prescribed oral, enteral and/or parenteral diets for children with CKD should include a balance of calories from carbohydrates and unsaturated fats within the physiological ranges recommended as the acceptable macronutrient distribution ranges of the DRI [5]. Recommended carbohydrate intake should account for 45–65% of total daily caloric intake, with less than 10% coming from added sugars. In addition, children on PD may receive up to an additional 8 ± 2.8 calories/kg/day from absorption of intraperitoneal dextrose [40, 41]. Diets with excess simple carbohydrates or fats may lead to potential increased risk of chronic diseases such as coronary heart disease, obesity and diabetes. Cardiovascular disease (CVD) is the leading cause of morbidity and death in children with CKD. After transplantation, glucocorticosteroids and immunosuppressive agents such as tacrolimus may cause impaired glucose tolerance, glycosuria, and a relative resistance to insulin that may lead to diabetes. The management of children who have or who develop diabetes should follow the recommendations of the American Diabetes Association, including the avoidance of simple carbohydrates, weight control, and physical activity [42].

Table 58.2 Examples of modular protein additives

Beneprotein powder	Nestle nutrition	Contains 6 g of protein, 25 kcal per 7 g scoop
Complete amino acid mix	Nutricia	Contains 7.8 g protein, 31 kcal per TBSP
Liquid protein fortifier	Abbott nutrition	Contains 1 g protein, 4 kcal per 6 mL

Fiber

Obtaining an adequate intake of dietary fiber has traditionally been difficult for patients following a renal diet, but with a shift towards recommending fewer processed and packaged foods, and more whole and plant-based foods, it is easier to include fiber in the diet of children with CKD. Among the benefits of consuming adequate dietary fiber are the reduction of constipation and improvement of hypercholesterolemia with a reduction in total and LDL cholesterol levels as well as a decrease in overall risk of CVD. Recent literature has promoted the benefits of dietary fiber in improving the composition and metabolism of gut microbiota, which has been linked in adults with reducing obesity, diabetes and dyslipidemia [43]. An increased intake of high fiber foods may require an increase in the use of phosphate or potassium binders or an adjustment of the potassium content of the dialysate, but it is important to avoid restricting fiber for the sole purpose of controlling labs. For children who have difficulty meeting their fiber needs from food, fiber supplements can be used to increase fiber intake; however, care should be

taken to ensure that these supplements do not contain potassium. For patients following a fluid restriction, fiber intake may need to be limited to prevent the hardening of stools and subsequent constipation.

Fat

Dietary fat is an important source of calories for growing children, but high fat diets are also associated with an increased risk of CVD events, a major contributor to morbidity and mortality in patients with kidney disease. The KDOQI guidelines recommended that fat intake account for 25–35% of caloric intake in children above age 4, with slightly higher intake of 30–40% in younger children. [1] Less than 10% of fat intake should come from saturated fats. In the CKiD cohort, while overall energy intake was higher than recommended, the median percentage of caloric intake from fat was within this range [19]. Supplemental fats can be used for children with poor weight gain and fat intake can be safely restricted to the lower end of this range in circumstances of excessive weight gain of dyslipidemia.

CVD is the leading cause of death in adults with childhood onset CKD [44, 45] and dyslipidemia is one of many factors that increase the risk of CVD. Lipid metabolism is disrupted in CKD, with up to 45% of children with in the CKiD cohort having dyslipidemia and dyslipidemia is more prevalent and more severe in children with lower glomerular filtration rate (GFR) and nephrotic range proteinuria [46]. Dyslipidemia in children with CKD has not been directly associated with increased CVD events; however, higher triglyceride levels and lower LDL have been associated with increased carotid artery intima-media thickness and reduced brachial artery flow-mediated dilation, both of which are associated with atherosclerosis [47]. There is consensus that lipid profiles should be monitored annually in children with CKD and that diet and lifestyle modification is warranted for children with dyslipidemia. These modifications include increased physical activity to achieve 5 h of moderate to vigorous physical activity and increasing dietary fiber, fruit, and vegetable intake while decreasing intake of saturated and trans fats. Increased intake of monounsaturated and polyunsaturated fatty acids (e.g. oils from canola, corn, flaxseed, safflower, soy, olives, and peanuts) should be encouraged as total and LDL cholesterol are decreased with increased intake.

Because CVD events are rare, even in high-risk children, there have not been sufficient data to show a decrease in CVD risk with statin therapy in children. However, while KDOQI/KDIGO guidelines do not recommend lipid lowering pharmacotherapy for children with CKD [48], the American Heart Association (AHA) [49] recommends that children with increased risk of CVD have target triglyceride level <150 mg/dL and LDL <100 mg/dL for highest risk, which includes end-stage renal disease (ESRD), and <130 mg/dL for moderate risk, which includes CKD. The AHA recommends initiation of lipid lowering therapy if these targets cannot be achieved with 3 months of diet and lifestyle modification.

Consumption of omega-3 fatty acids (ALA, EPA, and DHA) can have beneficial effects on cardiovascular risk factors including inflammation, thrombosis, triglycerides, vascular and cardiac hemodynamics and arrhythmias [50]. Omega-3 fatty acids can be found in fatty fish such as tuna mackerel, trout, salmon, herring, sardines, and anchovies. Adequate intake of omega-3 fatty acids is 0.5 gm for children less than 1 year and rises to 1.6 g for males and 1.1 gm for non-pregnant females by age 14. Most individuals meet this level of intake through dietary ingestion, and there has not been sufficient evidence to recommend supplementation in treatment of hypertriglyceridemia.

Omega-3 fatty acid supplementation has been shown to result in modest improvement in lipid profile in children on dialysis, but there has not been convincing evidence that supplementation mitigates CV risk in adults or children with CKD [51, 52]. A recent meta-analysis of studies evaluating the effects of omega-3 polyunsaturated fats in adult patients with CKD showed a modestly reduced risk of CV events in patients on dialysis, but no benefit in regards to reduction of CV events, progression to ESRD, mortality, or graft rejection in patients with pre-dialysis CKD [53].

Potassium

The PRNT recently provided updated recommendations for management of potassium intake in children with CKD 2–5 [54], recognizing that there are limited data to support many of the recommendations. Maintenance of normal serum potassium levels depends on potassium intake, urinary potassium excretion and transcellular shifts of potassium, which can happen rapidly in response to increased potassium intake, acid-base disturbances, and insulin. Because 98% of total body potassium is intracellular, even small transcellular shifts can result in significant changes in serum potassium levels. Dyskalemias are associated with cardiac arrhythmias and muscle dysfunction and can be fatal if severe and not treated emergently. Urinary potassium excretion decreases as GFR decreases, and with the use of medications such as calcineurin inhibitors and renin-angiotensin-aldosterone inhibitors. Daily dialysis (either HD or PD) will typically provide sufficient potassium clearance to permit a diet without potassium restriction; however, children with more standard HD prescriptions or with CKD 4–5 not yet on dialysis often require dietary potassium restriction. High dietary potassium has been associated with increased mortality in adult patients on dialysis [55]. Recommendations for dietary potassium intake should target normal serum potassium levels and must account for all of these contributing factors. The KDIGO recommended potassium restriction is 30–40 mg/kg/day for older children and 40–120 mg/kg/day for younger children, but there are minimal data to support this recommendation [1].

Sources of dietary potassium intake can be difficult to identify on dietary history and bioavailability of ingested potassium varies widely. Most dietary potassium comes from breast milk, infant formula, milk, potatoes, tomatoes and other fruits and vegetables. Providers must also ask about salt substitutes, which are commonly used to avoid high sodium intake. A food is considered to have high potassium content if it contains >200 mg/serving. Restricting high potassium foods or soaking high potassium foods and rinsing them prior to ingestion reduces potassium intake. For infants or children on enteral formula, specific renal formulas with low potassium formulas can substitute for standard formula. In older children, high potassium foods such as oranges, bananas, potatoes and potato products, tomatoes and tomato products, legumes, lentils, yogurt and chocolate should be limited. Additional carbohydrate or fat additive may be required to achieve adequate caloric intake while limiting potassium intake [21]. If dietary restriction is insufficient to control elevated serum potassium levels, potassium binders such as sodium polystyrene sulfonate or patiromer can be used to prevent hyperkalemia; however, both binders have gastrointestinal side effects that must be monitored. These binders can be given orally or enterally. Alternately, fluids such as nutritional supplements and formulas can be pre-treated with the binder to reduce their potassium content. The insoluble resin is allowed to sit in the formula for 30 min and potassium is exchanged for sodium (SPS) or calcium (patiromer). The potassium-depleted formula can then be decanted from the sediment at the bottom of the container. Along with the decrease in potassium content, the decanted portion of formula may also affect concentration of other electrolytes and minerals [56, 57].

Hypokalemia occurs in children who have excessive potassium losses due to frequent dialysis or high urinary potassium excretion due to renal tubular dysfunction. Severe hypokalemia requires emergent intravenous (IV) supplementation to avoid cardiac arrhythmia. Dietary increases in potassium intake and cessation of binders is usually sufficient to correct the hypokalemia chronically.

Sodium

Recommendations for sodium intake depend on the specific cause and severity of CKD. Serum sodium cannot be used to differentiate between sodium excess and sodium depletion because it reflects water balance rather than total body sodium. For children with salt-wasting syndromes and associated polyuria, including obstructive uropathy, renal dysplasia, tubular disease, and polycystic kidney disease, urinary sodium losses commonly exceed dietary sodium intake, result-

ing in need for sodium supplementation. Conversely, for children with glomerular disease or oliguria/anuria, high sodium intake is associated with fluid overload and hypertension.

Salt restriction in adults with CKD is associated with lower blood pressure and proteinuria, both risk factors for CKD progression and CV mortality, but studies evaluating the relationship between sodium intake and those outcomes have been variable [58]. For children who are pre-hypertensive, hypertensive or edematous, restriction of daily sodium intake to 1500 mg/day for children aged 2–3 years, 1900 mg/day for children aged 4–8 years, 2200 mg/day for children aged 9–13 years and 2300 mg/day for adolescents aged ≥14 years is recommended [1]. This can be difficult to achieve, but families can start by limiting processed foods, canned foods, and fast foods, which are all high in sodium. Families should be taught to read nutritional labels and strategies to increase consumption of fresh foods that are flavorful and appealing to the child.

For children with excessive sodium losses in the urine or through PD, sodium supplementation is required to prevent sodium depletion, volume depletion, and impaired growth. Supplementation should begin with a goal of providing at least the age related DRI of sodium and chloride and can be adjusted as needed [1]. Supplements can be given separately or added to breast milk or infant formula, provided that the infant reliably receives the full volume of breast milk of formula. As infants transition to table food, dietary sodium intake increases and adjustments to sodium chloride supplementation should be made accordingly.

For patients with calcium-based kidney stone formation, decreased sodium intake has been shown to decrease urinary calcium excretion, but has not definitively been proven to reduce the risk of calcium-based stones [59].

Salt and Sugar in Hypertension

Regulation of total body sodium content is an important homeostatic mechanism for maintaining normal blood pressure—as blood pressure rises, urinary sodium excretion increases. The underlying mechanisms resulting in the presumed causal relationship between higher dietary sodium intake and higher blood pressure have not been fully described. In studies in adults, lower dietary sodium intake is associated with lower blood pressure and lower CVD risk. Higher sodium intake is associated with higher systolic blood pressure and risk for pre-hypertension and hypertension in U.S. children and adolescents [60] and reduction in sodium intake over time was temporally associated with a decreased incidence of hypertension among children participating in the NHANES study [61]. A meta-analysis of studies assessing the effect of sodium restriction for 2 weeks or longer on children with elevated blood pressure showed reduction in blood pressure on a lower sodium diet [62]. Based on these data and consistent data from adult studies [63] indicating improved outcomes at lower sodium intake, the CDC recommends that dietary intake of sodium should be restricted to 2300 mg for adults and 1500 mg for children with hypertension. Epidemiologic studies have linked fructose intake with hypertension and elevated uric acid [64]. Reduction in sugar-added beverages consumption is strongly associated with reduction in blood pressure [65]. Indeed, an important component of the DASH (dietary approaches to stop hypertension) diet is limitation of added sugar (fructose).

Fluid Intake

Similar to sodium intake, recommendations for target daily fluid intake should be adjusted based on the child's clinical condition, taking into account the underlying cause of CKD, the volume of urine output, the GFR, and the presence of edema and hypertension. Infants and children with polyuria due to nephrogenic diabetes insipidus or salt losing nephropathy, typically have high obligatory fluid output and require high fluid intake to prevent chronic dehydration, malaise, and poor growth. High fluid intake may also be recommended for children with nephrolithiasis or urinary tract infections to prevent recurrence. There is currently a study underway to evaluate the potential benefit of prescribed increase in fluid intake on progression of renal disease in adult patients with autosomal dominant polycystic kid-

ney disease [66]. After kidney transplantation, high fluid intake is prescribed to maintain adequate perfusion of the transplanted kidney, to replace high urine volume, and regulate intravascular volume. For all of these conditions, water and calorie free fluids are encouraged to prevent excessive weight gain and hyperglycemia.

Once CKD progresses to oliguria or anuria, fluid limitation is often required to avoid fluid overload, hypertension, and subsequent cardiac, cerebrovascular, renal and respiratory complications. Children on PD may not need fluid restriction because higher glucose concentration dialysates can increase ultrafiltration to maintain euvolemia. Fluid limits are more often needed for patients on HD to limit interdialytic fluid gain to less than 5% of dry weight between dialysis sessions. The prescribed total fluid intake is based on insensible fluid losses, urine output, ultrafiltration capacity, other losses, and current amount of fluid overload. Insensible water losses for children and adolescents are 20 mL/kg/day or 400 mL/m^2 with maximum estimates of 800 mL/day in adults [1], and can be 1.5–2 times higher in neonates and preterm infants. In anuric children, fluid intake above this threshold can lead to hypervolemia. Higher interdialytic weight gain is associated with higher blood pressure and left ventricular mass index in children on dialysis [67] and higher ultrafiltration rates is associated with increased mortality for adults on HD [68]. These data emphasize the importance of fluid restriction between dialysis treatments.

Limiting fluid intake can be difficult for children and their families and requires careful education about reducing sodium intake and about the water content of many fruits and vegetables.

Calcium and Phosphorous

Calcium and Phosphorous Intake

Appropriate calcium and phosphorous management is critical to maintaining normal bone health and growth while reducing the risk of extraosseous calcification in the kidneys and vascular system. Elevated serum phosphorus levels occur early in CKD and can lead to elevated levels of parathyroid hormone (PTH) and FGF23, which are associated with high bone turnover calcium deposition in organs and arteries. The PNRT and KDOQI recommend daily intake of calcium and phosphorous within age adjusted ranges for healthy children, with a goal of maintaining normal serum calcium and phosphorous levels [68]. Periodic food frequency questionnaires and/or a 3-day food diary can be used to assess dietary phosphorous intake in children with CKD. Absorption of calcium and phosphate varies by food source and vitamin D status.

Calcium

Children have higher calcium requirements than adults due to increasing skeletal mass, particularly during the periods of most rapid growth including infancy and puberty. Suggested dietary intake of calcium, including from dietary and calcium based phosphate binders, should be 1–2 times the standard daily intaking, ranging from 220 mg/day in infancy to 1300 mg/day in adolescence [69]. Most dietary calcium comes from breast milk, formula, cow's milk, other dairy products and cereals. Absorption of calcium is affected by bioavailability. Abnormal bone mineralization is seen at all stages of CKD, with increasing frequency at lower GFR [70, 71]. Increased calcium intake in childhood is associated with increased bone density [72–74] and phosphate binder usage (primarily calcium containing), was found to be protective for incident fracture in the CKiD cohort [71].

Patients with insufficient calcium intake should be encouraged to consume food with high endogenous calcium content (such as dairy products) as well as calcium-fortified foods. This must be balanced with the goal of limiting phosphorous intake, which is also high in many dairy products. If dietary intake alone cannot meet the SDI, calcium supplementation should be recommended, and should be taken between meals to maximize absorption. Salts of calcium such as calcium gluconate, lactate, acetate, or carbonate are usually well tolerated by children and can be given in doses of less than 500 mg at a time to optimize absorption. Calcium chloride should not be used to supplement patients with CKD as it may cause metabolic acidosis, and calcium

citrate should be avoided due to concerns for increased aluminum absorption [1].

Serum calcium should be maintained in the normal range for age. When hypocalcemia is present, vitamin D supplementation should also be considered to increase intestinal calcium absorption, as low vitamin D levels impair calcium absorption [75]. Patients with hypocalcemia and persistently elevated PTH levels may require calcium intake above the 200% SDI upper limit, and this should be monitored closely to avoid hypercalcemia. Hypercalcemia should also be avoided as it can lead to extraosseous calcification. In general, mild to moderate hypercalcemia can be treated with serial reduction in calcium supplementation, calcium-based phosphate binders, and vitamin D supplements. A variety of calcium-free phosphorous binders (i.e. sevelamer) are available as substitutes. In addition, synthetic vitamin D analogues such as paricalcitol and doxercalciferol may have less hypercalcemic effect. For patients on HD or PD, dialysate calcium content can be decreased.

After transplantation, glucocorticoid therapy can induce osteoporosis; therefore, calcium supplementation may be needed for children who are unable to meet the SDI for calcium through dietary intake alone.

Phosphorous

Elevated serum phosphorous levels occur relatively early in CKD progression due to decreased urinary phosphorous excretion. This stimulates higher levels of the phosphaturic hormones PTH and FGF-23, which results in high bone turnover with bone calcium loss. This leads to calcium deposition in organs and small vessels.

Targeting a normal serum phosphorous for age and decreasing dietary phosphorous intake, even prior to onset of hyperphosphatemia, can help prevent both elevated serum phosphorous and PTH levels. Dietary phosphorous intake should be limited to the SDI for age starting early in CKD, with more strict limitations to the lower limit of SDI as CKD progresses, or in the setting of ongoing hyperphosphatemia or hyperparathyroidism [69]. For infants and formula fed children, whey based infant formulas and specific renal formulas with low phosphorous content can be used to reduce phosphorous intake. For children who are not formula fed, attention to sources of dietary phosphorous is particularly important, as intestinal phosphate absorption is affected by the source of phosphorous. Inorganic dietary phosphorous, found in processed foods, has much higher absorption (up to 100%) compared to phosphorous found in meats, fish, dairy, vegetables, and other plant-based foods.

For many children, dietary restriction is insufficient to control serum phosphate levels, and mealtime phosphate binders are required to impair intestinal phosphorous absorption to achieve normal serum phosphorous levels. Calcium-based binders (calcium carbonate and calcium acetate) are preferred in infants and young children due to their higher calcium requirements. Non-calcium based binders (e.g. sevelamer carbonate) can be added if calcium based binders are insufficient to achieve normal phosphorous levels. Sevelamer carbonate may also be used to pre-treat breast milk, infant formula or cow's milk in order to reduce phosphate content [76].

Despite the importance of normalization of serum phosphorous, achieving control of serum phosphorous levels is particularly challenging. For example, the majority of subjects in the CKiD study ingest more phosphorous than recommended, especially in the younger age group [19].

For children with renal tubular disorders and associated urinary phosphorous wasting or those with intensified dialysis regimens resulting in hypophosphatemia, phosphorous supplementation is often required as hypophosphatemia is associated with morbidity, mortality, and poor growth.

Acid Load

Progressive renal dysfunction resulting in decreased urinary ammonia excretion despite stable acid generation leads to increased prevalence of acidosis in children with impaired renal function. In the CkiD cohort, 25% of children with CKD had serum bicarbonate <22 mEq/L [77]. Children with tubular dysfunction from dysplasia or inherited tubular defects are at higher

risk for acidosis. In the 4C cohort, 43–60% of children had serum bicarbonate <22 mmol/L [78]. Normalization of acid-base status in children with glomerular disease has been associated with reduced progression of kidney disease [79]. While dietary acid load is more difficult to quantify, increased urinary acid excretion, a correlate of dietary acid intake, has been associated with CKD progression and hypertension in small pediatric studies [80, 81]. Reducing dietary acid load by increasing intake of fruits and vegetables may improve acidosis, minimize requirement for alkali supplementation, and slow kidney disease progression.

Vitamins, Minerals and Trace Elements

Children with CKD and ESRD are at increased risk for vitamin and mineral deficiencies due to multiple nutritional factors, including anorexia, poor intake, dietary restrictions, abnormal renal metabolism, drug-nutrient interactions, and poor intestinal absorption. In addition, there are concerns about potential losses of water-soluble vitamins during dialysis. Thiamine (B1), pyridoxine (B6), folate (B9), and vitamin C are all small to middle-sized molecules that are easily cleared on HD. PD is thought to result in less diffusive clearance of these molecules, but higher protein losses may result in greater losses of protein bound vitamins and trace elements [82]. A recent study of adults on thrice weekly HD and typical doses of vitamin B and C supplementation (given after dialysis), showed that almost all patients had high vitamin B levels at baseline, while over 80% of patients had normal vitamin C levels. When the dose of supplementation was reduced by 50%, vitamin B levels generally stayed normal; however, there were significant losses of B vitamins into the dialysate effluent [83]. While vitamin C clearance during dialysis is hypothesized, no difference in vitamin C levels over time were seen in adult patients receiving three times weekly versus six times weekly in the Frequent Hemodialysis Network Study [84]. Children with protein energy malnutrition may be particularly susceptible to thiamine deficiency because it is extensively protein bound.

Current practice, consistent with KDOQI guidelines, is to provide vitamin and trace element supplementation for children on dialysis to achieve DRI for healthy children, with the goal of avoiding complications of vitamin insufficiencies. There are minimal pediatric data available to support this recommendation [82]. Thiamine deficiency is rare in children, but can lead to severe lactic acidosis and neurologic complications in the acute phase; therefore, supplementation to avoid deficiency is recommended [82]. Folate (B9), pyridoxine (B6), and cobalamin (B12) supplementation may mitigate hyperhomocysteinemia, which potentially reduces cardiovascular risk [85]. In addition, folate deficiency can result in erythropoietin resistant anemia that can be corrected with supplementation [86].

Vitamin C is thought to be low in patients with CKD due to decreased intake to accommodate low potassium and low oxalate diets, low dose vitamin C supplementation due to concern for conversion to oxalate, and clearance of vitamin C during dialysis. Scurvy has been reported in a pediatric PD patient.

There are no pediatric renal vitamins available in the US. Adult B and C vitamin formulations can be used in older children and adolescent patients. Younger children can be prescribed liquid adult vitamins or can be offered split doses of adult renal vitamins or every other day dosing. These preparations contain no more that 6–100 mg of vitamin C to avoid oxalate retention.

Trace Elements

Trace elements are important components of many enzymatic pathways and proteins. Deficiencies of these micronutrients may have major adverse effects in growing children. Dialysis can lead to the accumulation of trace elements either because of insufficient elimination or because of contamination. It can alternately lead to excessive removal of trace elements. Zinc and selenium contents vary by formula type and, for breast-fed infants, are largely determined by maternal diet [82].

Low dietary intake of copper has been noted in children receiving dialysis and the KDOQI

guidelines recommend monitoring intake every 4–6 months [1]. Copper deficiency can cause anemia and neutropenia. Clinical signs of copper overload should also be assessed, as excess copper is associated with CKD [1].

Selenium acts as a cofactor for oxidation and reduction enzymes such as glutathione peroxidase. Low serum selenium levels have been found in patients with CKD and those receiving dialysis, although it is not removed by dialysis. Selenium depletion appears most likely in critically ill patients on continuous renal replacement therapy.

Zinc deficiency is relatively common in adult dialysis patients, resulting from poor dietary intake and removal by HD because it is not protein bound [82]. Zinc deficiency can present as failure to thrive, dermatitis, and inflammatory disease in children. Monitoring of trace elements in dialysis may be warranted, but there are no specific recommendations for children.

Supplemental oral or IV iron is usually required by all children on erythropoietin-stimulating agents to avoid depletion of iron stores and to maintain target hemoglobin concentrations. Current KDIGO guidelines recommend iron administration during erythropoietin therapy to maintain transferrin saturation greater than 20% and serum ferritin greater than 100 ng/mL to ensure adequate iron stores [87]. Iron stores in patients with kidney disease may be depleted by frequent blood draws and chronic blood loss in the gastrointestinal tract and HD circuit. In addition, iron metabolism is disrupted in patients with advanced CKD and other chronic inflammatory conditions due to increased levels of the regulatory protein hepcidin. Hepcidin prevents duodenal iron absorption and movement of iron into the circulation, making iron unavailable for erythropoiesis. This is thought to explain resistance to enteral iron supplementation for patients with CKD.

Enteral or parenteral iron supplementation are options. Oral supplementation at a dose of 3–6 mg/kg of elemental iron daily is often successful, particularly for patients with lower serum ferritin levels and who are earlier in their CKD course. For patients with significant iron losses on dialysis and with hepcidin mediated iron deficiency (i.e. those with normal or high ferritin levels), IV iron supplementation is preferred. There are several formulations of intravenous iron available, with the more recently developed formulations less likely to result in hypotension [88]. IV iron can be given as a loading dose with serial infusions or as maintenance therapy on a weekly to monthly basis depending on patient need. There are limited data on the use of IV iron in children with CKD and ESRD. Studies including adults on HD, demonstrated higher dose IV iron supplementation was non-inferior, with a statistical trend towards superiority for a composite endpoint of mortality and cardiovascular morbidity ascribed to lower exogenous erythropoietin dose [89]. In this population, higher dose IV iron supplementation was also found to be safe, without an increased risk of infection [90].

Vitamin D

Vitamin D is obtained from the diet (D2, ergocalciferol) and exposure to ultraviolet light (D3, cholecalciferol). The liver hydroxylates the vitamin D precursors to form 25-hydroxy vitamin D (25[OH]D). A second hydroxylation occurs in the kidney, leading to the formation of 1,25 dihydroxyvitamin D (1,25(OH)2D), calcitriol). Calcitriol regulates intestinal calcium absorption, bone resorption and renal excretion of phosphate and calcium. As GFR declines, plasma concentrations of calcitriol decrease. This leads to a decrease in intestinal calcium absorption and reduced renal phosphate excretion. The result is hypocalcemia and hyperphosphatemia, which stimulate PTH and FGF-23 secretion, leading to mineral bone disorder.

Clinical practice guidelines focused on optimizing bone health in children with CKD recommend defining sufficient 25[OH]D levels as >75 nmol/L (>30 ng/mL) and supplementation with vitamin D (cholecalciferol or ergocalciferol) for children with CKD who have levels below this threshold [90]. This is based on data demonstrating that children with CKD who were treated with ergocalciferol have longer time prior to developing hyperparathyroidism [91]. In addition, Stein et al. [92] reported 100 pediatric CKD patients and

found a high prevalence of hyperparathyroidism early in CKD which was associated with lower 25[OH]D levels, independent of calcitriol level. There may also be a relationship between vitamin D deficiency in CKD and CVD as measured by arterial stiffness and LVMI [93, 94].

Low 25[OH]D levels have been documented in patients with nephrotic syndrome and can be at least partially attributed to loss of vitamin D binding proteins in the urine. Supplementation with vitamin D3 in 43 children with nephrotic syndrome showed improved 25[OH]D vitamin D level. There was no change in bone mineral content or bone mineral density at 6 months [95]. There is a risk of nephrocalcinosis and urolithiasis with excess vitamin D supplementation, so levels should be monitored periodically.

For children with PTH elevated above target range for CKD stage, treatment with vitamin D analogs such as calcitriol [96] reduce PTH levels; however, the effect on bone outcomes including, fracture and growth is less clear. Daily calcitriol, at the lowest effective dose, with monitoring for hypercalcemia, is recommended.

Other Fat-Soluble Vitamins

Vitamin A is best known for its effects on vision. Vitamin A toxicity can cause dry skin, pruritus, weight loss, anorexia, taste disturbances, bone and joint pain, hypercalcemia, and neuropsychiatric symptoms [97]. Vitamin A is not removed by dialysis and patients with CKD and ESRD have been found to exhibit elevated serum levels without supplementation [1]. The total intake for vitamin A should be limited to the DRI and supplementation should only be used in patients with very low dietary intake. Vitamin K and E, the other primary fat soluble vitamins, are not removed by dialysis and intake should be limited to the DRI to avoid toxicity, which is less common for these fat soluble vitamins than for vitamin A.

Transplantation

When caring for children after kidney transplantation, it is important to view the post-transplant period as a dynamic phase of CKD. Factors such as electrolyte balance, linear growth, and weight gain remain important components of nutrition assessment. The primary goal of medical nutrition therapy post kidney transplant is to promote optimal nutrition status while helping patients offset or minimize the side effects of immunosuppressive medications. These side effects may include protein catabolism, hypertension, hyperglycemia, hyperkalemia, dyslipidemias, hypophosphatemia, hypomagnesemia, osteoporosis, increased infection risk and gastrointestinal disturbances. Patients should be closely monitored for changes in kidney function resulting from recurrence of disease, rejection episodes, lapses in taking medication, and gradual decline in graft function.

Immediately post-transplant, patients may require restrictions of sodium, potassium, phosphorus and/or fluid until full graft function is established. Assuming the kidney is functioning well, a balanced, heart healthy diet is recommended. Intake of saturated, trans-fats and sodium should be limited, although some toddlers may require sodium supplementation. For children receiving tube feeds, formula can typically be changed to a standard pediatric variety. Energy needs should be established based on age and gender, starting at 100% estimated energy requirement and adjusting for activity and body size [1]. Protein needs are increased for the first 3 months post-transplant to allow for healing of the surgical site. Subsequently, needs should be based on DRI or recommended daily allowance for age and gender.

Hydration is critical after transplant to ensure adequate perfusion of the graft. For infants and toddlers, 2500 mL/ 1.0 m^2 body surface area is recommended. For older children and adolescents, 1.5–4 L/day may be required [98]. Sugar-free, caffeine-free beverages should be encouraged. It is important to educate patients and families on strategies for ensuring that fluid goals are met. Cell phone apps, water bottles, watch alarms or incentive charts may all be helpful in reminding patients to drink throughout the day. Tube fed patients who are able to transition successfully to taking nutrition orally may continue to require the g-tube in order to meet fluid goals.

Children will benefit from a nutrition assessment to evaluate if their diets are balanced. Children who lack variety in their diets should be encouraged to take a daily multivitamin. Phosphorus and magnesium supplementation are often required immediately post-transplant as these minerals are often wasted in the urine. Calcium intake should be evaluated, and supplements should be encouraged if dietary intake is poor, particularly for children taking corticosteroids as part of their immunosuppressive protocol. Vitamin D levels should be evaluated routinely as supplementation should be provided if insufficiency or deficiency is present.

Weight management can be particularly difficult for children who are taking steroids as part of their post-transplant immunosuppressive regimen. The steroid-induced increase in appetite along with the liberalization of diet restrictions that may have been present prior to transplant can lead to rapid weight gain and obesity. Education on diet and lifestyle modification should be provided and repeated in order to help prevent this outcome.

Food safety and hygiene are important in order to prevent food borne illness in this immunosuppressed population. Families should be specifically instructed about washing hands and food preparation surfaces, separating foods to avoid cross-contamination, prompt refrigeration of foods and heating foods to the proper temperatures to kill pathogens. Additionally, foods such as grapefruit and pomegranate should be avoided because they can alter the absorption of certain medications, including some immunosuppressants and calcium-channel blockers.

Potential side effects of common transplant medications	Nutrition therapy
HTN/edema	Low-sodium diet
Hypotension	Encourage increased fluid intake, sodium if appropriate
Hyperkalemia/ hypomagnesemia/ hypophosphatemia	Adjust mineral intake with diet and/or with supplementation
Hyperglycemia	Carbohydrate control/ Insulin
Diarrhea/constipation	Add fiber/fluid/physical activity Polyethylene glycol/Docusate Sodium prescribed when needed
Nausea/vomiting	Small frequent meals/ medication if needed
Anemia	Supplement with iron
Growth suppression	Ensure adequate intake of protein, calories Growth hormone may be warranted but is typically note used within the first year post transplant
Osteoporosis	Calcium/Vitamin D, weight bearing exercise
Increased appetite	Calorie control, exercise
Hyperlipidemia	Low fat diet, exercise, Omega-3 supplementation
Anorexia	Small frequent meals

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Abbreviations

CKD	Chronic kidney disease
CRP	C-reactive protein
DA	Darbepoetin alpha
ESA	Erythropoiesis stimulating agent
FDA	Food and Drug Administration
GFR	Glomerular filtration rate
Hb	Hemoglobin
HD	Hemodialysis
HIF	Hypoxia inducible factor
IL-6	Interleukin-6
IV	Intravenous
KDIGO	Kidney Disease: Improving Global Outcomes
Kg	Kilogram
LVH	Left ventricular hypertrophy
MCV	Mean corpuscular volume
PD	Peritoneal dialysis
PEG	Polyethylene glycol
PHD	Propyl hydroxylase domain
PHIs	Prolyl hydroxylase inhibitors
PTH	Parathyroid hormone
rHuEPO	Recombinant human erythropoietin
SLE	Systemic lupus erythematosus
TIBC	Total iron binding capacity
TSAT	Transferrin saturation

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Introduction

Anemia is one of the most common problems in children with chronic kidney disease (CKD); it is almost universal in children with stage 5 CKD. The development of recombinant human erythropoietin (rHuEPO) revolutionized the treatment of anemia in CKD, but anemia management remains challenging. Many management issues remain uncertain, including the ideal target hemoglobin (Hb). There are guidelines for the management of anemia in CKD [1–3].

Pathophysiology of Anemia

A variety of factors contribute to anemia in CKD (Table 59.1). The principal etiology is decreased production of EPO by the kidneys. However, many children are still anemic despite administration of erythropoiesis stimulating agents (ESAs) [4, 5], emphasizing the multifactorial etiology of anemia in children with CKD.

Erythropoietin Deficiency

The kidneys produce EPO and kidney damage leads to decreased EPO production. In children with CKD, EPO levels are inappropriately low for the degree of anemia [6]. The degree of EPO deficiency generally worsens as the glomerular

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Table 59.1 Causes of anemia in CKD

Erythropoietin deficiency.
Blood loss
Hemolysis
Bone marrow suppression
Iron deficiency
Inadequate dialysis
Malnutrition
Chronic or acute inflammation
Infection
Hyperparathyroidism
B12 or folate deficiency
Aluminum toxicity
Carnitine deficiency
Medications (e.g. ACE inhibitors)
Systemic disease
Hemoglobinopathy
Hypothyroidism
Systemic lupus erythematosus
Malignancy

ACE angiotensin-converting enzyme

filtration rate (GFR) decreases, but the level of GFR at which inadequate EPO causes anemia varies between patients, partially due to the nature of the underlying kidney disease [7]. On average, a GFR below 43 mL/min/1.73 m^2 is associated with a decline in Hb in children with CKD [8].

Blood Loss

Excessive blood loss may directly cause anemia, or it may lead to iron deficiency (see below). Causes of blood loss in children with CKD include phlebotomy, blood lost in the dialyzer and tubing during hemodialysis (HD) [9], gastrointestinal losses [9], and increased menstrual bleeding due to the acquired platelet function defect of CKD. Children receiving HD have increased intestinal blood loss when compared to other children with CKD [9] and have lower Hb values than children receiving peritoneal dialysis (PD) [5]. Increased blood loss is associated with rHuEPO resistance in pediatric HD patients [10].

Decreased Red Blood Cell Survival

Red blood cells in children with CKD have a decreased lifespan [9]. This may be partially due to carnitine deficiency (see below) [11], and a direct consequence of EPO deficiency, since red cell survival increases in CKD patients after starting rHuEPO [12]. Red blood cells in patients receiving HD have an increased osmotic fragility. Hemolytic anemia may occur due to a child's primary disease [e.g. systemic lupus erythematosus (SLE)].

Bone Marrow Suppression

In an in vitro assay, serum from children with CKD directly suppressed red blood cell production [6]. The specific inhibitory substances have not been identified, but dialysis appears to effectively remove some of these molecules, allowing for decreased doses of rHuEPO [13]. In a study of teenagers receiving HD, the children with Hb less than 11 g/dL had a slightly lower Kt/V_{urea} (1.46 v.s 1.53), but dialysis adequacy did not predict anemia in the multiple regression analysis, perhaps due to the high overall Kt/V in this patient population [14]. Severe bone marrow suppression may occur in children after renal transplantation due to medications [15] or infections, especially parvovirus B19 [16].

Iron Deficiency

Iron deficiency is a significant cause of anemia in patients with CKD; it is multifactorial (Table 59.2). In a study of older children, a serum transferrin saturation (TSAT) less than 20% was an independent predictor of anemia [14]. However, serum ferritin was not predictive of anemia, perhaps because ferritin is often elevated in CKD patients with concurrent inflammation, which may inhibit red cell synthesis (see below). This is supported by a study of children receiving PD where Hb was inversely related to serum ferritin [17].

Table 59.2 Causes of iron deficiency in children with CKD

Blood loss
Phlebotomy
Hemodialysis
Menses
Gastrointestinal
Surgical procedures
Dietary iron deficiency
Poor absorption of enteral iron
Inflammation
Medications (phosphate binders, gastric acid inhibitors)
Iron depletion during ESA therapy

ESA erythropoiesis-stimulating agent

Iron deficiency often develops after initiation of an ESA because the increase in red blood cell synthesis depletes iron stores. In some patients, there is a functional iron deficiency following ESA treatment; there are adequate supplies of iron, but the transfer of iron from ferritin is not fast enough to meet the high demand for red blood cell synthesis.

Inadequate Dialysis

In adults receiving dialysis, there is evidence that anemia is associated with inadequate dialysis. An increase in dialysis dose leads to an improvement in Hb. In addition, there is an inverse relationship between Kt/V and ESA dose. Resistance to ESAs was associated with a lower Kt/V in a study of pediatric HD patients [10]. Dialysis is effective at removing hepcidin (see below) [18], suggesting a possible mechanism for the relationship between dialysis dose and anemia.

Malnutrition

Malnutrition may be another factor contributing to anemia in CKD. In one pediatric study, low albumin was one predictor of anemia [14]. There are many possible explanations for the relationship between malnutrition and anemia. Generalized malnutrition may be a marker for nutritional iron deficiency or for deficiency of other nutrients that influence red cell production or survival. Another possible explanation for this observation is the relationship between markers of malnutrition and markers of inflammation [19]. As described below, inflammation is another mechanism of resistance to rHuEPO. It is possible that inflammation causes malnutrition, and this directly causes resistance to ESAs. An alternative explanation is that inflammation directly causes rHuEPO resistance, and that malnutrition is a surrogate marker of inflammation. A malnutrition inflammation score predicted ESA resistance in a study of pediatric HD patients [10].

Inflammation

Acute and chronic inflammation are well-known causes of decreased red blood cell synthesis. Inflammation is one of the mechanisms of the anemia of chronic disease and of the decreased erythropoiesis that occurs during infections. Markers of inflammation are commonly increased in CKD patients. There are a variety of putative mechanisms. Surgical procedures and acute infections are more common in CKD patients, especially those who are receiving dialysis or have a kidney transplant. The impaired immune system in uremia may lead to an increase in nonspecific inflammation [20]. CKD patients may have underlying systemic diseases, such as SLE or granulomatosis with polyangiitis. HD may induce inflammation via complement activation, direct activation of inflammatory cells by the dialysis membrane, and diffusion of endotoxin into the patient from the dialysate. Use of ultrapure dialysis in HD patients decreases inflammatory markers, increases Hb levels, and decreases ESA use [21].

Hepcidin is an important mediator of ESA resistance [22]. Hepcidin, which is produced in the liver, inhibits intestinal absorption of iron and release of iron stores from the reticuloendothelial system. This is accomplished through downregulation of ferroprotein, the principal trans-

membrane transporter of iron. Hepcidin is normally down-regulated in anemia, increasing absorption of iron and release of iron stores. In contrast, hepcidin increases when iron stores are adequate. Hepcidin is also up-regulated by inflammation. Interleukin 6 (IL-6) induces production of hepcidin and is risk factor for ESA resistance [23, 24]. Hepcidin's effect on release of iron stores explains inflammatory block, a condition where body stores of iron are adequate, but there is ineffective delivery of iron to the bone marrow. Findings in patients with inflammatory blockade may include elevated C-reactive protein (CRP) levels, resistance to ESAs, high serum ferritin levels, and low levels of serum iron and TSAT [25]. This mechanism is common to the anemia of many chronic diseases.

Hyperparathyroidism

Hyperparathyroidism may decrease bone marrow production of red blood cells [26] and rarely causes pancytopenia [27]. Elevated parathyroid hormone (PTH) levels are associated with ESA resistance in pediatric HD patients [10] and PTH is inversely related to Hb in pediatric dialysis patients [5]. Treatment of hyperparathyroidism via parathyroidectomy may lead to an increase in Hb.

B12 or Folate Deficiency

Patients with CKD may rarely develop a megaloblastic anemia due to folate or vitamin B12 deficiency. Poor nutritional intake combined with dialytic losses may predispose CKD patients to deficiencies of these water-soluble vitamins. There is some evidence that routine folate supplementation improves the response to rHuEPO, even in the absence of low serum levels of folic acid [28].

Aluminum Toxicity

Aluminum overload may cause a microcytic anemia in patients with CKD [29]. Currently, aluminum overload is an uncommon cause of anemia due to the recognition of the dangers of aluminum-containing phosphate binders.

Carnitine Deficiency

Carnitine deficiency may occur in CKD, principally due to removal of carnitine by dialysis, although decreased dietary intake and endogenous synthesis may also contribute [11]. Renal losses of carnitine are significant in children with Fanconi syndrome. Carnitine deficiency may decrease red blood cell survival by reducing the strength of the red cell membrane [11]. Intravenous (IV) carnitine may reduce rHuEPO dose requirements in adults receiving HD, but there is disagreement regarding the strength of the evidence; carnitine should not be used routinely in children, if at all, outside of a research setting [1, 11, 30–32]. Oral carnitine should not be used in patients receiving HD [11].

Medications

A variety of medications can inhibit erythropoiesis, especially certain medications used in renal transplant recipients [15, 33]. Angiotensin-converting enzyme inhibitors and angiotensin receptor blockers are especially pertinent in CKD patients because of their widespread use [34].

Summary

Erythropoietin deficiency and iron deficiency are the most common causes of anemia in children with CKD. Inflammation and hyperparathyroidism are important mediators of ESA resistance.

Epidemiology of Anemia in Pediatric CKD

Anemia is common in children with CKD, even in patients with stage 3 CKD [5, 35]. In one study, anemia was more common in children

greater than 2 years old, males, and patients receiving antihypertensive medications [35]. In another cohort, African American race was associated with an increased risk of anemia in children with CKD [4].

In children receiving PD, anemia is quite common and varies by region [17]. Risk factors for anemia include inflammation, decreased residual urine output and hyperparathyroidism [17].

Anemia is common in children after kidney transplantation [36]. The principal cause of anemia is allograft dysfunction, although current immunosuppressive medications appear to be responsible for the increased prevalence of anemia in these patients. Iron deficiency is common in children who are anemic after renal transplantation.

Clinical Effects of Anemia

There are a variety of clinical effects of anemia (Table 59.3). The clinical consequences of anemia in patients with CKD are difficult to discern because anemia identifies patients with co-morbidities such as inflammation and malnutrition. A variety of studies demonstrated an association between anemia and morbidity and mortality, but these conclusions may be biased by co-morbidities.

Studies in adult CKD patients have shown an association between anemia and increased mortality and hospitalization rates [37–40]. In a pediatric analysis, anemia 30 days after initiation of dialysis was associated with a significant increase in mortality and hospitalization rate [41]. In analysis of children receiving PD, anemia was associated with increased mortality, hypertension, and left ventricular hypertrophy (LVH) [17], suggesting that in patients with ESKD low Hb may partially reflect fluid overload ("dilutional anemia"). In children with CKD, anemia was associated with an increased risk of hospitalization [42].

In a group of children receiving dialysis, treatment of anemia with rHuEPO was associated with an elevated cardiac index in 6 months and a significant reduction in left ventricular mass index by 12 months [43]. In one study [44], children with severe LVH had significantly lower Hb values than children without LVH. However, anemia did not predict LVH in the final multiple regression model.

There is uncertainty regarding the direct deleterious consequences of a lower Hb in adults with CKD. These include observational studies that have corrected for a variety of co-morbidities [45]. More importantly, randomized studies using ESAs to target different Hb levels have failed to demonstrate a benefit of higher Hb targets on morbidity or mortality [46, 47]. In fact, a higher Hb target has resulted in a significant increase in morbidity and mortality in randomized studies [48, 49].

In randomized studies in adults, a higher Hb improves quality of life [47], although this is not a consistent finding [48, 50]. In a placebo-controlled trial, randomization to rHuEPO resulted in less fatigue, improved exercise tolerance and improved scores of physical symptoms and depression [51]. There is evidence of the deleterious effects of anemia on child development. There is an association in children with CKD between anemia and lower scores of health-related quality of life [52]. Studies of the effect of rHuEPO in children with CKD have shown improvement in quality of life, exercise tolerance, appetite, peak oxygen consumption and treadmill time during exercise testing, Wechsler intelligence score, and ventilatory aerobic threshold [53–55]. There does not appear to be a beneficial effect of anemia correction on the growth retardation associated with CKD [56].

Table 59.3 Clinical effects of anemia

Cardiovascular
Left ventricular hypertrophy
Systemic
Fatigue
Depression
Decreased quality of life
Sleep disturbances
Decreased exercise tolerance
Impaired cognitive function
Loss of appetite

Clinical Evaluation of Anemia

Initial Evaluation

Alternative causes of anemia should be evaluated prior to treating patients with an ESA (Table 59.4). The diagnosis of anemia in children should be based on age and gender specific normal ranges.

The initial evaluation of children with CKD and anemia should include a complete blood count, reticulocyte count, ferritin, iron, total iron binding capacity (TIBC) and a TSAT. The TSAT is calculated by dividing the serum iron by the TIBC. Measurement of EPO levels generally does not have clinical utility. A cost-effectiveness analysis in adults argues against routine screening for aluminum overload or deficiencies of folate or B12 [57]. EPO deficiency causes a normocytic anemia; macrocytosis or microcytosis should lead to consideration of other etiologies (Table 59.4).

Table 59.4 Indications for additional evaluation in children with chronic kidney disease and anemia

Indication	Response
Macrocytosis	Consider B12 or folate deficiency, unless due to brisk reticulocytosis
Decreased platelets and/or white blood cells	Consider malignancy, acute infection, SLE, severe hyperparathyroidism or medications
History of using aluminum-containing phosphate binders or other symptoms of aluminum overload	Consider aluminum toxicity
Anemia despite adequate reticulocytosis	Consider excessive blood loss or hemolysis
Microcytosis	Consider iron deficiency, hemoglobinopathy, or inflammation
Iron deficiency prior to starting an ESA	Evaluate for causes of iron deficiency (see Table 59.2)
Low reticulocyte count and falling hemoglobin in a patient being treated with an ESA	Consider non-adherence, anti-erythropoietin antibodies, parvovirus B19 infection

ESA human erythropoiesis stimulating agent, *SLE* systemic lupus erythematosus

A low mean corpuscular volume (MCV) occurs with iron deficiency, thalassemia and in up to 50% of patients with anemia of chronic disease. A high MCV suggests the possibility of B12 or folate deficiency. Measurement of serum levels of B12 and folate is indicated if there is an elevated MCV or if there is anemia without an alternative explanation. RBC folate is useful when a serum folate level is inconclusive or if recent intake of folate may lead to a falsely normal serum folate level.

Concomitant depression of white cells or platelets raises the specter of malignancy, although an isolated low white blood cell count may be due to a transient viral infection or a medication. SLE may cause depression of the white blood cell count, platelet count and an autoimmune Coombs positive hemolytic anemia. EPO deficiency causes an inappropriately low reticulocyte count, and the presence of an adequate reticulocytosis suggests alternative explanations, such as blood loss or hemolysis. An elevated CRP, indicating inflammation, may provide an explanation for anemia refractory to ESAs.

Iron deficiency is common in children with CKD, even prior to starting an ESA. There are a variety of explanations for iron deficiency in children with CKD (Table 59.2). All children with CKD should be queried about gastrointestinal blood loss and, when appropriate, menstrual losses. A more aggressive work-up (e.g. testing stool for occult blood or endoscopy) is appropriate in children with significant, unexplained iron deficiency prior to receiving ESA therapy. Along with low serum ferritin and TSAT, children with iron deficiency typically have a low MCV. Because it is a marker of inflammation, serum ferritin may be misleadingly normal in children with CKD despite iron deficiency.

Evaluation of the ferritin and TSAT establishes a baseline, since iron deficiency is likely to develop during ESA treatment. In addition, while all patients starting an ESA should receive oral iron supplementation unless iron overload is present, iron deficiency prior to starting ESA therapy may significantly attenuate the response to therapy. Such patients are candidates for IV iron.

Chronic Monitoring

Routine monitoring in children with anemia due to CKD includes periodic assessment of Hb, MCV, and iron stores. The development of macrocytosis in a patient after starting an ESA is usually due to the expected reticulocytosis; an increasing Hb, arguing against a nutritional deficiency anemia, supports this explanation. Iron overload may also cause an increased MCV [58]. Microcytosis is usually due to iron deficiency.

A decrease in Hb is expected during acute infections [59] or after surgical procedures [60]. ESA dose requirements increase following blood loss that causes a fall in Hb; this persists until the Hb returns to the target range. Depleted iron stores are the usual explanation for a poor response to ESA therapy. Some children have a functional iron deficiency, and may respond to IV iron, even though the ferritin levels are adequate. Additional evaluation is indicated in children who have an unexplained increase in ESA dose requirement, need unexpectedly large doses of ESA, or have a decreasing Hb (Table 59.4).

A reticulocyte count is the usual first step in evaluating unexplained anemia or an excessive ESA requirement. An appropriately elevated reticulocyte count (corrected for the degree of anemia) argues that the patient is anemic due to blood loss or hemolysis. Blood loss is also suggested by a minimal increase in ferritin and TSAT despite the use of multiple doses of IV iron. The child should then have stool tested for occult blood; an evaluation for hemolysis may also be appropriate. Inadequate reticulocytosis suggests that there is a defect in red cell production. This may be due to poor adherence or technique failure in a patient receiving home ESA. There may be a readily identifiable explanation, such as severe secondary hyperparathyroidism. Alternatively, additional testing may be necessary. A serum aluminum level is an appropriate test in the child with a history of using aluminum-containing phosphate binders. One of the most common causes of a poor response to ESA is an inflammatory block due to acute or chronic inflammation. An elevated CRP supports this diagnosis [20]. Other testing, depending on the patient, may include screening for anti-EPO antibodies (see below), and serum levels of folate and B12. A hematologist should evaluate refractory anemia with no identifiable explanation [31, 61].

Treatment of Anemia

Treatment with an ESA is necessary in many children with CKD, including children with chronic allograft dysfunction [62]. Almost all children receiving dialysis are treated with an ESA. In addition, almost all treated patients require oral or IV iron. Other underlying causes of anemia should be corrected (Table 59.1). Blood transfusions should be reserved for children with symptomatic anemia or with worsening anemia due to blood loss, hemolysis, or unresponsiveness to ESAs [1].

Target Hemoglobin

A number of randomized studies in adult patients with CKD have demonstrated that targeting higher Hb levels leads to increased morbidity and mortality [46, 48, 49, 63]. In one study, targeting a higher Hb led to a decrease in dialysis adequacy and a higher use of IV iron [46]. The TREAT trial compared placebo and darbepoetin alpha (DA) in adults with CKD. The risk of stroke was almost doubled in the patients randomized to receive DA to achieve a Hb of 13 g/dL, although there was a modest improvement in fatigue in the DA group [49]. Stroke was also increased when adult HD patients were randomized to a higher Hb target [63]. In the CREATE trial, the group randomized to a higher Hb had more episodes of hypertension and headache [47]. In the CHOIR trial, the group randomized to a higher Hb had an increased risk of death or cardiovascular event and there was no difference in quality of life between the groups [48]. In a retrospective cohort study of adult kidney transplant recipients, patients receiving an ESA with a Hb level above 12.5 g/dL had an increase in mortality [64].

The reason for the increased morbidity and mortality associated with a higher Hb target is

unresolved. It could be attributed to a higher Hb level or to the need for higher doses of ESAs, which have been postulated to have untoward effects beyond increasing the Hb. High ESA dose is associated with increased morbidity and mortality, but this appears to be partially related to resistance to ESAs due to co-morbidities [65–67]. However, in studies that randomized CKD patients to ESA or placebo, the groups receiving ESAs had an increased risk of stroke [49] and access clotting [51]. Moreover, in a randomized study of rHuEPO in patients with ischemic stroke, the group receiving rHuEPO had significantly increased mortality [68]. Dialysis patients who have high Hb levels without the use of ESAs do not have increased mortality [69]. A higher Hb target could lead to more variation in Hb levels, which has been associated with increased mortality [70].

The negative consequences of targeting higher Hb concentrations in the trials of ESAs has led the US Food and Drug Administration (FDA) to recommend that ESAs be given at the lowest dose possible to avoid blood transfusions and that ESAs should be reduced or withheld if the Hb exceeds 11 g/dL and withheld if the Hb exceeds 12 g/dL. Moreover, all ESAs have a black box warning and patients must receive education regarding the possible adverse effects of ESAs. A combination of these concerns, and possibly a change in the payment structure for ESAs, has led to a decrease in the dose of ESAs and Hb levels in US dialysis patients [71].

There are no data on the ideal target Hb in children or whether the target should be adjusted based on age and gender. Kidney Disease: Improving Global Outcomes (KDIGO) recommends an upper limit Hb of 11.5 g/dL in adults, but recommends targeting pediatric CKD patients between 11–12 g/dL. The British NICE guidelines recommend a target of 10–12 g/dL for all patient 2 years and older, with a target of 9.5–11.5 g/dL in children less than 2 years [2, 3]. The Canadian Society of Nephrology recommends a target of 10–11 g/dL for adults, but considers 9.5–11.5 g/dL an acceptable range [72]. The US KDOQI commentary on KDIGO advocates the FDA-recommended upper cutoff of 11 g/dL for adults, but a range of 11–13 g/dL for children [73]. There are clearly clinical situations that require different target Hb values. For example, specific children may require a higher target Hb (e.g. a child with underlying cyanotic heart disease) or a lower target (e.g. a child with sickle cell disease).

Some patients remain anemic or develop refractory anemia despite ESA therapy and correction of other etiologies of anemia. This is especially common in patients who have inflammation. Ongoing escalation of ESA dose in these patients has been associated with adverse outcomes in adults [49], suggesting that ESA dose should not be increased without limit in patients who are hyporesponsive. Patients who do not respond to an ESA or stop responding to an ESA should have an evaluation of possible etiologies of anemia (Tables 56.1 and 56.4).

Hemoglobin Monitoring

Hb monitoring is preferred over hematocrit because Hb measurements are more standardized and consistent. For patients receiving HD, blood samples should be taken immediately prior to dialysis. This may lead to a falsely low Hb value due to hemodilution from fluid gain between dialysis sessions. Hence, this should be considered in children with significant interdialytic weight gain. It is reasonable to measure Hb prior to a HD session after a short interdialytic period (2 days) since the effect of hemodilution on Hb is generally less significant [31].

The frequency of monitoring varies depending on the patient. Children who are being given a stable dose of ESA and within their target Hb can have a Hb level performed as infrequently as monthly in a dialysis patient, and even less often in a predialysis patient (minimum of every 3 months). After the initiation of ESA or after a dosing change, a Hb should generally be obtained at least monthly until the Hb has stabilized within the target range. Protocols that use less frequent monitoring and dose adjustments may reduce costs and minimize Hb cycling, large variations in Hb values that are associated with increased morbidity in adults [74, 75].

Erythropoiesis Stimulating Agents

rHuEPO was the first ESA, but a number of other preparations are now available. Two preparations, DA and methoxy polyethylene glycol (PEG)-epoetin beta, are modified forms of EPO. The main advantage of other preparations is a longer half-life, which permits less frequent dosing. This is especially advantageous in children who require subcutaneous dosing given the discomfort and fear associated with injections. Less frequent dosing may also decrease provider burden in a dialysis unit and provide cost savings [76]. Hypoxia-inducible factor (HIF) prolyl hydroxylase inhibitors (PHIs) have a different mechanism of action than the other ESAs, including both stimulation of EPO production and increasing iron bioavailability. HIH-PHIs have the advantage of oral administration, but regulatory approval has been slow due to safety concerns; they have not been systematically studied in children.

There are no studies directly comparing different ESAs in children, and limited studies in adults except for comparisons of the HIF-PHIs with other ESAs. Availability of the different preparations varies by country.

Recombinant Human Erythropoietin

Multiple studies in adult patients demonstrated the efficacy of IV and subcutaneously administered rHuEPO for correcting the anemia of CKD. A placebo controlled trial demonstrated that rHuEPO is effective in children with CKD [55], and studies in adults demonstrate that use of ESAs decreases the need for transfusions [49, 51, 77].

Pharmacokinetics

The pharmacokinetics of rHuEPO in CKD has been studied in children and adults. There are clear differences based on the route of administration, with less complete absorption of subcutaneous rHuEPO, but a significantly longer half-life when compared to IV administration. In studies of children with CKD, the measured mean half-life of rHuEPO is 5.6–7.5 h for IV dosing and 14.2–25.2 h for subcutaneous dosing. For IV dosing, there is evidence in adults that the half-life of rHuEPO increases as the dose increases.

Dosing

There are dramatic differences in the dosing needs of children with CKD who are receiving rHuEPO, even when adjusted for patient size [78, 79]. Some have recommended dosing children independent of weight and utilizing "adult" dosing [80]. A variety of variables influence the dosing needs of patients (Table 59.5), but it remains difficult to predict the dosing needs of an individual patient. Factors affecting the necessary dose per kilogram (kg) of rHuEPO in children with CKD include the stage of CKD (higher in stage 5), the mode of dialysis (higher in HD due to increased blood loss) [81, 82], the age of the patient (higher in younger patients) [78, 79, 82], the route of administration (higher with IV versus subcutaneous) [79], and the dosing frequency (higher with less frequent dosing regimens). Concurrent causes of poor response to rHuEPO, such as iron deficiency, inflammation, or hyperparathyroidism, often result in higher doses. Blood loss, due to HD, blood draws, and other sources, increase the need for rHuEPO. Blood draws can be especially problematic in the youngest patients, because they often need more frequent monitoring and the relative losses per kg of body weight tend to be higher. Finally, residual renal production of EPO can decrease the need for rHuEPO.

In children receiving PD or predialysis patients, an appropriate starting dose for subcutaneous rHuEPO is 100 units/kg/week divided into two doses, although once weekly dosing may be appropriate in a child with mild anemia. Children less than 5 years are likely to need a higher dose,

Table 59.5 Factors influencing erythropoietin dosing

Route of administration
Mode of dialysis
Initial and target hemoglobin
Endogenous erythropoietin
Patient age
Dosing frequency
Presence of other causes of anemia (see Table 59.1)

and a starting dose of 150 units/kg/week may be appropriate in such patients, especially if severe anemia (Hb < 8 g/dL) is present. Alternatively, a study of children receiving PD demonstrated that the average weekly dose of EPO was 4300 IU/m^2 independent of age [17], suggesting that body surface area can be used to dose EPO in children independent of age or weight. For children receiving HD and IV rHuEPO dosing, a starting dose of 150 units/kg/week divided into theree doses is reasonable, again with the caveat that higher doses are likely necessary in children less than 5 years. A starting dose of 200–300 units/kg/week may be more appropriate in such patients, especially if there is concomitant severe anemia.

In children receiving chronic subcutaneous dosing of rHuEPO, the majority can be maintained on weekly dosing to minimize the number of painful injections. However, some patients require more frequent injections. Less frequent dosing regimens of rHuEPO are effective in adults with pre-dialysis CKD [83].

When children receive IV dosing during HD, it is important to inject rHuEPO via the bloodlines. Use of the venous drip chamber may result in reduced drug delivery due to "trapping" of rHuEPO, although this appears to be somewhat machine dependent.

For children receiving subcutaneous dosing, the site of injection should be rotated. The discomfort of subcutaneous dosing can be reduced by utilizing the multidose vial, which contains the local anesthetic benzyl alcohol as a preservative.

Frequent dose adjustments are typically necessary in patients receiving rHuEPO. This is probably due to variations in the factors that cause anemia (Table 59.1) and that influence rHuEPO dosing (Table 59.5). In addition, more active erythropoiesis is needed to increase a patient's Hb. Hence, the dose that patients need to increase their Hb level into the target range is often more than the dose needed to maintain a stable Hb. Patients may need higher doses of rHuEPO at the start of therapy or after a decrease in Hb due to blood loss or a transient illness.

Most children, when they initiate HD, are converted to IV dosing of rHuEPO, which should then almost always be given thrice weekly. Based on adult studies, the total weekly dose of rHuEPO should be increased by 50% when a patient changes from subcutaneous to IV dosing. Similarly, patients changing from IV dosing to subcutaneous dosing should have their weekly dose decreased by 33%. However, most pediatric patients who convert between IV and subcutaneous dosing are also changing dialysis modality. Given the higher needs for rHuEPO in children on HD [78], patients changing to IV dosing because they are initiating HD may need an additional increase in their dose. In children less than 10 years and certainly those less than 5 years, rHuEPO dosing requirements during HD are very high [79]. This suggests that these patients may need an increase in their rHuEPO dose after beginning HD, irrespective of any change in route of administration. Young children should have careful monitoring of the Hb when initiating HD, increasing the dose of rHuEPO further if necessary. Even in older children, there is extreme variability in the dose requirements when converting to IV dosing; dose requirements may increase or decrease. The ability to more aggressively treat iron deficiency in children receiving HD (see below) may result in a decrease in rHuEPO requirements.

The goal of rHuEPO therapy is to maintain patient Hb within a desired target range. Overly rapid increases in Hb can be associated with hypertension, and should be avoided. In patients with a Hb below the target, the goal is to increase the Hb by 1–2 g/dL per month. The dose of rHuEPO should be increased by 25% if the patient is below the target Hb and has not increased at least 1 g/dL over the last month. The dose should be reduced by 25% if the Hb is greater than the target Hb or the Hb has increased by more than 2 g/dL over the last month. The rHuEPO should be temporarily held if the Hb is more than 1 g/dL over the target Hb or the Hb has increased by more than 2 g/dL over the last month and is above the target Hb.

Complications

An increase in blood pressure after starting rHuEPO therapy may occur in children [55, 84].

This appears to be more common in children who receive higher doses of rHuEPO, and have a consequent more rapid increase in Hb [85]. Hence, rapid increases in Hb should be avoided. While the increase in red cell mass appears to be one mechanism of the hypertension, there also appears to be a direct effect of rHuEPO on the vasculature.

An increase in vascular access clotting following rHuEPO treatment has been attributed to the increase in Hb. There may also be a small negative effect on dialytic clearance, but this is not clinically significant.

Iron deficiency may develop in children treated with rHuEPO [55, 84]. This is secondary to iron utilization for red blood cell synthesis. Consequently, unless iron overload is present, all patients treated with rHuEPO should receive iron supplementation and be screened for iron deficiency before and during therapy.

A rare complication of rHuEPO is the development of anti-EPO antibodies [86]. These antibodies neutralize both endogenous EPO and rHuEPO, resulting in red cell aplasia. Immunosuppressive therapy, including after renal transplantation, results in hematologic recovery in many patients [87]. Patients with undetectable anti-EPO antibodies may subsequently respond to rHuEPO [87].

Darbepoetin Alpha

DA (Aranesp™) is a genetically engineered molecule with a longer half-life than rHuEPO, permitting less frequent administration. The longer half-life of DA is due two additional N-glycosylation sites.

Efficacy

Studies in adults demonstrate comparable efficacy of rHuEPO and DA, despite less frequent dosing of DA [88, 89]. One study demonstrated that many CKD patients do well when receiving DA subcutaneously as infrequently as once every 3–4 weeks [90].

In a prospective study, children receiving rHuEPO were randomized to rHuEPO or DA at a less frequent dosing interval (0.42 μg of DA per week for each 100 units/week of rHuEPO). There was no significant difference in Hb or side effects between the groups at the end of the 20-week study; the median weekly dose of DA was 0.41 mcg/kg, with 25th and 75th percentiles of 0.25 and 0.82 mcg/kg, respectively [91]. A small prospective study evaluated the response to converting seven children receiving HD from thrice weekly rHuEPO to weekly DA (1 mcg of DA per week for each 200 units/week of rHuEPO). Especially in the younger children who were receiving high doses of rHuEPO, there were problems initially with elevated Hb levels and associated hypertension. This was corrected by reducing the dose of DA, suggesting that this dose conversion ratio may be inappropriate in younger children, and that careful monitoring of the initial response is necessary when converting to DA. The mean steady-state dose of DA after 3 months was 0.51 mcg/kg/week [92].

In a large prospective study, children with CKD and anemia were given DA at a starting dose of 0.45 μg/kg/week. There was a significant improvement in Hb and it was sustained during the 28 weeks of the study. By the end of the study, slightly more than half the patients were receiving DA at dosing intervals of at least 2 weeks [93]. A small study has described the successful use of DA in infants, with a starting dose of 0.5 μg/kg/week [94]. The dose was able to be reduced and the dosing interval was increased to 3–4 weeks in some of the infants [94]. In another study in children, the initial dose in naïve patients was 0.45 mcg/kg and patients previously treated with rHuEPO were converted using a dose of 1 mcg of DA per 200 IU of rHuEPO [95]. The final dose was higher in the patients converted from rHuEPO, perhaps because most of these patients were receiving dialysis and none of the naïve patients were dialysis patients [95]. In a prospective registry study in children, including dialysis and pre-dialysis patients, the geometric mean dose range was 1.4–2.0 mcg/kg/month (0.3–0.5 mcg/kg/week) [96].

Pharmacokinetics

One study evaluated the half-life of DA in pediatric patients with CKD [97]. Each patient received one

dose of DA (0.5 mcg/kg) intravenously and subcutaneously. The half-life of DA with IV administration was 22.1 h (SD = 4.5 h). The half-life was 42.8 h (SD = 4.8 h) with subcutaneous administration. The pharmacokinetics were comparable to a similarly designed study in adults except for increased bioavailability (54% vs. 37%) and an earlier T_{max} (36 h vs. 54 h) in the pediatric patients when DA was administered subcutaneously [97, 98]. Hence, DA may be absorbed more rapidly in pediatric patients [97]. More rapid absorption was also seen in pediatric studies of rHuEPO [99].

Dosing

Based on protein mass, 1 mcg of DA is equivalent to 200 units of rHuEPO. Nevertheless, the recommended DA dose by the manufacturer when converting patients from rHuEPO to DA is not a direct conversion based on the 1 mcg of DA to 200 units rHuEPO ratio (Table 59.6). The recommended conversion ratios are based on an analysis of the dose conversion clinical trials [100]. This analysis indicates that proportionally less DA was needed in patients who began the trial on higher doses of rHuEPO [100]. The explanation for this observation is unclear. It is possible that the efficacy of DA increases at higher doses. Alternatively, there may simply be a "regression to the mean" in those patients who were on very high doses of rHuEPO. These patients may have had a transient reason (e.g. inflammation) that led to high ESA dose requirements that subsequently resolved, allowing lowering of the DA dose during the study.

One challenge with DA administration in children is the lack of a multidose vial. First, many small pediatric patients are likely to need less than 25 μg, the smallest available single-dose vial. This results in wasting of the unused medication. Second, pediatric patients may not tolerate the discomfort of 1 mL injections or may require multiple injections in order to tolerate the full 1 mL volume of the single-dose vials. A useful alternative is to utilize DA in more concentrated single-dose prefilled syringes. Thus, dosing of DA necessitates knowledge of the available preparations (Table 59.7), and requires creative adjustments of doses and dosing intervals to minimize wasting of medication.

Recommendations for converting patients from rHuEPO to DA based on adult data are available (Table 59.6). Patients who are receiving rHuEPO twice or thrice weekly should receive DA weekly, and patients who are receiving weekly rHuEPO should receive DA every other week.

Based on the pediatric literature, a reasonable starting dose of DA in ESA naïve patients is approximately 0.5 mcg/kg given weekly. Alternatively, the same total dose could be given every 2 weeks (i.e. 1 mcg/kg every 2 weeks). Every 2-week dosing at initiation should be

Table 59.6 Starting dose of darbepoetin alpha based on previous dosing of rHuEPO

Previous weekly rHuEPO dose (units/week)	Weekly darbepoetin alpha dose (mcg/week)
<2500	6.25
2500–4999	12.5
5000–10,999	25
11,000–17,999	40
18,000–33,999	60
34,000–89,999	100
≥90,000	200

Table based on manufacturer's recommendations
rHuEPO recombinant human erythropoietin

Table 59.7 Available preparations of darbepoetin alpha (single use vials)

*25 mcg	*40 mcg	[a]60 mcg	[a]100 mcg	[a]150 mcg	[a]200 mcg	[a]300 mcg	500 mcg

[a]Preparations that are available in low volume prefilled syringes (0.3–0.6 mL, depending on the dose)

Table 59.8 Dose adjustment table for darbepoetin alpha

6.25.	10	15	20	30	40	50	60	80	100	130	150	200

Doses are in micrograms. The dose to the left of the current dose should be used for dose decreases and the dose to the right of the current dose for dose increases

reserved for patients with a Hb that is only mildly below target. Close monitoring of the Hb is essential for all patients due to the variable response to DA.

As occurs with rHuEPO, frequent dose adjustments of DA are often necessary [88]. Since DA has a long half-life, it is important not to increase the dose too quickly to avoid overshooting the target Hb. Many patients require lower doses after their Hb reaches the target range. When adjusting DA dosing, it is desirable to round doses based on the available preparations (Table 59.7) to avoid excessive wasting of the medication. Nevertheless, excessive rounding is not appropriate; some patients will need to discard some of their medication. Table 59–8 presents one system for dose adjustment. It is unclear whether the DA is evenly distributed in the prefilled syringes. Hence, gentle mixing of the medication and transfer to a 1 mL syringe has been recommended for patients who do not need a full dose [94].

Dose frequency of DA can be gradually reduced from weekly to every other week to every 3 weeks to every 4 weeks [101]. Not all patients will tolerate decreased dose frequency, especially beyond every 3 weeks. The dose frequency can be reduced whenever the patient has a Hb level that would normally mandate decreasing the dose. Alternatively, the dose frequency can be reduced in patients who are on a stable DA dose and have a Hb in the target range. The total weekly dose should remain the same. For patients receiving DA less often than weekly, consideration should be given to increasing the dose frequency if a patient requires more than 1 dose increase, especially if the total weekly dose is relatively high.

Complications

Side effect profiles have been similar in studies comparing IV DA with IV rHuEPO [88, 89]. In one study, there was a statistically significant increase in pruritus in the DA group [89]. Injection site pain is more severe in children with subcutaneous DA than with rHuEPO [102]. There have been cases of antibodies developing to DA leading to pure red cell aplasia [103].

Methoxy Polyethylene Glycol-Epoetin Beta

Methoxy PEG-epoetin beta is made by linking EPO with a large polymer chain via amide bonds between amino acids and methoxy PEG butanoic acid. Methoxy PEG-epoetin beta is also called Continuous Erythropoietin Receptor Activator (C.E.R.A.). A long half-life permits less frequent dosing [104].

Efficacy

A number of studies have demonstrated the effectiveness of methoxy PEG-epoetin beta in adult patients with CKD [105–111]. In a direct comparison with DA in adult HD patients, methoxy PEG-epoetin beta was superior at maintaining Hb at the prescribed target [112]. Methoxy PEG-epoetin beta given either every 2 weeks or every 4 weeks was comparable to rHuEPO in adult dialysis patients [107, 109].

Methoxy PEG-epoetin beta is effective in adult kidney transplant recipients [113, 114]. In a randomized study, once-monthly methoxy PEG-epoetin beta was compared with every 1–2 weeks DA, with similar efficacy and side effect profiles [115].

Two clinical trials have demonstrated that methoxy PEG-epoetin beta is effective in children with CKD [116, 117]. Children with stable anemia undergoing HD were converted from EPO or DA to methoxy PEG-epoetin beta, and Hb remained stable [116]. Similar efficacy was seen in a clinical trial that included children with pre-dialysis CKD and children receiving PD or HD [117].

Pharmacokinetics

The half-life of methoxy PEG-epoetin beta was 134 h and 139 h in adult PD patients following IV and subcutaneous dosing, respectively [118]. The peak reticulocyte count occurred at a mean of

8 days in both groups. Injection site location does not influence the pharmacokinetics, with half-lives between 160 and 164 h [104].

Dosing

For adults, the starting dose is 0.6 mcg/kg every 2 weeks, with conversion to monthly dosing once the Hb is within the target range. The every 2 week dose is doubled to calculate the monthly dose. An initial dose of 1.2 mcg/kg every 4 weeks was effective and comparable to DA every 1–2 weeks in a randomized clinical trial in ESA naïve non-dialysis patients [110]. In one study, the dose conversion ratio for HD patients converted from DA to methoxy PEG-epoetin beta was 1.21 mcg of methoxy PEG-epoetin beta for each 1 mcg of DA [119]. In a small study of pediatric kidney transplant recipients, the patients received a median dose of 2.5 mcg/kg every 4 weeks [120]. In a study of children 6–17 years receiving HD, a conversion factor of 4 mcg every four weeks of methoxy PEG-epoetin beta for each weekly dose of 125 U of EPO or 0.55 mcg of DA maintained a stable Hb [116]. In a registry study of patients receiving HD or PD, monthly doses per kg/month were much higher in younger children than older children, although the difference was minimal when analyzed using body surface area (approximately 100 mcg/m^2/month) [121].

Methoxy PEG-epoetin beta is available in single use vials and single use prefilled syringes in a variety of doses. The prefilled syringes are more concentrated and hence preferable in patients who require subcutaneous injection.

Complications

In adult HD patients, conversion from DA to methoxy PEG-epoetin beta led to more transfusions [119]. No safety concerns have been noted in the pediatric studies [116, 121].

Hypoxia-Inducible Factor Prolyl Hydroxylase Inhibitors

Hypoxia-inducible factor (HIF) prolyl hydroxylase inhibitors (PHIs) have a different mechanism of action than the other ESAs, and they have the advantage of oral administration. HIF-PHIs activate the normal response to hypoxia. HIF-α is constitutively produced; however, in the absence of hypoxia, HIF-α is degraded via the actions of propyl hydroxylase domain (PHD) enzymes (PHD1, PHD2 and PHD3), which are oxygen sensors. These PHD enzymes hydroxylate proline residues in HIF-α, leading to ubiquitylation and proteasomal degradation of HIF-α [122, 123].

Hypoxia or HIF-PHIs decrease the degradation by PHD enzymes of HIF-α, which can then combine with HIF-β, and translocate to the nucleus, where the resulting heterodimer increases transcription of a variety of hypoxia-responsive genes, including EPO in kidney and liver cells, glycolytic enzymes, angiogenic factors, and proteins involved in iron metabolism, absorption, mobilization and transport [122, 123]. The induction of EPO in liver cells permits effective use in anephric patients, though it is unknown whether such patients will require higher doses.

Theoretically, HIF-PHIs could also increase Hb levels by increasing the availability of iron for red blood cell synthesis, especially in patients with decreased iron availability due to inflammatory blockade. HIF decreases synthesis of hepcidin, which is increased by inflammation and decreases iron availability for red blood cell synthesis. Moreover, HIF directly increases ferroprotein, which increases the release of absorbed and stored iron into the circulation, and transferrin, which transports iron to the bone marrow [122, 123].

There are multiple HIF-PHIs, including daprodustat, roxadustat, molidustat, vadadustat and enarodustat. They have different pharmacologic and pharmacodynamic properties [122, 123]. Roxadustat, daprodustat and enarodustat are primarily metabolized by cytochrome P450 enzymes, while molidustat and vadadustat are primarily metabolized by uridine 5′-diphosphoglucuronosyltransferases. Roxadustat is administered thrice weekly; the others are given daily [123].

The HIF-PHIs have been studied in predialysis and dialysis-dependent adult patients.

These studies demonstrate dose-dependent increases in Hb when compared to placebo, and generally equivalent hematologic responses when compared to other ESAs [122, 123]. There is not yet compelling evidence for a clinical benefit of HIF-PHIs on iron metabolism in patients with CKD (e.g. decreased need for IV iron). There is also a hope that patients with inflammation may respond better to HIF-PHIs than other ESAs. There remains concern regarding the multiple actions of HIF-PHIs beyond stimulating erythropoiesis could lead to adverse effects (e.g. upregulation of vascular endothelial growth factor could theoretically enhance tumor growth or worsen retinal disease), though there is also the possibility of beneficial effects (e.g. improved lipid metabolism). One study suggested a cardiovascular safety concern with vadadustat when compared to DA in non-dialysis-dependent adults with CKD [124]. Both positive and negative effects may differ by preparation [122, 123].

Monitoring Iron Stores

Serum ferritin and TSAT are the most widely used tests for monitoring iron stores. However, the United Kingdom National Institute for Health Care Excellence (NICE) guidelines recommend initiating iron therapy based on the measurement of either the percentage of hypochromic red blood cells (iron if >6% unless ferritin >800 mcg/L) or the reticulocyte Hb count (iron if <29 pg unless ferritin >800 mcg/L) [3, 125], though pediatric reference ranges may be needed [126, 127]. Per these guidelines, ferritin and TSAT should only be used if these tests are not available or the patient has thalassemia or thalassemia trait [3]. There is some information on the use of these measures in children, but studies are quite limited [128, 129]. Test availability remains a barrier in many settings, especially since samples may need to be processed relatively quickly (<6 h for percentage of hypochromic red blood cells).

Per KDIGO, TSAT and serum ferritin should be measured at initiation of ESA therapy in all children with CKD [1]. Subsequent monitoring should be at least every 3 months [1]. More frequent monitoring is appropriate in a variety of clinical situations, including after initiation of ESA therapy, when there is a poor response to ESA therapy, after a course of IV iron, or during administration of chronic IV iron therapy. Children receiving IV iron doses of more than 1.5 mg/kg or more than 100 mg should have a delay of at least 1 week before checking serum iron parameters.

Diagnosis of Iron Deficiency

The gold standard for diagnosing iron deficiency in patients with CKD is bone marrow assessment of iron stores, a test that is impractical on a routine basis. An alternative definition is the response to IV iron. An increase in Hb or a decrease in ESA dose after receiving IV iron suggests that the patient was iron deficient. This definition is not perfect—the "response" to IV iron may be coincidental or the patient may not respond for other reasons—but it has been widely used in clinical research and clinical practice.

The traditional criterion for iron deficiency, the combination of a low serum ferritin and a low TSAT, are not applicable in patients with CKD. The serum ferritin is especially problematic because ferritin is an acute phase reactant, and it is therefore often elevated in CKD patients because of infection and non-specific inflammation. Moreover, treatment with an ESA can induce functional iron deficiency. This occurs because the high rate of red blood cell synthesis depletes the readily available iron, even though total body iron stores may be adequate. Patients with functional iron deficiency due to rapid erythropoiesis may have a normal ferritin, but a low TSAT. Often the ferritin level decreases in these patients, yet it remains in the normal range, and it is therefore not as useful a predictor of iron deficiency as the TSAT.

A TSAT below 20% and a serum ferritin below 100 ng/mL is consistent with an absolute iron deficiency. A TSAT below 20% and a serum ferritin above 100 ng/mL suggests a functional iron deficiency. This same scenario can also

occur with an inflammatory block, a condition where inflammation prevents effective delivery of iron for erythropoiesis. Clinical signs of infection, a low serum iron, an elevated CRP, and an increasing ferritin support a diagnosis of an inflammatory block.

KDIGO recommends treating adult CKD patients with IV iron when the TSAT is <30% and the ferritin is <500 ng/mL, assuming there is an indication (desire to increase the Hb or decrease the ESA dose). It is clear that some patients respond to IV iron despite an elevated ferritin [130]. This has led to controversy regarding the upper limit of ferritin in the KDIGO guidelines, with some suggesting that there is no evidence base for an upper limit for ferritin of 500 ng/mL [72, 73]. There has been an increase in the use of IV iron worldwide [131], and in the US the mean serum ferritin in HD patients increased to close to 800 ng/ml [71].

The KDIGO cutoffs for initiating IV iron in pediatric patients are a TSAT <20% and a ferritin <100 ng/ml. This is quite conservative and many pediatric nephrologists treat patients with TSATs <20–30% despite ferritin values >100 ng/ml. The upper limit of ferritin for holding IV iron for pediatric patients varies among clinicians, with some practitioners using cutoffs of 500–800 ng/ml. This variation is due to the lack of evidence supporting a specific cutoff.

Iron Therapy

After EPO deficiency, iron deficiency is the leading cause of anemia in children with CKD. Treatment of iron deficiency often allows achievement of the target Hb with a lower dose of ESA. Iron therapy should not be given to patients who have iron overload, which is commonly defined as a ferritin greater than 800 ng/mL or a TSAT>50%.

Oral Iron

Only a small percentage of oral iron is absorbed, limiting its efficacy in patients who have high iron requirements due to blood loss, such as children receiving HD [9]. Adherence to therapy may be problematic due to problems with gastrointestinal side effects, including abdominal discomfort and constipation [132]. In HD patients, oral iron appears to be of limited benefit and is poorly tolerated [133].

There is an up-regulation in oral iron absorption in patients who have a low serum ferritin or decreased marrow iron stores. However, HD patients have decreased absorption of oral iron when compared to normal controls; inflammation, which is common in HD patients, decreases iron absorption. This is partially mediated by hepcidin, which inhibits iron absorption from the intestines, and is increased by inflammation [22].

Oral iron absorption is decreased when given with food; hence, iron should be given either 1 h before or 2 h after a meal. Calcium carbonate and calcium acetate decrease iron absorption; oral iron should not be given at the same time as these phosphate binders. Sevelamer seems to have little effect on oral iron absorption [134]. H2-receptor antagonists and proton pump inhibitors may also adversely affect iron absorption.

Oral iron may be adequate therapy in children who are not receiving HD. In children receiving HD, oral iron is often not sufficient to correct absolute or functional iron deficiency [135, 136]. Children receiving chronic IV iron should not receive oral iron given the limited benefits and high rate of side effects of oral iron.

Ferrous sulfate, an iron salt, is the most commonly prescribed oral iron preparation; other iron salts include ferrous fumarate, ferrous gluconate and polysaccharide iron complex. Iron salts rapidly release iron ions, which increases the risk of gastrointestinal side effects. The iron salts are inexpensive options. Children should receive a dose of 3–6 mg/kg/day of elemental iron (maximum dose: 150–300 mg/day).

There are a variety of alternative options to iron salts, but many are expensive, with prices that range from 20 to more than 500 times the cost of ferrous sulfate. The possible advantages include increased effectiveness due to increased bioavailability, and improved tolerability with some preparations.

Ferric citrate was originally approved as a phosphate binder, but has been shown to be effective in treating iron deficiency, though gastrointestinal side effects are common [137–139]. In a randomized study in adults with CKD, ferric citrate was superior to ferrous sulfate for correcting iron deficiency [140]. There is limited pediatric experience with ferric citrate as a phosphate binder [141].

Ferric maltol is an oral iron preparation that was approved by the FDA and European Medical Agency for iron deficiency based on studies of patients with inflammatory bowel disease and CKD [142, 143]. It is better tolerated than iron salts [142]. Because of its improved bioavailability, doses of 30 mg twice daily are effective in adults. A randomized study in adult inflammatory bowel disease patients showed comparable 26 and 52 week efficacy when compared to IV ferric carboxymaltose [144]. There is no published pediatric experience.

Sucrosomial® iron has improved bioavailability and gastrointestinal tolerance compared to iron salts [144]. It is effective in adults with CKD at a dose of 30 mg daily, and had comparative long-term efficacy in increasing hemoglobin in adult CKD patients not on dialysis when compared to 125 mg of IV iron gluconate given weekly, though iron stores improved more with IV iron gluconate [145]. It is much less expensive than the other alternatives to iron salts (ferric citrate and ferric maltol) and is cost-effective when compared to IV iron preparations [146]. It is available over the counter in the US, though only via on-line sources. There is no published pediatric experience.

Intravenous Iron

IV iron is more effective than oral iron in correcting anemia in adult patients with CKD, although the benefit is relatively small in pre-dialysis patients [132, 147, 148]. IV iron is believed to be cost effective in HD patients due to decrease ESA utilization [149, 150]. Studies in children, including a meta-analysis [151], have shown the efficacy of IV iron in correcting iron deficiency, improving Hb levels, and reducing rHuEPO dose requirements [135, 136, 152–155]. There is limited experience using IV iron in pediatric kidney transplant recipients [156].

Acute dosing of IV iron is used for patients who have evidence of iron deficiency, which is most commonly defined based on measures of iron stores. The dose given is relatively large. Chronic dosing utilizes smaller doses to maintain iron stores and provide a regular source of iron for erythropoiesis. Acute dosing results in a more significant change in Hb and iron stores [157]. For children receiving PD or who are predialysis, the goal is usually to minimize the need for IV line placement by maximizing the dose given during a single infusion. A chronic dosing strategy is generally only utilized in patients receiving HD due to the burden of IV placement.

Preparations

There are a variety of IV iron preparations available (Table 59.9). The European Pediatric PD Working Group recommended not using iron dextran due to concerns about life-threatening anaphylactic reactions [30]. These preparations have different side effect profiles. In addition, there is no preparation that is ideal in every situation. Iron sucrose and ferric gluconate have limitations on individual doses and must be given over an extended period when higher doses are utilized. This somewhat limits their utility in patients who are not receiving HD. In contrast, ferric carboxymaltose, ferric derisomaltose and ferumoxytol can be given more rapidly at higher doses. However, ferric carboxymaltose, ferric derisomaltose and ferumoxytol are not designed to be given in a chronic dosing strategy due to product packaging in very high doses.

Ferric gluconate is generally well-tolerated [158, 159], and an effective treatment for CKD patients with iron deficiency [130, 160–163]. Ferric gluconate is available in 62.5 mg vials. Acute dosing in adult HD patients is typically 125 mg administered over 10 min given over 8 consecutive HD sessions (total dose = 1000 mg). The recommended acute dose in children receiving HD is a maximum of 1.5 mg/kg over 10 min. In adults, ferric gluconate doses of 250 mg, over

Table 59.9 Intravenous iron preparations[a]

Compound	Adult dosing	Adult administration
LMW Iron dextran[b]	500–1000 mg	Test dose (25 mg) needed; Remainder of dose over >60 min
Ferric gluconate	125 mg	Diluted infusion over 1 h Undiluted up to 12.5 mg/min
Iron sucrose	100 mg	Injection: 2–5 min (≤200 mg) Infusion: ≤200 mg: 15 min; 300 mg: 1.5 h; 400 mg: 2.5 h; 500 mg: 3.5–4 h
Ferric carboxymaltose	≥50 kg: 750 mg × 2 (≥1 week apart) or 15 mg/kg (max 1 g) × 1 dose <50 kg: 15 mg/kg (once or twice ≥1 week apart)	Injection: 100 mg/min (≤750 mg) or over 15 min (1 g dose); infusion (diluted) over ≥15 min
Ferric derisomaltose	≥ 50 kg: 1 g <50 kg: 2 mg/kg	Infusion: ≥30 min
Ferumoxytol	1020 mg (one dose) or 510 mg (2 doses) ≥ 3 days apart	Infusion: 510 mg: ≥ 15 min; 1020 mg ≥ 30 min

LMW low molecular weight

[a]Dosing and administration recommendations may vary by country and change over time. Review current, local recommendations prior to using

[b]Smaller doses may be used for maintenance therapy, typically in hemodialysis patients

60 or 90 min, were well tolerated [164], as were infusions over 1–4 h) [165]. In children, one study reported administration of doses ranging from 1.5 to 8.8 mg/kg, with the child receiving the highest dose having a significant adverse event [152]. Thus, acute doses of ferric gluconate in patients not receiving HD should not exceed 4 mg/kg (250 mg if >60 kg), which should be given over at least 90 min.

Iron sucrose is generally well-tolerated [166, 167] and effective in correcting iron deficiency in patients with CKD [168–170]. Iron sucrose is available in 50 mg vials. Adult HD patients are usually given 50 or 100 mg over 5 min [166]. Infusions of 100 mg over 10 consecutive dialysis sessions is used for acute dosing to provide a total of 1000 mg. For chronic dosing in adult HD patients, doses of 50 or 100 mg avoids wasting medication and can be given weekly or every other week [166].

In adult PD or predialysis pat over 1.5–2 h, appear to be well tolerated. Doses of iron sucrose as high as 500 mg have been given, but the infusion time must be extended to avoid side effects, and this dose may not be tolerated in smaller adults [169, 171, 172]. In children, a dose of 2 mg/kg (maximum 100 mg) over 3 min or 5 mg/kg (maximum 300 mg) over 90 min is well-tolerated [173]. The 5 mg/kg dose can be repeated the next day [173].

Ferric carboxymaltose is effective in treating iron deficiency and is approved for use in non-dialysis CKD patients [174–178]. When ferric carboxymaltose was directly compared to iron sucrose, there was more hypertension in the ferric carboxymaltose group and more hypotension in the iron sucrose group [177]. There are recommendations for acute dosing in adults: 15 mg/kg (maximum 750 mg) with a second dose at least 1 week later. It is administered over 15 min. There is substantial pediatric experience with ferric carboxymaltose, but limited experience in children with CKD [179–183]. In one study, the usual dose was 15 mg/kg, with a maximum dose of 750 mg [179]. In another study, the usual dose was 20 mg/kg, with a maximum dose of 1000 mg in children >35 kg and 500 mg in children <35 kg [183]. Ferric carboxymaltose is available in 750 mg vials, precluding its use in a chronic dosing strategy (see below) or in small children without wasting medication. Hypophosphatemia is a complication in adults with CKD and children who receive ferric carboxymaltose for other indications [177, 184–187].

Ferumoxytol, approved for CKD patients, is effective when given to adults at a dose of 510 mg, followed by a second dose 3–8 days later, though single doses of 1020 mg also appear safe [188–191]. The infusion rate should not exceed 30 mg of iron/second. The large vial size (510 mg) limits

its utility in small children or for use in a chronic dosing strategy. There is limited pediatric experience, albeit not in children with CKD [192, 193]. In one study, ferumoxytol at a dose of 10 mg/kg (maximum 510 mg) was administered over 60 min and 15 min for the first infusion and subsequent infusions, respectively [193].

Ferric derisomaltose is approved and marketed in more than 30 countries worldwide, including the US, the EU, Canada, and Australia for treating iron deficiency. It has comparative efficacy to iron sucrose in patients without CKD and patients with pre-dialysis CKD. There is no published pediatric experience [194, 195].

Acute Dosing

Acute doses of IV iron are given when the patient has evidence of iron deficiency (see criteria above). The goal of acute IV iron dosing is to normalize the serum ferritin and the TSAT. In some cases, an acute dose may be used as a trial of IV iron in a patient with normal iron studies, but a poor response to an ESA. In these patients, the goal of acute IV iron is a reduction in ESA dose or correction of resistant anemia.

In adult HD patients, studies suggest that a total dose of 1000 mg of iron, divided over multiple consecutive dialysis sessions, is appropriate, since smaller doses are not as effective [162]. A total dose of 1000 mg has been used in older children with good results [136]. A randomized study of children receiving HD compared 2 acute dosing regimens of ferric gluconate (1.5 mg/kg/dose and 3.0 mg/kg/dose; maximum dose, 125 mg/dose) given during 8 consecutive HD sessions. The patients had a TSAT <20% and/or a ferritin less than 100 ng/mL at baseline. Both doses led to an increase in Hb and normalization of iron indices. Since there was no difference in the response, the authors recommended a dose of 1.5 mg/kg/dose (maximum of 125 mg/dose) for 8 consecutive HD sessions [196]. This provides a total dose of 12 mg/kg (maximum dose of 1000 mg). Based on the available evidence, the total dose for acute pediatric dosing should be between 12 and 25 mg/kg (1000 mg maximum) divided over up to 12 HD sessions, depending on the dose and iron preparation (see above discussion of specific preparations).

Chronic Dosing

Acute dosing is effective in correcting iron deficiency, but especially in HD patients there is a risk of ongoing episodes of iron deficiency due to continued blood loss. Transient iron deficiency may lead to decreased red blood cell synthesis. This has led to more frequent chronic IV iron use. Observational studies in adults suggests that maintenance therapy may be associated with better outcomes than an acute dosing strategy [197, 198].

In one pediatric study, 1 mg/kg of ferric gluconate for 12 weeks led to a significant increase in Hb [135]. In another pediatric study, chronic IV iron sucrose (2 mg/kg [max = 200 mg] weekly) produced a reduction in rHuEPO dose [153]. A randomized 16-week study in children receiving HD compared maintenance IV iron dextran (doses of 25, 50 or 100 mg/week based on weight; doses therefore ranged from 1.25–2.5 mg/kg/week) with oral iron (4–6 mg/kg/day). The patients receiving IV iron had a significant increase in ferritin when compared to the oral iron group. There was a trend toward a reduction in rHuEPO dose in the IV iron group when compared to the oral iron group [155].

Another study randomized children receiving HD to intermittent IV iron versus maintenance IV iron. There was a higher rate of iron overload in the children receiving intermittent IV iron [199]. This observation may be secondary to a decreased ability to utilize stored iron in children receiving HD due to an inflammatory block. The low doses of maintenance IV iron are immediately employed for red cell synthesis, avoiding an excessive accumulation of stored iron. This contrasts with intermittent IV iron; the high doses cannot all be utilized immediately, increasing the risk of eventual iron overload.

One pediatric study prospectively followed children who were started on a maintenance dose of 1 mg/kg/week of ferric gluconate and then adjusted the dose of ferric gluconate based on iron studies. The majority of the patients completing the study required a dose of 1.5 mg/kg to maintain adequate iron stores [200].

Maintenance IV iron in children receiving HD should be started at about 1 mg/kg/week, usually given as a once/week dose. The maintenance

dose is titrated to keep the TSAT above 20% and the ferritin above 100 ng/mL; IV iron should be held if the TSAT is greater than 50% or the ferritin is greater than 500 ng/mL.

Complications

There are some complications of IV iron that are specific to the particular preparation. Iron dextran may cause an acute anaphylactic reaction, which is potentially fatal [201]. Iron sucrose [159] and ferric gluconate [162] have a safer side effect profile, although all IV iron preparations have the potential to cause serious adverse reactions. Children and adults who have had anaphylactic reactions to iron dextran have tolerated other iron preparations [152, 202, 203]. High doses of iron dextran may cause patients to develop arthralgias and myalgias [204].

There are reports of laboratory findings and clinical symptoms that may be due to acute iron toxicity during the use of iron sucrose and ferric gluconate. This effect is related to the dose and infusion rate and is presumably secondary to rapid release of free iron. Symptoms with ferric gluconate have included loin pain, hypotension, emesis and paresthesias [205]. Iron sucrose side effects have included rash, flushing, and hypotension, which were rapidly reversible [206]. These side effects limit the maximum single dose of these compounds when compared to iron dextran, which releases free iron at a slower rate.

Use of IV iron may increase generation of reactive oxidative species, which has the potential to impair endothelial cell function, promote atherosclerosis, cause inflammation, and decrease immune function [207–210]. There is evidence of an association between higher iron dose and increased adverse outcomes, recognizing a variety of potential confounders [211]. These adverse events may be less likely with iron preparations that release iron more gradually (e.g. ferric carboxymaltose, ferumoxytol and ferric derisomaltose) than iron sucrose or ferric gluconate. This was seen in a randomized study comparing iron sucrose with ferumoxytol [177]. Yet, there is not yet enough evidence to reach a conclusion on this issue given the limitations with the extant literature [212].

Ferumoxytol is associated with higher rates of adverse events than iron sucrose or ferric gluconate [167]. Adverse reactions include injection site reactions, hypersensitivity reactions and hypotension that can be severe [213]. Patients should be observed for at least 30 min after receiving ferumoxytol. Its use may also transiently affect MRI interpretation since it is also used as a contrast agent for MRIs [213].

Iron overload is a potential complication of IV iron therapy, and may occur in CKD patients receiving IV iron [214–216]. There is concern that current IV iron protocols may lead to more problems with iron overload, which has been seen in children receiving acute or maintenance IV iron [153, 199].

IV iron may cause hypophosphatemia, which appears to be mediated by increased levels of FGF-23 and urinary phosphate wasting [217, 218]. Clinically significant, sustained hypophosphatemia is associated with ferric carboxymaltose [186, 187, 219].

IV iron may increase the risk of infection [220]. A multivariate analysis did not find a relationship between IV iron and infection, although there was a trend toward more infections among those patients who received large amounts of IV iron versus those who received lower doses [221]. Given this potential complication, IV iron should be held in patients with acute infections.

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60 Disorders of Bone Mineral Metabolism in Chronic Kidney Disease

Claus Peter Schmitt and Rukshana C. Shroff

Introduction

Disturbances of bone and mineral metabolism almost inevitably develop in the course of chronic kidney disease (CKD). These comprise altered calcium and phosphate homeostasis, abnormal synthesis and secretion of parathyroid hormone (PTH) and vitamin D, and alterations in bone metabolism and function. If not treated appropriately, severe and sometimes disabling complications may occur. Alterations of bone and mineral metabolism originating in childhood contribute not only to degenerative bone disease, but also to vascular morbidity and mortality in young adult life. Hence, adequate control of bone and mineral metabolism is one of the major challenges in the treatment of pediatric patients with chronic renal failure.

With the growing awareness that mineral dysregulation in CKD is closely linked to abnormal bone pathology, and that these in turn lead to extra-skeletal calcification, Kidney Disease Improving Global Outcomes (KDIGO) have proposed a broad and encompassing term chronic kidney disease - mineral and bone disorder (CKD-MBD) to describe this clinical entity [1]. CKD-MBD is defined as a systemic disorder of mineral and bone metabolism that is manifested by either one or a combination of the following:

- Abnormalities of calcium, phosphorus, PTH, or vitamin D metabolism
- Abnormalities in bone turnover, mineralization, linear growth, or strength
- Vascular or other soft tissue calcification

A proposed framework for classifying CKD-MBD divides patients into four types based on the presence or absence of abnormalities in the three primary components used in the definition of the disorder: laboratory abnormalities, bone disease, and calcification of extraskeletal tissue. In this chapter we discuss the pathophysiology, clinical presentation and treatment of CKD-MBD in children.

Epidemiology

Metabolic derangements begin as early as in CKD stage II (glomerular filtration rate [GFR] of 60–90 ml/min/1.73 m^2), plasma levels of active 1,25-dihydroxyvitamin D [1,25$(OH)_2D_3$] decline and parathyroid hormone [PTH] and fibroblast growth factor-23 [FGF23] levels start to increase [2] (Fig. 60.1), with Fibroblast growth factor 23 (FGF-23), a phosphaturic hormone, being the earliest marker of disrupted mineral homeostasis

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[3]. Mineralization defects have been demonstrated in 29% of children with CKD stage 2 [4]. When end stage renal disease (ESRD) is reached, most patients have abnormal bone histology. The specific features of bone disease depend on the degree of hyperparathyroidism and the therapeutic measures taken to control the disease, whereas the mode of dialysis therapy, PD or HD, does not appear to play a major role [5]. Adynamic bone disease has a high prevalence, being observed in 40–50% of adult and almost 30% of pediatric ESRD patients (Fig. 60.2) [5–7]. Amongst children, CKD related bone disease can manifest as bone pain and deformities, growth retardation and fractures and have long-term consequences. In a cohort of 249 young Dutch adults with onset of end-stage renal failure before the age of 14 years 61% had severe growth retardation, 37% bone disease (defined by at least one of the following conditions: deforming bone abnormalities, chronic pain related to the skeletal system, disabling bone abnormalities, aseptic bone necrosis and atraumatic fractures and 18% disabilities resulting from bone impairment [8].

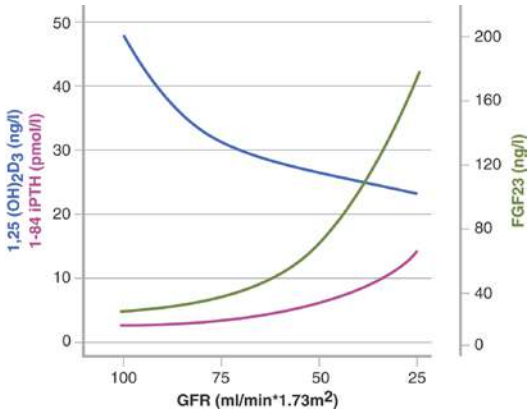

Fig. 60.1 Mean plasma intact PTH, $1,25(OH)_2D_3$ (left y-axis) and FGF23 concentrations (right y-axis) in patients with different degree of CKD. Individual values may vary considerably especially in patients with advanced renal failure

There is a complex interplay between CKD-related bone disease and cardiovascular disease that begins early in the course of CKD, is seen even in children [9], and leads to a significant decrease in life expectancy [10, 11]. Several large national registries have published similar findings for pediatric dialysis recipients. The United States Renal Data Systems (USRDS) has reported that 23% of all deaths in dialysis patients were from cardiovascular causes [12], and 50% of all deaths in young adults who received dialysis as children were from cardiovascular or cerebrovascular causes [10]. Encouragingly, recent reports suggest that there is a substantial decrease in mortality rates over time among US patients [13]. Cardiovascular disease in CKD is discussed in greater detail in Chap. 61.

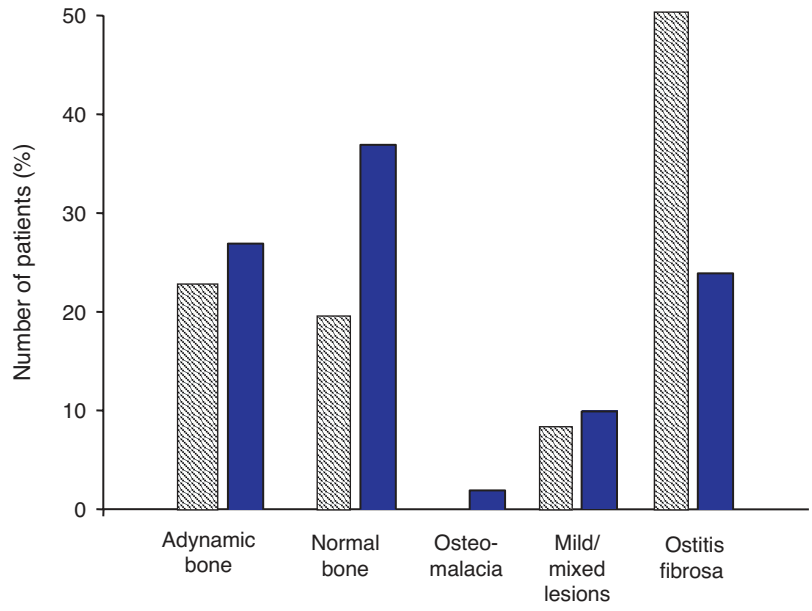

Fig. 60.2 Spectrum of renal osteodystrophy in children with CKD stage 5 diagnosed in the USA in the early 1990s (hatched bars, adapted from [6]) and in Poland in the late 1990s (filled bars, adapted from [5])

Pathogenesis

Bone and mineral homeostasis are regulated in a complex network of local and systemic factors. Patients with CKD develop major disturbances in calcium, $1,25(OH)_2D$ and phosphate homeostasis and subsequently abnormal parathyroid gland function, which ultimately drives the course of the disease (Fig. 60.3). FGF23 has added a new dimension to the Ca-P-PTH axis and advanced our understanding of mineral dysregulation in CKD [3]. FGF23, along with its membrane receptor klotho, acts through an intricate negative feedback system involving PTH and vitamin D to regulate serum calcium and phosphate levels.

Disorders of Calcium Homeostasis

In healthy individuals 99% of total body calcium is stored in the bone, 0.975% in soft tissues and only 0.025% is circulating in blood. Plasma ionized calcium levels are tightly controlled by PTH and $1,25(OH)_2D$. The parathyroid gland senses changes in ionized calcium by a G-protein coupled membrane receptor. Acute hypocalcemia is counteracted by an instantaneous and marked increase in PTH release from storage vesicles, rapidly normalizing plasma calcium levels, and by an increased PTH gene transcription and synthesis rate, an adaptive response which takes several hours to occur. In addition, hypocalcemia stabilizes PTH mRNA by increased binding of a cytosolic adenosine-uridine-rich protein (AUF1) in the 3′ untranslated region of the PTH mRNA [14]. A subsequent increase in $1,25(OH)_2D$ synthesis further stabilizes plasma calcium levels via stimulation of gastrointestinal calcium absorption. A recent study has shown that 76% of children with CKD4-5D had a dietary Ca intake <100% Reference Nutrient Intake, largely because a restriction of dairy foods as part of a P controlled diet limits Ca intake, and additional Ca from medications is required to meet the KDOQI guideline of 100–200% normal recommended Ca intake [15].

In CKD, reduced $1,25(OH)_2D$ synthesis impairs intestinal calcium resorption, resulting in an activation of the regulatory circuits described above and a resetting of ionized calcium at low or low-normal levels. The calcemic response of bone to PTH is reduced and higher PTH levels are required to maintain calcium homeostasis and bone turnover. Uremic toxins, low levels of $1,25(OH)_2D$, accumulation of inactive PTH fragments and osteoprotegerin, and altered PTH receptor expression have been implicated in the skeletal resistance to PTH, providing yet another

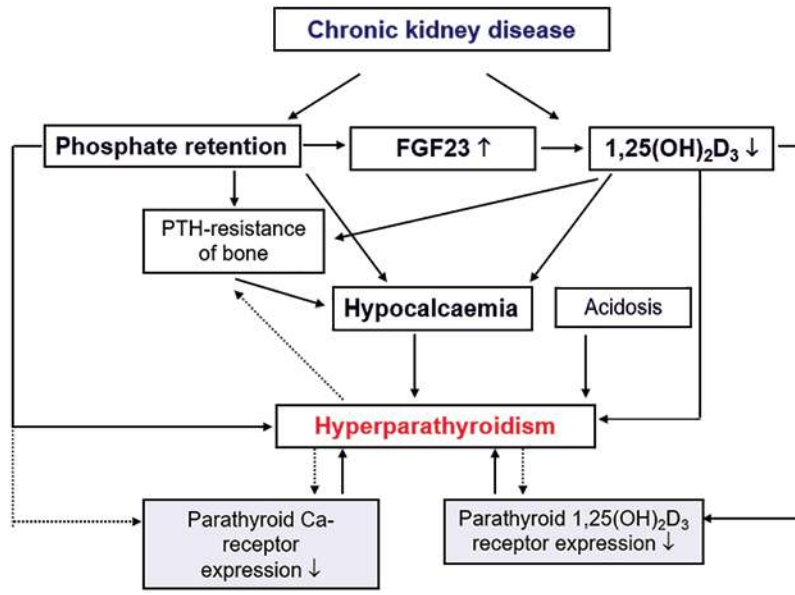

Fig. 60.3 Pathophysiology of secondary hyperparathyroidism

mechanism contributing to the development of hyperparathyroidism (Fig. 60.3) [16].

Plasma ionized calcium is the major regulator of the parathyroids at the level of gene expression, secretion and cell proliferation. Induction of hypocalcemia stimulates PTH release, PTH peptide synthesis via stabilization of PTH mRNA and, if sustained, induces profound parathyroid cell proliferation [17]. Hypocalcemia appears to be a more important regulator of the parathyroid than vitamin D, as suggested by the efficient control of hyperparathyroidism in vitamin D-receptor knock-out mice by a selective increase of dietary calcium content [18]. Moreover, calcimimetic agents suppress PTH by up to 80%, independent of plasma phosphate and $1,25(OH)_2D$ levels.

Abnormalities of $1,25(OH)_2D$ Metabolism in CKD

25(OH)D is converted to the systemically active $1,25(OH)_2D$ by the enzyme 1-alpha hydroxylase. Progressive loss of intact renal parenchyma, low 25(OH)D levels and increased FGF-23 release from the bone result in low circulating $1,25(OH)_2D$ levels. This in turn leads to reduced intestinal calcium absorption and hypocalcemia; it is estimated that the intestinal calcium absorption increases from ~45% to 65% in the presence of adequate vitamin D. Hypocalcaemia triggers PTH release in order to maintain calcium homeostasis and to stimulate 1-alpha hydroxylase.

$1,25(OH)_2D$ controls parathyroid gland function not only via modulating plasma ionized calcium, but also directly by suppressing PTH gene transcription [19] and by upregulating its own receptor in parathyroid cells. Moreover $1,25(OH)_2D$ binds to a response element in the promoter region of the calcium receptor, resulting in increased calcium receptor abundance, thereby increasing the sensitivity of the parathyroid gland to ionized calcium [20]. Hypocalcemia on the other hand compromises vitamin D action by upregulating calreticulin, a repressor of the vitamin response element in the parathyroid glands [21]. In addition, $1,25(OH)_2D$ regulates parathyroid cell proliferation, with low levels promoting parathyroid gland hyperplasia.

FGF23 controls $1,25(OH)_2D$ production through a complex feedback mechanism [22]. FGF23 suppresses the renal 1-alpha-hydroxylase and thereby reduces conversion of 25(OH)D to $1,25(OH)_2D$. In addition, FGF23 increases the activity of 24,25 hydroxylase to degrade $1,25(OH)_2D$.

$1,25(OH)_2D$ has numerous additional important functions outside the bone and parathyroid. The hormone is an important regulator of the immune system and affects the contractility, growth and migration of vascular smooth muscle cells (VSMCs) as well as the evolution of vascular calcifications. Both endothelial and VSMCs express high-affinity Vitamin D3 receptors. $1,25(OH)_2D$ deficiency may contribute to cardiovascular disease by unrepressed production of proteins involved in arterial calcification such as bone morphogenetic protein-2 [23, 24] or by suppressed production of local inhibitors of mineralization, e.g. matrix GLA protein [25]. High doses of $1,25(OH)_2D$ on the other hand promote vascular calcification via an increased calcium phosphate product and transition of vascular smooth muscle cells to an osteoblast like phenotype. Of note in this context, $1,25(OH)_2D$ is not only an endocrine factor exclusively secreted by the kidney. Extrarenal 1-α-hydroxylase expression has been demonstrated in various tissues such as bone, smooth muscle cells and parathyroid glands [26, 27], suggesting an additional paracrine mode of action independent of renal conversion.

25-Hydroxy Vitamin D Deficiency

The widespread deficiency of 25(OH)D is even more pronounced in CKD patients than in the general population, related to multiple potential causes [28]: (1) Patients with CKD may be less active and have less sunlight exposure; (2) The endogenous synthesis of vitamin D in the skin is reduced in CKD; (3) Ingestion of foods that are natural sources of vitamin D may be diminished; (4) Proteinuria may be accompanied by high urinary losses of vitamin D binding protein (VDBP),

leading to increased renal losses of all vitamin D metabolites; (5) 25(OH)D and VDBP may be lost in peritoneal dialysis fluid. There is debate about what levels of vitamin D can be considered adequate. Current clinical practice recommendations for children with CKD suggest a target level of 30–48 ng/ml (75–120 nmol/L) [29].

Low 25(OH)D levels result in muscle weakness [30] and bone pain and aggravate renal bone disease [31, 32]. Bone histomorphological changes are correlated with plasma 25(OH)D levels [31, 33]. *In vitro*, a concentration of 40 ng/ml 25(OH)D is as efficient in suppressing PTH as calcitriol at a maximally PTH suppressive dose. A randomized trial in 47 children with CKD2–4 demonstrated a longer time to development of secondary hyperparathyroidism with vit. D supplementation as compared to those children on placebo [34].

Abnormalities of Phosphate Metabolism and Phosphaturic Hormone FGF23

Phosphate excretion declines early with failing renal function and this is a driving force of CKD-MBD. When the GFR falls to <50 ml/min/1.73 m^2, phosphate accumulates [2]. In early stages, the decline in renal phosphate excretion is counteracted by the phosphaturic hormones Fibroblast Growth Factor 23 (FGF23) and PTH. Clinical studies have identified increased FGF23 levels as the earliest biological marker of deranged calcium - phosphate homeostasis in CKD, even before PTH levels start to rise [3, 35, 36] (Fig. 60.2). FGF23 is mainly secreted by osteocytes and acts on the type IIa and IIc sodium-phosphate co-transporters (NaPi) in the apical membrane of proximal tubular cells to increase urinary phosphate excretion. It suppresses $1,25(OH)_2D$ synthesis by suppressing 1-α hydroxylase (CYP27B1) and increasing $1,25(OH)_2D$ degradation to 24,25(OH)2D by promoting CYP24A1 [37]. Thus, by reducing circulating $1,25(OH)_2D$ levels, FGF23 reduces phosphate absorption from the gut. Also, FGF23 suppresses PTH mRNA and decreases serum PTH [38]. In turn, FGF23 secretion is stimulated by phosphorus, $1,25(OH)_2D$ and PTH. Mutations in the FGF23 gene result in severe hypo- and hyperphosphatemic disorders (see Chap. 34). Recent data suggests that FGF23 may in fact play a key role in calcium homeostasis and increased FGF23 is associated with higher calcium levels [39].

FGF23 signaling requires the presence of the transmembrane protein klotho on its target cells. Klotho is highly expressed in the kidneys where it serves as a high-affinity receptor for FGF23 [40]. When its extracellular domain is shed from the cell surface, it enters the circulation as soluble klotho and functions as a humoral factor that regulates ion channels and transporters (including NaPi and the calcium channel TRPV5 in the gut). Klotho deficiency develops with declining GFR and leads to a state of relative FGF23 resistance. However, FGF23 may be a 'double edged sword': although elevated FGF23 levels increase phosphate excretion in early stages of CKD, they have been associated with increased cardiovascular mortality both in CKD patients [41] and the general population. FGF23 exerts a direct toxic effect on the myocardium and is associated with left ventricular hypertrophy [42, 43].

A decline in circulating P concentrations can be observed in very early CKD when circulating FGF23 levels and bone FGF23 expression are increased. In contrast, in advanced stages of CKD hyperphosphatemia does contribute to increased FGF23 and treatment with active vitamin D analogs further contributes to increasing FGF23 levels. In all CKD stages, non-mineral metabolism factors such as iron status, erythropoietin and inflammation may also contribute to increased FGF23 production. In murine models, both absolute and "functional" iron deficiency increase bone FGF23 expression [44]. Erythropoietin can also stimulate FGF23 production and, conversely, FGF23 may suppress erythropoiesis [45]. FGF23 levels are higher in patients with glomerular diseases when compared to those with congenital anomalies of the kidneys and urinary tract (CAKUT) [46]; inflammation increases bone and circulating FGF23 levels [44].

Hyperphosphatemia has multiple deleterious effects. It contributes to hyperparathyroidism

independently of plasma calcium and $1,25(OH)_2D$ [47, 48] via increasing PTH gene transcription, PTH peptide secretion and parathyroid cell proliferation. Furthermore, hyperphosphatemia reduces renal $1,25(OH)_2D$ synthesis, inhibits the suppressive action of $1,25(OH)_2D$ on the parathyroid glands and promotes resistance of bone to PTH. Another indirect mechanism by which high phosphate drives hyperparathyroidism is via physicochemical precipitation of calcium-phosphate salts, a process aggravating hypocalcemia. High phosphate levels are very difficult to control as phosphate binders are one of the most difficult medications to comply with. The International Pediatric Peritoneal Dialysis Network has shown that 45% of all children have hyperphosphataemia, with the prevalence increasing to >80% amongst adolescents [49].

Secondary Hyperparathyroidism

The multiple effects of $1,25(OH)_2D$ deficiency, hypocalcemia and hyperphosphatemia in CKD lead to the development of secondary hyperparathyroidism, with a progressive demineralisation of the bone. Pre-pro PTH gene transcription rate, mRNA stability, protein synthesis and secretion of the mature protein are increased. Persistent hyperparathyroidism induces distinct changes in parathyroid gland morphology and function. Parathyroid cell proliferation results in diffuse and polyclonal and eventually monoclonal cell growth, associated with the formation of adenoma. Regulatory systems include endothelin, TGF-alpha, epidermal growth factor receptor and the cell cycle inhibitor p21 [50–53]. Monoclonal parathyroid cell growth is the result of an array of possible genetic aberrations such as gene deletions, loss of heterozygosity, clonal rearrangement and /or oncogene overexpression and tumor suppressor gene inactivation. Polymorphisms in the PTH, Vitamin D receptor and calcium receptor genes may also be involved and explain some of the clinical variability of the disease [54]. Reduced expression of the parathyroid calcium sensing receptor and of the vitamin D receptor

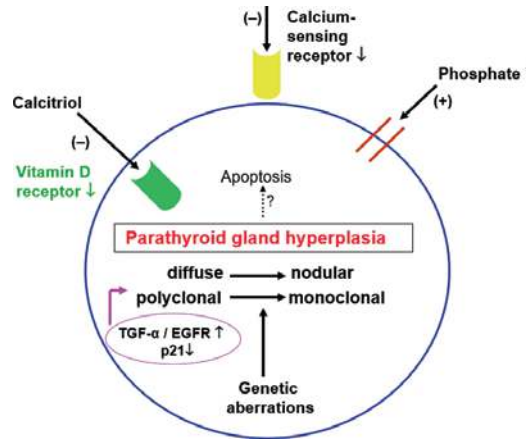

Fig. 60.4 Cellular alterations of parathyroid gland function in CKD

leads to a progressive escape from the two key physiological control mechanisms and ultimately to parathyroid gland autonomy (Fig. 60.4).

Minute to minute analyses of PTH secretion have revealed distinct alterations of the dynamics of oscillatory PTH release in uremic patients, including a markedly reduced secretory capacity to counteract changes in ionized calcium by modulation of the frequency and mass of PTH secretory bursts [55, 56].

Apoptosis is a rare event in normal parathyroid tissue; parathyroid cells can survive for >25 years, so, although uremia is associated with increased apoptosis of parathyroid cells, this mechanism is insufficient to counterbalance enhanced proliferation [57]. Whether an inversion of this imbalance, i.e. regression of parathyroid hyperplasia, occurs in uremic patients, or after correction of uremia, remains controversial [58].

In uremia, *fragments of the intact 1–84 PTH* peptide accumulate in the circulation and exert distinct biological activity, most notable 7–84 PTH fragments that are thought to be biologically inactive are present in the circulation and offset the classic biological actions of PTH [59–61]. 7–84 PTH internalizes the PTH type-1 receptor without prior activation. This may be one explanation for the reduced PTH1R level in uremia, the resistance of bone to PTH and the dissociation of phosphorus and calcium homeo-

stasis in CKD patients. Interindividual variation of 7–84 PTH /1–84 PTH ratios is high. Alternatively, PTH fragments may also signal via receptors distinct from the PTH1R, e.g. through a C-terminal PTH receptor [62] and by this impact on bone. Up to now, there is only limited evidence that the differentiation of PTH fragments is clinically helpful for defining low and high turnover bone disease. The use of PTH assays measuring 7–84 in addition to 1–84, intact-PTH is therefore currently not recommended for routine clinical practice.

Oxidative stress is increased in CKD, and the amino acid methionine at two positions in the PTH peptide is prone to oxidation. A large proportion of circulating PTH measured by standard assay systems is oxidized and may thus not be biologically active. Non-oxidized PTH concentrations are 1.5–2.25 fold higher in patients in renal failure as compared to health controls [63]. Oxidation blunts the biological activity of the peptide. Oxidized PTH (oxPTH) loses its PTH receptor-stimulating properties, whereas non-oxidized PTH is a full agonist of the receptor [63]. Measurements of non-oxidized PTH should reflect the hormone status more precisely, but a recent bone biopsy study in 31 patients with CKD suggests no additional benefit [64]. Non-oxidized PTH measurements are currently not recommended for routine clinical practice.

The Impact of Metabolic Acidosis

Metabolic acidosis, an almost inevitable covariate in patients with failing renal function, has a number of untoward effects on bone. These include physicochemical dissolution of bone with inhibition of osteoblast and activation of osteoclast activity leading to a net calcium efflux from the bone. This is associated with impaired bone mineralization and an increased incidence of osteomalacia, which can be improved by correction of metabolic acidosis [65]. Moreover some evidence suggests that metabolic acidosis increases PTH levels in CKD patients and enhances the peripheral actions of PTH on bone by increasing the expression and ligand affinity of the PTH receptor [66]. The relative quantitative contribution of metabolic acidosis to bone disease in CKD patients remains uncertain.

Further Mediators of CKD Bone Disease

The kidney-bone-vessel interaction is not confined to the homeostasis of calcium-phosphate and $1,25(OH)_2D$ synthesis, but a number of other key molecules are involved.

Bone morphogenetic protein-7 (BMP-7) is produced and secreted in postnatal life mainly by renal collecting tubule cells. CKD is associated with a marked deficiency of circulating BMP-7, most likely due to reduced renal synthesis. In the uremic rat model, adynamic bone disease develops if serum calcium, phosphate, vitamin D and PTH levels are maintained in the normal range. This observation has led to the hypothesis that the variable histopathological appearance of uremic bone may reflect the net balance of hyperparathyroidism inducing a high-turnover and BMP-7 deficiency causing a low-turnover state. At least in the uremic rat, exogenous administration of BMP-7 can reverse both adynamic and high turnover bone disease by improving osteoblast number and bone formation activity [67] . Moreover, BMP-7 reduces vascular calcifications in uremic animals, possibly by increasing the skeletal deposition of phosphorus and calcium [68] . The Diabetes Heart Study showed that single nucleotide polymorphisms in the BMP-7 gene have significant and reciprocal effects on vascular calcification and bone density [69]. Despite these notable discoveries BMP-7 has not entered the clinical arena so far; neither have circulating BMP-7 measurements been established as a biomarker of bone health nor have therapeutic studies progressed beyond the preclinical phase to date.

Osteoprotegerin (OPG) is a soluble protein of the tumor necrosis factor (TNF) receptor superfamily and a decoy receptor for the *receptor activator of nuclear factor kappa B ligand (RANKL).* By binding RANKL, OPG inhibits nuclear kappa

B, which is an essential transcription factor for immune and inflammation related genes, cell survival and differentiation. OPG inhibits the differentiation of osteoclast precursors into mature osteoclasts and regulates the resorptive action. OPG-deficient mice exhibit media calcifications. In humans, circulating OPG levels associate with vascular calcifications and in CKD patients in particular with aortic and coronary calcifications [70, 71]. Low levels of soluble RANKL indicate a higher risk for cardiovascular events [72] and predict CV mortality in patients with CKD [73]. Prospective clinical trials with the OPG analogue, RANKL inhibitor denosumab suggest improved bone mineral density in early stages of CKD [74], the impact on vascular calcifications is yet uncertain.

Sclerostin is a glycoprotein secreted by osteocytes. It binds to a transmembrane complex (consisting of the frizzled receptor and the low-density lipoprotein receptor-related protein 5 or 6 coreceptor) and inhibits the Wingless-type mouse mammary tumor virus integration site (Wnt) pathway. The Wnt pathway stimulates stem cell and pre-osteoblast proliferation, induces osteoblastogenesis, inhibits osteoblast and osteocyte apoptosis and attenuates osteoclastogenesis. Sclerostin is produced by osteocytes and has an anti-anabolic effect on bone. Ageing, mechanical unloading of the skeleton, low PTH levels and progression of CKD have been associated with high circulating sclerostin levels. In HD patients, sclerostin levels correlate negatively with histomorphometric parameters of bone turnover, osteoblast number and function [75]. Sclerostin expression is increased during vascular smooth muscle cell calcification, where it potentially inhibits local arterial Wnt signalling and thus may represent a defense response against calcification. High circulating sclerostin levels have been associated with improved survival in hemodialysis patients [76].

Bone Histology in CKD-MBD

The term "renal osteodystrophy" is reserved for the spectrum of histological changes in CKD-associated bone disease, including changes in bone turn-over, mineralization and volume (TMV). The type of renal osteodystrophy in an individual patient depends on the therapeutic interventions taken to counteract an otherwise progressive disease (Fig. 60.5). Changes in bone turnover develop early in the course of CKD, prior to measurable changes in mineral homeostasis, possibly effected by osteocyte cytokines such as sclerostin [77]. In untreated children with CKD histological signs of fibrosis and demineralization prevail, whereas aggressive long-term treatment with calcium and vitamin D is associated with low bone turn over. At present, an adequate balance with normal or mild alterations in bone morphology is achieved only in a minority of the children [1]. A histological classification of renal osteodystrophy is given in Table 60.1, and respective histopathological illustrations are given in Fig. 60.6.

Children with some forms of rare inherited kidney diseases may have an element of bone disease independent of the degree of kidney failure and CKD-MBD, due to potential functions of the mutated genes in bone homeostasis. Exacerbated skeletal disease is prevalent in patients with cystinosis, primary hyperoxaluria and some primary ciliopathies [78–80]. Understanding the impact

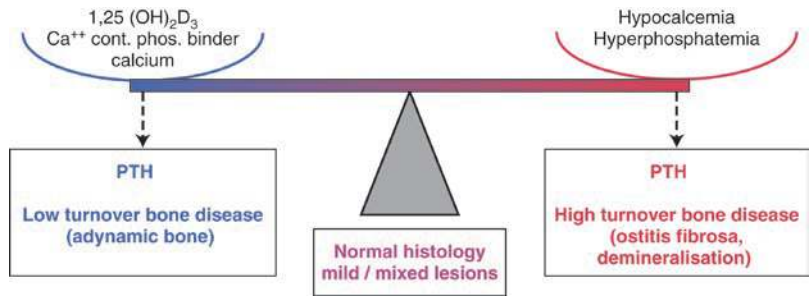

Fig. 60.5 The type of renal bone disease depends on therapeutic interventions

Table 60.1 Histological classification of renal osteodystrophy

Type	Etiology	Description	Comments
High turnover bone disease (Ostitis fibrosa)	Hyperparathyroidism	– Increased bone formation, resorption and osteoid deposition/seams – Disorganized collagen (woven bone), marrow fibrosis	– Frequent in untreated patients – Skeletal deformities, bone pain, epiphysiolysis
Low turnover: I Adynamic bone	– Relatively low PTH – Ca^{++} load, vit. D metabolites – Uremic toxins – Altered cytokines/ growth factors	– Low bone formation and resorption rate – Decreased osteoid deposition	– Most common type – Increased fracture risk (?) – Extraosseus calcifications (?)
II Osteomalacia	– Aluminium – Unknown factors	– Accumulation of osteoid – Inhibition of the mineralization process	– Incidence ↓ with adequate dialysate purification and withdrawal of aluminium cont. Phosphate binders
Mixed disease	– Hyperparathyroidism – Aluminium – Unknown factors	– Increased remodelling, resorption and osteoid, – Areas of low bone formation	

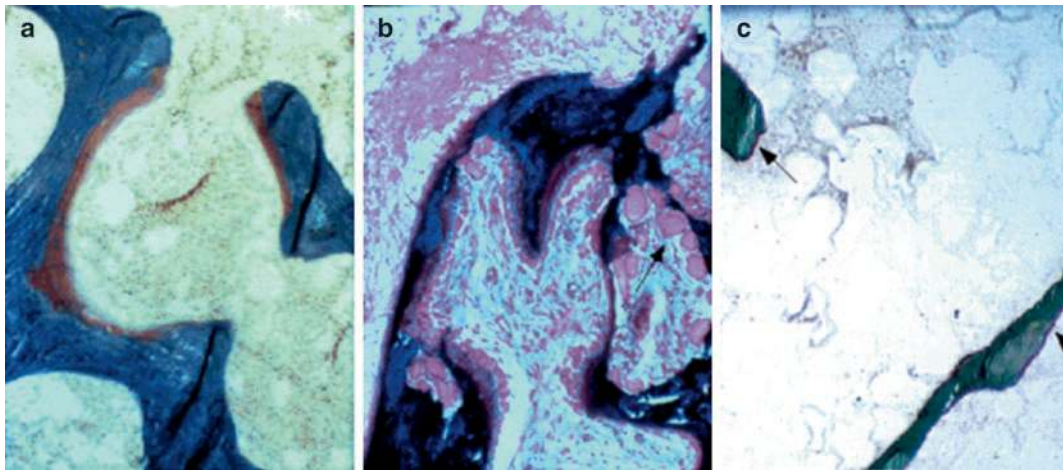

Fig. 60.6 Representative examples of different types of bone histology in patients with CKD (courtesy of LD Quarles, Department of Nephrology and Hypertension, University of Kansas Medical Center, Kansas City, Kansas, USA). (**a**) Histologic appearance of normal bone. Goldner Masson trichrome staining shows mineralized lamellar bone in blue and adjacent nonmineralized osteoid in red-brown. Osteoid comprises less than 25% of bone surfaces. The cellular area between the osseous structures is the marrow space. (**b**) Goldner Masson trichrome staining showing osteitis fibrosa due to secondary hyperparathyroidism with increased number of osteoclasts (arrow) and extensive bone marrow fibrosis (as shown by the light blue staining of the marrow). The increased resorption results in a thin and scalloped appearance of mineralized trabecular bone. Osteoid and bone formation are relatively increased, too. (**c**) Adynamic bone disease, characterized by reduction in bone formation and resorption. The osteoid seams are thin (red lines at the bone surface; arrows), and there is little evidence of cellular activity

of the different genetic defects on the growing skeleton will likely lead to targeted therapeutic strategies.

Bone Mineral Accrual and Peak Bone Mass

Bone mass is mainly genetically determined [81], but exogenous factors also play a major role. Bone mass markedly increases during childhood, reaching a peak at 25–30 years of age [82]. The growing skeleton of children is uniquely vulnerable to factors that can impair bone accrual including poor growth, delayed maturation, muscle deficits, decreased physical activity, abnormal mineral metabolism, and secondary hyperparathyroidism. Skeletal calcium increases from 25 g in newborns to 900 and 1200 g in adult females and males, respectively. Increased $1,25(OH)_2D$ levels coincide with an increased rate of skeletal calcium accumulation during puberty [83]. In healthy adolescents approximately 25% of total skeletal mass is laid down during the 2-year interval of peak height velocity. This gives rise to an increased calcium and phosphate requirement in children, especially during periods of rapid growth, and the enormous buffering capacity of the growing skeleton. Calcium balance studies have shown that in healthy children on a high calcium diet the amount of calcium incorporated into the skeleton increases up to a threshold dietary intake above which no further bone accumulation occurs [84]. Calcium requirements are highest in the first year of life (503 ± 91 mg/day) and during pubertal growth (396 ± 164 mg/day), dropping thereafter to normal adult requirements (114 ± 133 mg/day) [84].

The mineral requirements of the growing skeleton have two important consequences: the normal range for Ca and P varies substantially with age (Fig. 60.10), and secondly, the high Ca and P requirements of the bones can 'buffer' increased dietetic calcium and phosphate loads, preventing extra-skeletal mineralization and ectopic calcification. Normal serum calcium and phosphate levels fall steeply from birth until the age of 1–2 years and then gradually reduce up to the age of 7 years when they reach adult levels. Children with CKD are highly prone to develop mineralization defects. Almost one third of children with CKD stage 2 and more than of 90% of children on dialysis have deficient mineralisation [4], while this is the case in only 3% of adult dialysis patients [85]. Hence, calcium balance studies are urgently required in children with CKD to determine balance at different levels of calcium intake and at different stages of puberty, ideally correlating this data with bone histology.

Osteomalacia in animals with FGF23 deficiency suggests that FGF23 may play a direct role in skeletal mineralization; both overexpression [86] and ablation of FGF23 [87] in mice with normal renal function is associated with abnormal mineralization of osteoid, although by different mechanisms. The phosphaturic effect of increased FGF23 may cause rickets and osteomalacia through an insufficiency of mineral substrate.

Both, bone mass and bone geometry are altered in children with uremic bone disease, depending on the degree of hyperparathyroidism, disturbed vitamin D metabolism and therapeutic counteractions taken to control the disease. In CKD patients DXA (dual energy x-ray absorptiometry) scans are not a reliable measure of bone mineral density (BMD), but peripheral quantitative CT (pQCT) scans provide a useful measure [88]. In children with CKD low calcium and high PTH levels have been associated with a decrease in tibial BMD on pQCT [89]; this study also showed that a 1 SD decrease in BMD was associated with a twofold increase in fracture risk.

Linear Growth

Longitudinal growth is a unique feature of childhood. Growth occurs through the modelling of new bone by skeletal accretion and longitudinal growth in the growth plate. Bone

formation in children occurs by two distinct mechanisms: skeletal remodelling of existing mineralized tissue that is controlled by osteoclasts and osteoblasts and modelling of new bone by skeletal accretion and longitudinal growth from the growth plate, through the action of chondrocytes [4, 90]. The growth plate is an avascular tissue between the epiphyses and metaphyses of long bones. During endochondral bone formation it is progressively replaced by bone. Bone formation in the endochondral growth plate is regulated by growth hormone (GH) and the PTH/PTH-related protein-receptor axis that together promote chondrocyte proliferation, matrix synthesis and chondrocyte differentiation into osteogenic cells [91].

Clinical Manifestations

Symptoms of renal osteodystrophy can develop early, especially in children with CKD from infancy. Initial signs are often vague and nonspecific and may not come to the attention of the caregivers. Especially young infants require meticulous monitoring, since the high growth velocity, the high mineral demand and the enhanced pressure load to joints with increasing mobility can rapidly lead to severe deformations.

Bone Pain

Bone pain is a common manifestation in children with CKD. Initial symptoms may be difficult to distinguish from other causes of pain, but become more specific with progressive disease and localize to the weight bearing joints. Since symptoms vary considerably among individual patients, they do not allow for conclusions regarding the type and severity of the underlying bone disease. Limping, bone deformities and axial displacement require a prompt and thorough diagnostic process, including biochemical and radiographic studies. A recent study reported that significant bone pain hindered the daily activities of 58% of children with advanced (i.e. stages 4–5) CKD [88].

Myopathy

Patients with severe bone disease may also show muscular symptoms such as muscle weakness and wasting, exercise limitation, and waddling gait. The pathogenesis seems to be complex. One major reason is vitamin D deficiency, but inefficient clearance of uremic toxins, insulin resistance, carnitine deficiency, malnutrition and anemia may also contribute [92]. Muscle fat mass is increased in CKD children and negatively correlated with physical performance [93]. Some studies indicate an abnormal oxygen conductance from the muscular microcirculation to the normal functioning mitochondria [94]. Myopathy may further reduce bone strength 96).

Skeletal Deformities

Bone deformities include bowing of the weight bearing bones, with genua valga being most frequent. Genua vara, coxa vara, ulnar deviation and ankle deformations may also be seen, whereas avascular necrosis rarely occurs unless glucocorticoids are given. In infants, skeletal abnormalities often resemble vitamin D deficient rickets. Widening of the metaphyseal regions may develop in all long bones. The degree is dependent on the severity of metabolic bone disease and metaphyseal growth which varies with age. Particular attention has to be paid to deformities in small children when they start to bear weight. Physical examination should always include a detailed bone status and in case of suspicious findings be followed by radiological studies. According to the International Pediatric Peritoneal Dialysis Network clinical symptoms and/or radiological signs of bone disease are seen in about 15% of children on PD including 5% with limb deformities, with the latter possibly necessitating corrective orthopedic procedures [49].

Slipped Epiphyses

Pronounced secondary hyperparathyroidism, hypocalcemia and severe osteitis fibrosa cause disintegration of the growth plate and increase the risk of epiphysiolysis. Fibrotic alterations develop in the region connecting the epiphysis and the metaphysis. The growth cartilage columns are disorganized and partly substituted by fibrous tissue predisposing to local displacement with shear stress. Slipped epiphyses are more common in children with severe hyperparathyroidism, especially when insufficiently controlled for an extended period of time. The most frequently affected site is the proximal femur, followed by distal radius, ulna, distal femur, humerus, tibia and fibula, often depending on the mechanical stress put on the joints. Virtually any epiphysis may be involved (Figs. 60.7 and 60.8). Since epiphyseal slipping is a potentially severe and eventually incapacitating complication resulting in osteonecrosis, severe deformities, and degenerative joint disease, one should always be aware of the characteristic clinical symptoms. These include pain, limping, waddling gait, inability to walk and limited range of motion on examination. In infants the deformities may develop within few weeks. Diagnosis is established by radiography, treatment includes correction of factors involved in the metabolic bone disease, in particular control of secondary hyperparathyroidism, reduced weight bearing and conservative orthopedic measures. If these measures are not successful, surgical intervention for stabilization may be required. Surgery should only be performed after control of secondary hyperparathyroidism has been achieved, if necessary by cinacalcet therapy or even parathyroidectomy. Even though prospective studies are missing, a failure of orthopaedic corrective measures has been documented in patients with uncontrolled high turnover bone disease.

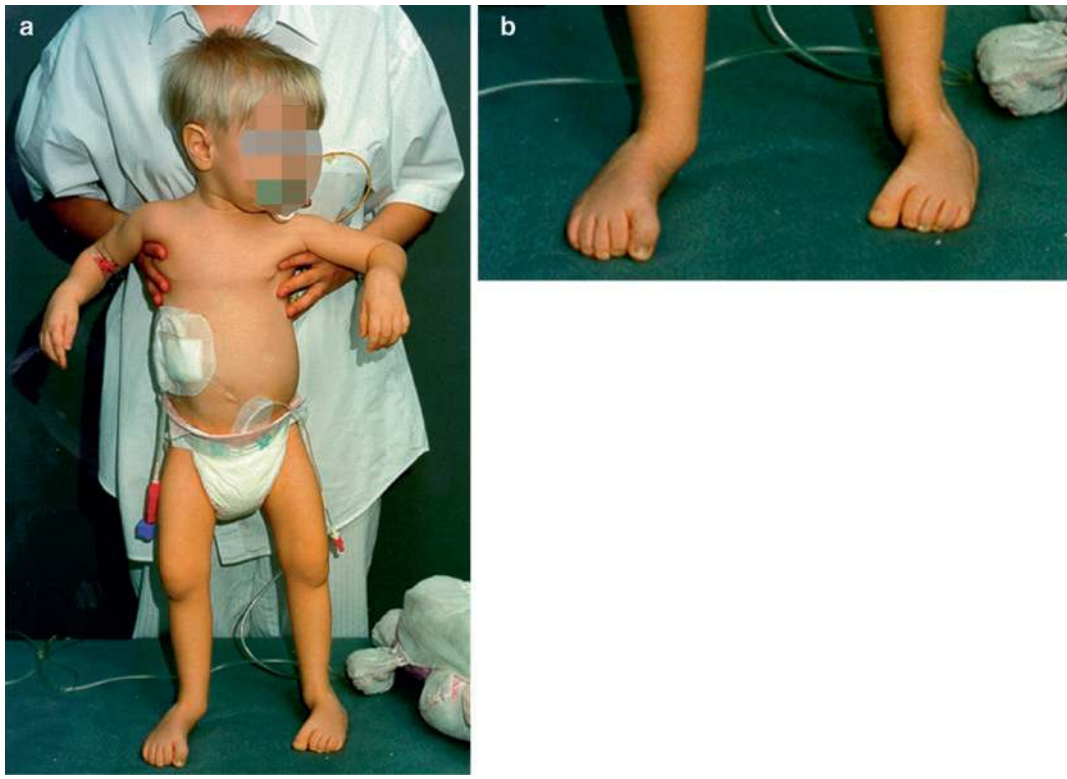

Fig. 60.7 3-year-old boy with ESRD at age 1 year due to prune belly syndrome. He rapidly developed severe renal osteodystrophy with epiphyseal slipping of multiple joints after non-adherence to calcitriol therapy

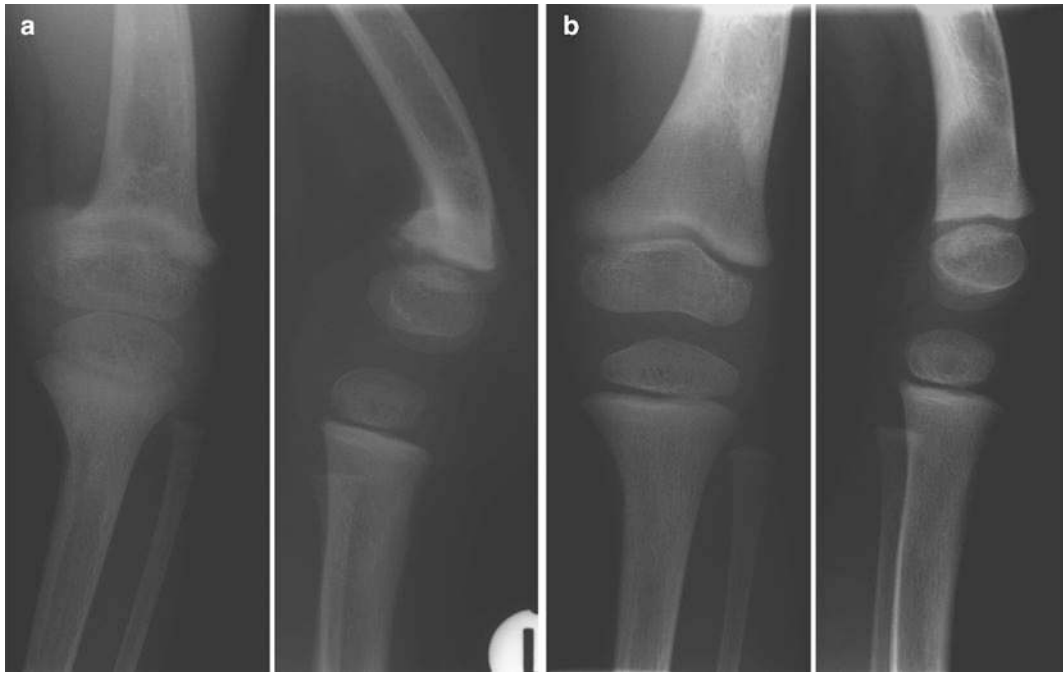

Fig. 60.8 X- rays of the left knee of the same boy as in Fig. 60.7. (**a**) Prior to therapy. (**b**) After 1 year of treatment with calcitriol and calcium i.v. and i.p. and parathyroidectomy with autotransplantation of parathyroid tissue. The severe deformities have substantially improved, surgical intervention was not required

Fractures

Healthy children have an incidence of 14 fractures per 1000 person years, mainly localized to the limbs [95]. In children with CKD fracture risk is increased [96]. Besides complete fractures, bone deformities, slipped epiphyses and microfractures are common. While microfractures may occur and be rapidly repaired at high bone turnover, subjects with low bone turnover may be at increased risk of macrofractures; however this concept has not been proven consistently [97]. In large prospective studies of children with CKD, including the North-American Chronic Kidney Disease in Childhood (CKiD) cohort, a 2.4- and three-fold higher rate of fractures have been reported in boys and girls, respectively, with predialysis CKD as compared to healthy children [98]. Fracture risk in pediatric CKD is associated with baseline-walking difficulty, Tanner stages 4–5 pubertal development, lower height Z-score, higher parathyroid hormone (PTH) levels, lower Ca and 25-hydroxyvitamin D (25D) levels and team sports participation [98]. Phosphate binder use, and particularly Ca-based phosphate binder use has been associated with decreased fracture risk in children with pre-dialysis CKD, suggesting that improved phosphate control and/or increased calcium intake may exert some protective action [98].

Studies using peripheral quantitative CT scans have demonstrated that children with CKD have significant deficits in cortical volumetric bone mineral density. A reduction in tibial cortical BMD z-score over 1 year follow-up was associated with low serum calcium and high PTH levels [89] as well as uremic myopathy [96]. Growth hormone appears to have a protective effect [96].

Bone biopsies in adults demonstrate that both low and high bone turnover are associated with distinct abnormalities contributing to the diminished mechanical competence of bone in CKD. Low turnover bone is characterized by lower cancellous bone volume and reduced trabecular thickness, high turnover bone by a reduced mineral to matrix ratio and lower stiff-

ness [85]. The fracture risk of adult dialysis patients correlates with plasma iPTH in a U-shaped manner, with the lowest risk observed at average PTH levels around 300 pg/ml [99]. After pediatric kidney transplantation the incidence of fractures is increased five-fold as compared to healthy children, i.e. to 76 fractures per 1000 patient years, two thirds of which affect the vertebrae [95].

Growth Retardation

Growth failure, a regular feature of children with CKD, is mainly due to endogenous growth hormone and IGF1 resistance, malnutrition (especially in infants) and metabolic acidosis. The effects of altered Vitamin D and PTH metabolism on growth are not entirely clear.

In children with CKD a short-term improvement of growth rate has been reported with 1,25-OH_2-D treatment [100]; this however was not confirmed in long-term observations. Excessive vitamin D treatment may even result in adynamic bone disease and growth retardation [91]. *In vitro*, calcitropic and somatotropic hormones interact with the proliferation of growth plate chondrocytes [101]. PTH-rP, which acts via the type I PTH receptor, is an important local inhibitor of growth plate chondrocyte maturation [102]. In rats, intermittent bolus injection of PTH increases growth rate whereas this is not seen with continuous PTH infusion, indicating that fluctuations in the level and not the PTH plasma concentration alone impact on bone [103].

From clinical observation it is not clear which PTH range is optimal for growth. In two studies a positive correlation between mean plasma PTH levels and growth was observed, suggesting that higher PTH levels promote growth [91, 104]. Others reported a normal growth rate in children with CKD stage II-IV with PTH levels in the normal range, as well as in CKD stage V with mean plasma PTH concentrations only 50% above the upper limit of normal. In these children particular attention was paid to adequate nutritional support and a strict control of serum phosphate levels within the normal range [105]. Whether normal bone turnover was maintained in these children is unclear since bone biopsies were not performed [104–106]. In 214 children followed prospectively in the International Pediatric PD Network (IPPN) Registry, the annual change in height SDS tended to be inversely correlated with mean plasma PTH levels. Patients with mean PTH > 500 ng/ml exhibited a significant loss in height SDS [43].

Cardiovascular Calcifications

Cardiovascular mortality is markedly increased in uremic patients. Vascular calcification is one of the main mechanisms underlying this most important long-term complication of renal replacement therapy. In a study published in 2000 36 of 39 young adults with childhood onset of CKD already had significant coronary artery calcifications [10]. Alterations of the morphological and functional properties of arteries have been reported as early as in the second decade of life in children on dialysis [9, 10, 107, 108]. The presence of vascular calcification on CT scan is directly related to hyperphosphatemia, the average calcium x phosphate product over time, intake of calcium containing phosphate binders and plasma PTH levels [9, 10]. In a post mortem analysis of 120 children with CKD, soft tissue and vascular calcification was associated with the use of active vitamin D and calcium containing phosphate binders [109]. Both low and high levels of 1,25(OH)2D have been associated with coronary artery calcification: very low 1,25(OH)2D levels were associated with high turnover bone disease as well as with greater inflammation whereas very high 1,25(OH)2D increase gut calcium absorption and suppress bone calcium and phosphate uptake with adynamic bone disease [110]. Although calcium balance studies have not been performed in children with CKD. Calcium supply should be higher with rapid growth, but it is suspected that the calcium load is often too high in children with advanced CKD treated with active vitamin D, calcium containing phosphate binders and dialysis solutions containing unphysiologically high calcium lev-

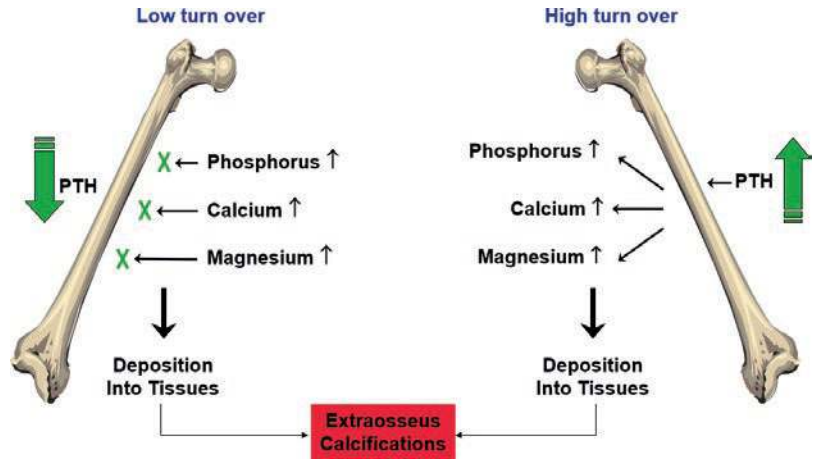

Fig. 60.9 Bone disease and soft tissue calcification. Low PTH levels (low turnover bone disease) prevent mineral deposition into bone, high PTH levels result in mineral release from bone. Excessive minerals precipitate in soft tissues

els. On the other hand, the calcium buffering capacity of bone is reduced in patients with low- and high-turn-over bone disease (Fig. 60.9). Noteworthy, calcifications are not merely a passive process of precipitation but triggered by oxidative stress, advanced glycation endproducts and regulated by locally and systemically acting proteins [71]. The plasma protein fetuin is significantly reduced in CKD patients and correlates with cardiovascular mortality [111] and vascular calcification even in children on dialysis [112].

Calcifications may also occur in other sites than the vascular wall, such as the lung or and in periarticular areas. Calciphylaxis, a severe form of extra-skeletal calcifications, has been described in children with tertiary hyperparathyroidism [113]. It is characterized by painful nodules that become mottled or violaceous, indurated and ultimately ulcerated. Accurate diagnosis and optimal treatment of disturbance in bone and mineral metabolism may be crucial in preventing these life-limiting sequelae in children with CKD. Vascular calcification is discussed in detail in chap. 58.

Post-transplant Bone Disease

Successful renal transplantation should reverse all pathologic conditions that lead to CKD-associated mineral and bone disease. In both adult and pediatric kidney recipients, bone histologic changes associated with secondary hyperparathyroidism resolve within 6 months after kidney transplantation; bone mineral content Z-scores, however, remain significantly reduced, especially in cortical bone [114]. Interestingly, a disconnect is observed between circulating PTH levels and bone turnover post transplantation; some patients have persistently elevated rates of bone turnover while others develop adynamic lesions, despite moderately elevated serum PTH levels [115].

Immunosuppressive treatment, in particular glucocorticoids, and any degree of CKD developing in the post-transplant course interferes with bone metabolism. Persistent hypophosphatemia is a common finding in renal allograft recipients, especially within the first months post-surgery. Tubular dysfunction secondary to any toxicity, persistent HPT and increased plasma FGF23 levels are considered the main causes of exaggerated renal phosphate secretion after transplantation. Of note, plasma phosphate levels can be normal despite reduced total body phosphate content. In these patients increased skeletal phosphate removal due to increased PTH and inappropriately low calcitriol levels may counterbalance the phosphatonin (FGF23)-induced persistent renal phosphate loss [116]. Likewise, magnesium, an integral part of the hydroxyapatite structure of the bone and essential for osteoblast and osteoclast function, is wasted secondary to calcineurin inhibitor use.

According to bone biopsy studies in adults, loss of bone volume and mineralization leading

to low turn-over bone disease is frequent in adults after successful transplantation [117, 118]. The risk of fracture is particularly high the first several months following transplantation. Skeletal lesions may improve subsequently, depending on renal function and pharmacological treatment, but do not resolve completely in the majority of patients [115]. Assessment of BMD by means of DXA shows conflicting results, possibly due to the inadequate standardization and the failure to correct for height and muscular mass. PQCT results demonstrate nearly normal BMD for height and muscle mass, while cortical thickness is reduced [119]. The fracture risk is increased (see above). Avascular necrosis is reported to develop in 4% of children after organ transplantation, most often in the femoral head, but it may also develop in the talus, the humeral neck and other skeletal sites [120]. Avascular necrosis has become less common in recent years, due to steroid sparing with newer immunosuppressive protocols.

Assessment of Renal Bone Disease

Apart from thorough physical examination, the diagnosis of the uremic bone mineral metabolic disorder is mainly based on repeated biochemical analyses (Table 60.2). Even though no single biochemical marker is able to provide a complete assessment of renal osteodystrophy, bone biopsies are rarely performed for the clinical management of patients. In a study of 161 children undergoing chronic PD, high PTH and low serum calcium levels were seen in those with defective mineralization, irrespective of their bone turnover rate [122]. As recommended by KDIGO guidelines, a thorough assessment of CKD-MBD parameters requires serial assessment of phosphate, calcium and PTH levels, considered together, with trends in levels being most useful in guiding clinical decision making [123]. The 2010 Cochrane review on 'Interventions for Bone Disease in Children with CKD' [124] and the 2013 NICE guideline on hyperphosphataemia management in adults and children with CKD and on dialysis [125, 126] emphasize the lack of pediatric information and the need for more research in children with CKD.

Biochemistry

Serum Calcium

Measurement of total calcium is the mostly used method to assess serum calcium levels. Normal serum concentrations depend on age (Fig. 60.10). Of note, normal total serum calcium may not indicate normal serum ionized calcium levels, and should be corrected for serum albumin concentration if this is low. pH corrected ionized calcium levels are the best reflection of free (biologically available) calcium. Importantly, serum calcium levels are not a reflection of the total body calcium stores, since calcium levels are tightly regulated by multiple negative feedback systems as described above.

Serum calcium is usually normal or low in untreated patients with advanced CKD, with the maintenance of calcium homeostasis being exerted mainly by a compensatory increase in PTH. Once treatment with active vitamin D and calcium containing phosphate binders is initiated, serum calcium levels increase and PTH

Table 60.2 Suggested monthly intervals for the frequency of measurements for biochemical markers of renal osteodystrophy [121]. More frequent measurements may be required in young children, in patients treated with severe renal osteodystrophy, non-compliant patients, and after renal transplantation

CKD stage	GFR (ml/min*1.73 m^2)	Calcium/Ca^{++}, phosphate, AP, PTH	Total ALP	PTH	Serum $25(OH)_2D_3$	Bicarbonate
2	60–89	6	12	12	12	6
3	30–59	6	6	6	6–12	6
4	15–29	3	3	3	3–12	3
5	<15, dialysis	1	1–3	1–3	3–12	1

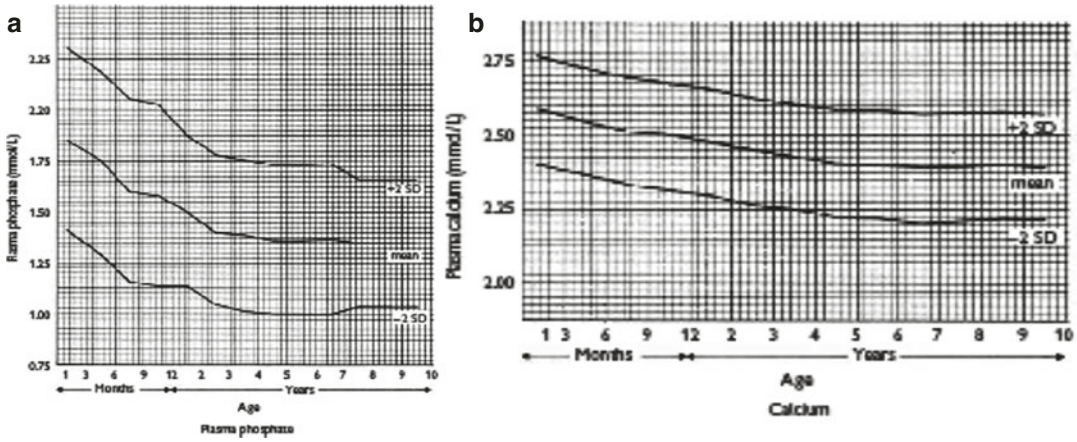

Fig. 60.10 Age-dependent calcium and phosphate percentiles in the plasma of healthy children. (**a**) Calcium, (**b**) Phosphate. *SD* standard deviation. Permission obtained from Blackwell Scientific © Claytob B.E. et al. Pediatric chemical pathology; clinical tests and reference ranges (1980)

declines. Hypercalcemia is still rare in calcitriol treated children with stage IV CKD [104], but seen in about 6% of measurements in children on peritoneal dialysis treated with calcitriol, calcium containing phosphate binders and PD solutions with calcium concentrations of 1.75 mmol/L [127]. Children with low turn-over bone disease and children with tertiary hyperparathyroidism treated with calcitriol are particularly prone to hypercalcemia, because the calcium buffering capacity of bone is reduced. An important differential diagnosis of hypercalcemia in children on dialysis is volume depletion. This condition is usually characterized by a high total but normal ionized calcium concentration. Alternative causes of hypercalcemia to consider include immobilization and, rarely, paraneoplastic release of PTHrP and extrarenal calcitriol production in granulomatous diseases such as tuberculosis and sarcoidosis.

Serum Phosphate

Dietary phosphate intake usually exceeds renal phosphate removal if GFR drops below 40 ml/min/1.73 m^2 but may be noted earlier if phosphate intake is high. The serum concentration of phosphate should be kept in the normal range. Of note, the renal phosphate threshold and serum phosphate concentrations are age-dependent with an upper limit of normal of 2.4 mmol/L in small infants, 2.1 and 1.9 in preschool and school children, and 1.44–1.9 mmol/L in adolescents according to different reference tables [128]; (Fig. 60.10). The physiological range depends on whether a child is growing or postpubertal. In non-fasting adolescents serum phosphate is subject to significant circadian variations following an M-shaped curve with peaks at 4 PM and 3:30 AM and a maximal diurnal amplitude of 1 mmol/L [129]. These fluctuations are not related to food intake and largely disappear with advancing CKD [130].

Parathyroid Hormone

Plasma PTH levels are a key element in the diagnosis and therapeutic monitoring of renal osteodystrophy and should be measured together with calcium, phosphate, alkaline phosphatase and blood bicarbonate at least every 6 months when GFR drops below 60, every 3 months at a GFR below 30 ml/min/1.73 m^2 and every month when end stage renal disease is reached (Table 60.2).

However, the interpretation of PTH measurements is compromised by significant variation between PTH assays and the variable presence of biologically inactive PTH fragments and oxidized

PTH as discussed above. The target range for PTH which allows for normal bone turnover and growth and which is not associated with increased vascular calcifications is not clear. In children with CKD stage 5, values of iPTH below 200 pg/ml have been suggested to indicate adynamic bone disease whereas values above 500 pg/ml are associated with osteitis fibrosa; however, considerable overlap exists [6]. A study of the IPPN registry found a markedly increasing risk of symptomatic bone disease with PTH exceeding 300 pg/ml and impaired longitudinal growth at mean PTH concentrations above 500 pg/ml, whereas the risk of hypercalcemia increased at levels below 100 pg/ml [49]. These findings support a PTH target range of 100–300 pg/ml in the pediatric age group (Table 60.3). In another study, children with PTH levels below twice the upper limit of normal had less vascular calcification compared to those with PTH levels above twice the upper limit of normal [9].

Alkaline Phosphatase

Measurements of serum total alkaline phosphatases, a marker of osteoblast activity, can be helpful in predicting bone turnover in conjunction with PTH [131]. Values are strongly age dependent with higher activity present during periods of rapid growth. Bone specific alkaline phosphatase may further increase predictability of bone turnover and may be advantageous in subgroups of patients, e.g. with hepatic diseases, to exclude measurement of non-skeletal enzyme. The potential benefits have to be weighed against the considerably higher costs.

Table 60.3 Target iPTH range in children with CKD 2–5 (adapted from 133;135). Recommendations for CKD stage 2–4 are opinion based; stage 5 recommendations are evidence based but still debated. Whole-PTH assays provide values which are only 50–60% values obtained with iPTH assays; this however may vary considerably

CKD stage	GFR (ml/min*1.73 m^2)	Target iPTH range (pg/ml)
2	60–89	35–70
3	30–59	35–70
4	15–29	70–110
5	<15, dialysis	100–300

25(OH)D and 1,25(OH)$_2$D

Serum levels of 25(OH)D give an estimate of vitamin D body stores; it's serum half-life is 3 weeks. There is debate about what levels of vitamin D can be considered adequate, but most authorities agree that a level of 20–30 ng/ml is sufficient to maintain normal serum calcium levels and prevent hyperparathyroidism. Recently published ESPN guidelines suggest that serum 25D levels be maintained above 75 nmol/L (30 ng/ml), and below 120 nmol/L (48 ng/ml) [29]. These levels maintain optimal gut Ca absorption and prevent seasonal fluctuations that can predispose even healthy children to nutritional rickets [28]. Serum levels <5 ng/ml have been categorized as severe deficiency, 5–15 ng/ml as mild deficiency and serum levels of 16–30 ng/ml as vitamin D insufficiency. Since 25(OH)D may have numerous physiological functions, vitamin D deficiency must be avoided at all stages of CKD. 25(OH)D serum levels should be measured 6–12 monthly depending on CKD stage in children not on vitamin D treatment. In case vitamin D supplementation is required, levels should be measured again after 3 months [29] (Table 60.2). Oral 1,25(OH)2D is rapidly absorbed and peak plasma levels are reached within 3 h in children with CKD [89]. Due to the short plasma half-life of 1,25(OH)$_2$D (4–6 h), the clinical usefulness of 1,25(OH)$_2$D measurements in plasma is limited. However, plasma 1,25(OH)$_2$D levels have been correlated with the presence of vascular calcification in children on dialysis [110].

Additional Serum Markers of Bone Turn Over

Biomarkers of bone formation, e.g. osteocalcin and procollagen type I carboxyl terminal peptides, and of bone resorption, e.g. type I collagen cross linked telopeptide and pyridinoline, have not been studied widely to date in children with CKD. Most of them are eliminated via the kidney and accumulate with reduced renal function. TRAP-5b and osteoprotegerin may prove as useful indicators of bone metabolism, based on the

central role of RANK ligand/osteoprotegerin in bone metabolism [71]. FGF23 has been correlated with indices of bone mineralization in children [4] and sclerostin, an inhibitor of bone formation, with bone turn-over rates [75]. Further studies correlating the circulating levels of these potential biomarkers to bone histology, growth and established biochemical markers will be required to determine their added value in the monitoring of uremic bone disease.

Aluminum

Aluminum containing phosphate binders are not recommended in children and dialysis water purification has generally improved. Therefore, aluminum related bone disease and encephalopathy should not develop anymore, and plasma aluminum levels do not need to be determined on a regular base. Exposure to aluminum, however, may still occur in some countries.

Imaging

Radiography of the Skeleton

Conventional radiographs of the skeleton are relatively insensitive in detecting renal bone disease, and only grossly evaluate bone structure and mineralization. They should be performed in patients if results are expected to impact on treatment, in case of bone pain and suspicion of fracture and in children with genetic diseases with specific bone involvement [121]. Hand and wrist X-rays can demonstrate severe hyperparathyroidism induced hyperosteoclasia and osteitis fibrosa as subperiostal resorption zones (Fig. 60.11), subperiostal erosions of the phalanges, and acroosteolyses at the end phalanges and at sites of ligament endings. In the skull, hyperparathyroidism results in ground-glass- or granular appearance, focal radiolucencies and sclerotic areas. In children with CKD widened radiolucent areas of the growth zones also indicate accumulation of fibrous tissue, in contrast to nutritional rickets where the radiolucent areas are mainly due to accumulation of unmineralized growth cartilage. Brown tumours also present accumulation of hyperosteoclastic tissue. They can typically be seen at the metaphyses of long bones but also at other skeletal sites such as the jaw. In contrast to hyperosteoclastic patients, there are no specific signs of osteomalacia. Even Looser zones, straight wide radiolucent bands within the cortex, can represent osteomalacia lesions but also fibrous tissue.

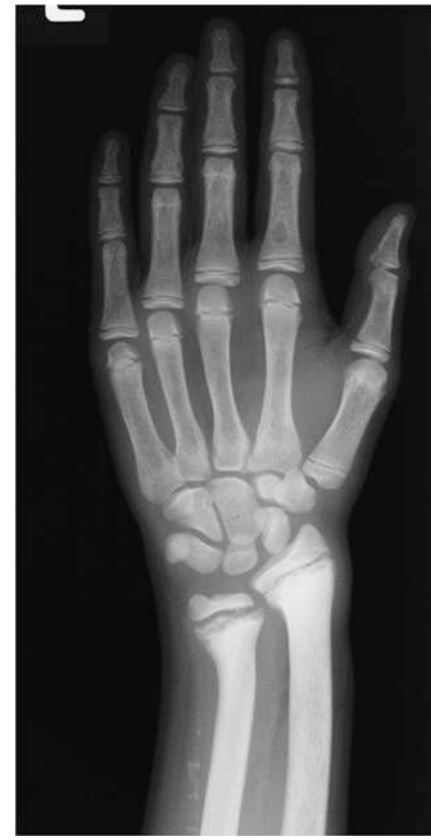

Fig. 60.11 Left hand X-ray in 18-year-old boy with signs of severe renal bone disease: subperiostal erosions, especially of the middle phalanges, brown tumor in the second digit, "ricket-like" lesions and vascular calcifications at the forearm (Courtesy of J. Troeger, University Hospital for Pediatric and Adolescent Medicine, Heidelberg, Germany)

Measurements of Bone Mineral Density

Measurements of bone mineral density are not routinely used in the monitoring of CKD-associated bone disease. Prospective interven-

tional studies are lacking and problems in interpreting results are common. Dual-energy X-ray absorptiometry (DXA) is the most widely used method for measuring bone mineral content and bone mineral density (BMD). BMD (g/cm^2) is often falsely taken as a surrogate parameter of bone strength and fracture risk. BMD increases physiologically in childhood with age and, more closely, with measures of body size such as height and weight. As a consequence, measurements should not be normalized to age but to body height, bone size or muscle function [132]. The interpretation of DEXA measurements is further complicated by the lacking distinction between cortical and cancellous bone. The ISCD 2007, the KDIGO 2017 guideline update and the European Pediatric Clinical Practice Points [121] recommend against routine DXA BMD testing in CKD3–5 since BMD does not predict the type of renal osteodystrophy. This is because PTH excess has generally catabolic effects on cortical bone with decrease in cortical volumetric BMD and cortical thickness whereas it exerts anabolic effects on trabecular bone. A recent study showed that combination of routine biomarkers were better predictors of cortical BMD evaluated by pQCT, and BMD measurement by DXA did not correlate with biochemical data or pQCT measures [88]. Thus, these imaging techniques are not recommended for routine screening tools of bone health or fracture risk prediction in pediatric CKD patients [121].

Peripheral Quantitative Computer Tomography (pQCT)

Peripheral Quantitative Computer Tomography (pQCT) is an alternative technology which permits resolution of cancellous and cortical bone. pQCT of the tibia provides a detailed picture of bone health, including volumetric bone density, separate evaluation of cortical and trabecular components, and measurement of bone dimensions, parameters of bone strength and muscle mass. The reported measurement error in children and adolescents is <2%. pQCT has been widely used in children with CKD and is a good predictor of future fracture risk [89]. In children with CKD, pQCT revealed reduced cortical area and a decline in cortical BMD with time, in association with high PTH plasma levels [96]. pQCT also unmasks differences in total bone density between patients with high turnover and adynamic bone disease [118]. Despite these promising findings, routine application of pQCT has not yet been recommended due to a lack of prospective information regarding an impact of pQCT monitoring on treatment outcomes.

Bone Biopsy

Micromorphometric analysis of the bone is the gold standard for characterization and quantification of renal bone disease. The biopsy procedure is safe and well tolerated by most children. It provides information on the current histologic status and, if double tetracycline staining is performed, the dynamics of bone formation and mineralization can be assessed. Bone biopsies are not needed for routine diagnosis and treatment of renal osteodystrophy, but may be considered if the clinical and biochemical findings do not explain underlying bone disease, e.g. severe bone deformity or pain, low-energy fracture, persistent hypercalcemia or hypophosphatemia, despite optimized treatment [121]. Since few bone biopsies have been performed in children with CKD in the last 20 years, cooperation with the few remaining centres with respective experience regarding the technical procedure and histomorphometric analyses is recommended. Bone biopsies may have a role in clinical trials in which the effect of therapeutic interventions require histological verification.

Imaging of the Parathyroid Glands

In children with severe secondary hyperparathyroidism, sonography of the parathyroid glands should be performed. Parathyroid glands usually cannot be detected by ultrasound unless they are enlarged. The indication of advanced imaging procedures in patients with refractory hyperpara-

thyroidism is controversial. MIBI scan and MRI may give additional information in cases where parathyroidectomy is considered, e.g. an ectopic gland, especially prior to re-exploration of unsuccessful parathyroidectomy. Preoperative imaging by ultrasound, and even by MIBI scan or MRI, may fail to detect adenomas. Therefore, a negative result should not prevent thorough surgical exploration if clinically indicated.

Imaging of Vascular Alterations

The intima media thickness (IMT) of the common carotid artery as assessed by high resolution sonography is a sensitive marker of early vascular lesions, and in adults with CKD it is a strong predictor of future cardiovascular events and death [133]. In children with CKD, carotid IMT has been correlated with the time averaged serum Ca x P product, the cumulative dose of calcium-based phosphate binders, and the mean calcitriol dose [9, 107, 108]. In experienced hands, sequential sonographic IMT assessments can be a valuable diagnostic tool of vasculopathy secondary to altered bone and mineral metabolism.

In cases of severely disturbed bone and mineral metabolism, plain x-rays may be performed to screen for vascular and soft tissue calcifications (Fig. 60.11).

Coronary artery calcifications can be assessed quantitatively by electron-beam or ECG-gated computer tomography, and vessel stiffness by applanation tonometry, ultrasound based or newer oscillometric techniques. These are discussed in detail in Chap. 58.

Treatment

CKD-MBD management in children needs to focus on three key areas. First, to maintain normal calcium - phosphate homeostasis so as to obtain acceptable bone quality and cardiovascular status, second to provide optimal growth in order to maximize the final height and third, to correct all metabolic and clinical abnormalities that can worsen bone disease, growth and cardiovascular disease, i.e. metabolic acidosis, anaemia, malnutrition and 25(OH)vitamin D deficiency.

Many of the factors contributing to the development of secondary hyperparathyroidism and renal osteodystrophy are present early in the course of renal disease (Fig. 60.1). Since patients are usually asymptomatic, insufficient attention is often paid to progressive alterations in bone and mineral homeostasis. With advancing renal failure, parathyroid glands become hyperplastic and less sensitive to therapeutic interventions. Therefore, **prevention** should always be the primary objective in children with CKD in order to delay the development and its osseous and cardiovascular sequelae. An overview of key preventative strategies is given in Table 60.6. A scheme summarizing the treatment in patients with established hyperparathyroidism is given in Fig. 60.12.

Optimal control of serum **phosphate** levels is probably the crucial element of preventive management. Hyperphosphatemia is an independent risk factor for secondary hyperparathyroidism, renal osteodystrophy and vascular calcifications. As early as in CKD stage II, regular dietary counseling should be performed and drug therapy may already be considered. On the other hand, hypophosphatemia, as commonly seen in children with associated tubulopathies even in CKD stage II–IV or after renal transplantation, may induce hypophosphatemic osteomalacia and should also be avoided.

Serum **calcium** must be maintained in the normal range, taking care of maintaining a normal serum calcium phosphorus ion product. The upper limit for the serum calcium × phosphorus ion product recommended for adults (55 mg^2/dl^2) is applicable in adolescents, whereas the upper normal limit is higher in children below 12 years of age (65 mg^2/dl^2) and even higher in infants. The calcium × phosphate product is rarely used in clinical practice now since it is not a 'biological' value and does not represent the true physic-chemical coupling in hydroxyapatite crystals (chemical formula $Ca_{10}(PO_4)_6(OH)_2$). Clinicians are advised to follow serum calcium and serum phosphate levels, particularly trends in both of these, rather than the calcium × phosphate product.

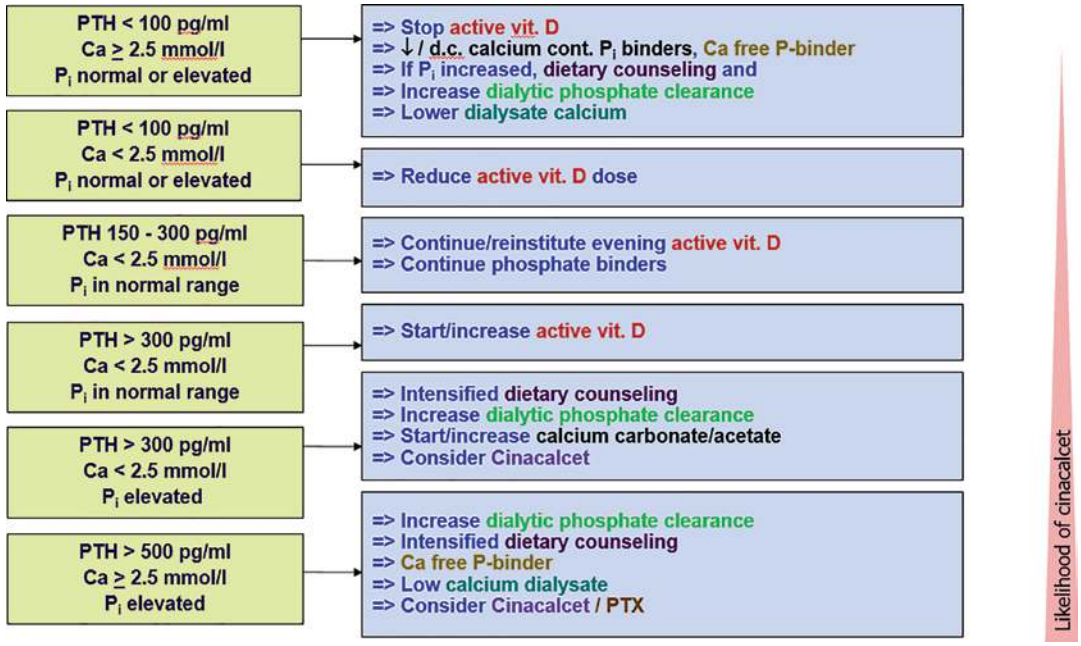

Fig. 60.12 Therapeutic algorithm for treatment of hyperparathyroidism in children with CKD

In CKD stage 5, serum calcium levels in the upper normal range should be avoided [128, 134] and hypercalcemia must be prevented. In case of hypercalcemia, all calcium containing phosphate binders must be withheld, vitamin D therapy be stopped and, if hypercalcemia persists, PD fluid with 1.25 mMol/L calcium be administered. Even lower dialysate calcium concentrations may be used in HD patients. On the other hand, pediatricians must keep in mind that growing infants and children need a positive calcium balance. In growing children hyperparathyroidism may often be triggered insufficient calcium supply. The routine use of dialysate calcium concentrations of 1.25 mmol/L may be inadequate in patients with major UF associated calcium losses. Thus 1.5 and 1.75 mMol/L dialysate calcium concentrations are often needed, depending on the oral calcium load and active vitamin D treatment.

The sensitivity of the skeleton to **PTH** effects decreases with declining renal function. Therefore, an increasing PTH target level has been suggested. These target levels are given in Table 60.3. For CKD stage II–IV target PTH recommendations only reflect expert opinion. For patients with CKD stage V there is some biopsy-derived evidence for the indicated target ranges, which, however, is challenged by recent studies [105, 135]. If plasma PTH levels are below the target range and serum calcium levels are increased vitamin D therapy and calcium containing phosphate binders should be reduced or even discontinued, a reduction in dialysate calcium concentration may be justified. Of note, growth hormone should not be given to patients with uncontrolled hyperparathyroidism, symptomatic high turn-over bone disease or slipped epiphysis.

Diet

Regular dietary counseling is an essential part of CKD-MBD management. Optimal control of serum P concentration is a crucial element of preventative management, starting early in the course of CKD. K/DOQI and the Pediatric Renal Nutrition Taskforce [136] have published guidelines on the dietary management of Ca and P in children with CKD2–5D. They recommend regular dietary counseling from early CKD and limit-

ing dietary P intake to within the age-appropriate normal ranges for children in the mild-moderate stages of CKD and to the lower limit of the normal range in patients with advanced CKD who have persistent hyperphosphatemia or hyperparathyroidism [128, 136, 137]. For detailed dietary recommendations regarding dietary calcium and phosphorus intake, please refer to Chap. 55.

Importantly, processed foods are likely to contain inorganic P additives (such as phosphoric acid and sodium phosphate), which are almost completely absorbed, unlike organic phosphates that have a lower bioavailability. Aggressive dietary P restriction is difficult since it may compromise adequate intake of other nutrients, especially protein and Ca [15]. P intake is directly linked to protein intake, with 10–12 mg of P accompanying each gram of protein; any reduction in P intake should not compromise protein intake. In a recent study 67% of children with CKD stages 4–5 had a dietary Ca intake below the recommended nutrient intake, and additional Ca from medications, mainly in the form of P binders, was required to maintain nutritional Ca requirements [15].

Dialysis

The efficacy of dialysis in removing excess phosphate is modifiable to some extent but overall limited [138]. E.g., an anuric child of 20 kg body weight and a daily protein intake of 1.4 g/kg ingests 430 mg phosphate per day. Concomitant intake of calcitriol and phosphate binders will result in absorption of roughly 50% of phosphate (215 mg/d). This amount of phosphate is the maximum that can be eliminated by conventional dialysis. At a protein intake of 2 g/kg*d, the phosphate balance will be positive by more than 60 mg per day on a conventional PD or hemodialysis schedule. A weekly creatinine clearance of more than 80 L/1.73m^2 would be required to compensate the dietary phosphate load. This efficacy can only be achieved by frequent [139] or long nocturnal hemodialysis [140]. Slow-flow nocturnal hemodialysis performed over 6–9 h per night five to six times per week dramatically improves phosphate clearance and may even require phosphate supplementation p.o. or in the dialysis bath to prevent phosphate depletion and aggravation of bone disease [141, 142]. If such intensified hemodialysis is not available or not feasible, repeated dietary counseling and optimized oral phosphate binder management are essential (see below). In children receiving automated peritoneal dialysis, total PD fluid turnover should be maximized using maximally tolerable fill volumes, maximally acceptable cycler times and additional day time exchanges [138]. In a prospective multi-centre study serum phosphate levels were similar between HD and HDF patients but PTH levels declined in the HDF cohort over 12 months while remaining static in those on HD [143]. A further study has shown that FGF23, a middle molecular weight substance, if cleared more efficiently on HDF, with 30% lower levels than conventional HD [144].

Phosphate Binders

Restriction of dietary phosphorus intake may be sufficient in early stages of CKD. However, even with some dietary restriction of phosphate intake, normophosphatemia may only be maintained at the expense of increased PTH and FGF 23 plasma levels, both of which decrease tubular phosphate reabsorption. As renal function deteriorates, dietary control of phosphate becomes more difficult and overt hyperphosphatemia usually develops. Oral phosphate binding agents should be added. Calcium salts are used as first line phosphate binders. They have limited phosphate binding capacity (Table 60.4). The dose required depends on the oral phosphorus intake, and often reaches several grams per day. While in hypocalcemic and rapidly growing children the additional calcium load still is beneficial, many patients tend to develop hypercalcemia with prolonged administration. Significantly more calcium is absorbed and little phosphorus retained when calcium containing phosphate binders are not given with meals. Calcium acetate contains 25% of elemental calcium, calcium carbonate 40%. Calcium acetate binds more phosphorus per unit of calcium content and thus allows for higher doses and improved phosphate

Table 60.4 Phosphate binding agents

Compound	Calcium content (%)	Calcium absorbed (%)	Phosphate bound per g compound	Phosphate bound per Ca^{++} absorbed	Comment
Calcium Carbonate	40	20–30	39 mg / g	≈ 1 mg/8 mg	High Ca^{++} load, inexpensive, GI side effects
Calcium Acetate	25	22 (between meals 40)	45 mg / g	≈ 1 mg/3 mg	GI side effects, less Ca^{++} load than $CaCO_3$, inexpensive
Mg + Ca carbonate	Variable	20–30% of Ca	NA	≈ 1 mg/2.3 mg	Less Ca^{++} load, GI side effects, long term effects?
Sevelamer HCl/– Carbonate	0	0	Similar to calcium acetate[a]	NA	Ca^{++} and Al^{+++} free, cholesterol ↓, costs ↑, acidosis ↑ (in case of HCl)
Aluminum cont. Binders	0	0	Similar to calcium acetate	NA	Effective but toxic, not recommended

NA not applicable or no data
[a]See Ref. [145]

Table 60.5 Suggested treatment for vitamin D supplementation in children with CKD and on dialysis. With permission from [29]

Age	25(OH) D serum (nmol/L)[a]	Vitamin D supplementation dose (daily)	Monitoring
Intensive replacement phase			
<1 year		600 IU/day[b]	– Serum calcium and urinary calcium levels 1–3 monthly based on CKD stage
>1 year[b]	<12	8000 IU/day	– 25(OH)D levels: after 3 months
	12–50	4000 IU/day	
	50–75	2000 IU/day	
Maintenance phase			
<1 year	>75[d]	400 IU/day	– 25(OH)D levels: after 6–12 monthly
>1 year[c]		1000–2000 IU/day based on CKD stage	

[a]To convert nmol/L to ng/ml divide by 2.5
[b]In infants <1 year, a fixed dose is recommended irrespective of the level of 25(OH)D
[c]Consider adjusting dose by body size (weight or body surface area)
[d]If levels remain <75 nmol/L, then give doses as per the 'intensive replacement' schedule for a further course of intensive replacement and recheck levels

control. If given at similar doses, calcium acetate results in a reduced incidence of hypercalcemia as compared to calcium carbonate (Table 60.4). On the other hand, less gastrointestinal side effects have been reported with calcium carbonate. An individual choice is required to assure optimal patient compliance and control of calcium and phosphate uptake.

Calcium citrate is also effective, but citrate increases aluminum resorption and should therefore be avoided (Table 60.5).

Magnesium Salts

Magnesium salts have a relatively weak intestinal phosphate binding activity and require higher dosing, but avoid calcium exposure. An inverse relationship between serum Mg, hyperparathyroidism and vascular calcification has been demonstrated in adult dialysis patients and thus potential benefits have been attributed to magnesium salts [146]. Interestingly, in addition to the beneficial effects of intestinal phosphate binding of magnesium, experimental studies suggest inhibition of vascular smooth muscle cell transition into an osteoblast like phenotype, and inhibition of nanocrystal formation from calcium and phosphate ions, a key mechanism in the formation of ectopic calcification in CKD [147]. Diarrhea, hyperkalemia and hypermagnesemia are their main side effects. The long-term effects of an increased magnesium load are not clear. At present, magnesium containing phosphate binders may be given as adjuncts to calcium containing binders.

Table 60.6 Measures for prevention of MBD in children with CKD

• Active screening for hyperparathyroidism—measurement of PTH begins when the GFR falls to <90 ml/min/1.73 m^2
• Control of hyperphosphataemia
– Diet
– Phosphate binders:
first line—calcium-based phosphate binders
second line or if hypercalcaemia—calcium-free phosphate binders
(in addition to calcium-based binders or as a substitute)
• Control of hypocalcaemia using lowest possible dose of vitamin D
– native vitamin D in CKD 1–3
– active vitamin D analogues in CKD 4–5 and 5D
• Improve dialysis efficiency for phosphate removal
• Pre-emptive renal transplantation where possible
• Avoid vitamin K antagonists like warfarin to prevent inactivation of calcification antagonist MGP
• Cinacalcet, especially in case of high doses of active vitamin D and high serum calcium concentrations
• Parathyroidectomy—in case of hyperparathyroidism refractory to all therapeutic measures including cinacalcet

Calcium Free Phosphate Binders

Growing evidence for a role of the oral calcium load in the progression of cardiovascular calcifications urged the development of calcium free phosphate binders. They are especially indicated in patients with a calcium intake exceeding twice the recommended daily intake (which increases from 210 mg elemental calcium per day in the first 6 months of life to 1250 mg/d in adolescents), reduced PTH levels (with likely adynamic bone disease), hypercalcemia or even emerging soft tissue calcifications.

Sevelamer hydrochloride is a hydrogel of polyallylamine, which is resistant to digestive degradation and therefore not absorbed. It binds dietary phosphate and releases hydrochloric acid. This may reduce plasma bicarbonate levels [145], a problem which can be circumvented by the use of sevelamer carbonate [148]. Intestinal binding of bile acids significantly lowers LDL cholesterol. The major advantage of sevelamer is that in contrast to calcium containing phosphate binders, sevelamer reduces serum phosphate without increasing serum calcium levels. Hypercalcemia occurs less frequently: in an 8-week cross-over study in children, sevelamer and calcium acetate were equally effective at reducing serum phosphate levels, but significantly less hypercalcaemia occurred in the sevelamer group [145].

Aluminum Containing Phosphate Binders

Aluminum containing phosphate binders are efficient phosphate absorbers but may result in aluminum intoxication. Their administration is not recommended in children. If their use cannot be avoided, e.g. in case of a severely increased calcium phosphate product not manageable otherwise, dosage should be limited to 30 mg/kg*d. Aluminum containing binders should only be given if other calcium free alternatives (see below) are not available. Regular monitoring of aluminum blood levels is needed. In case of persistent hyperparathyroidism cinacalcet and even parathyroidectomy should be considered (see below) to avoid extended exposure to aluminum containing binders.

Lanthanum carbonate binds intestinal phosphate more effectively than calcium carbonate and sevelamer. However, patients on extended treatment show increased lanthanum serum levels. Significant tissue accumulation has been demonstrated in rats. In light of the past experience with aluminum toxicity, lanthanum containing phosphate binders are currently not recommended in children with CKD. Nonetheless, according to the International Pediatric PD Network 1.6% of all children monitored worldwide until 2020 have been receiving lanthanum containing phosphate binders.

Iron-Based Phosphate Binders

Iron-based phosphate binders reduce serum phosphate and FGF 23 concentrations while simultaneously increasing serum iron stores in

patients with CKD and on dialysis [149, 150]. A recent randomized open-label study has shown that sucroferric oxyhydroxyde is as effective as Ca acetate in controlling serum P levels in children with CKD [151]. Similar to other P-binders, adverse events are primarily GI-related.

Newer P-Binders

Intestinal P absorption is regulated by sodium-dependent P co-transporter type 2b (NPT2b). Treatment with niacin (vitamin B3) or its metabolite nicotinamide/niacinamide, both of which modulate NPT2b expression, result in a sustained reduction in serum P concentrations in adult dialysis patients [152]. However, in a randomized trial of adult HD patients, NPT2b inhibition was not effective in reducing serum P concentrations [153]. Targeting salivary P using P-binding chewing gum has also been proposed [154]. These agents have not yet been studied in children.

Long-term Safety of Phosphate Binder Therapy

The long-term cardiovascular safety of phosphate binder therapy is still controversial. A randomized clinical trial in adults with a GFR of 20–45 ml/min/1.73 m^2 demonstrated an increase in arterial calcification with calcium, lanthanum and sevelamer binder therapy, but not in placebo treated patients [155]. Others showed progressive arterial calcification in predialysis patients on low-phosphorus diet alone. Progression was less in calcium carbonate-treated patients, and absent in sevelamer-treated patients [156]. The Independent trial demonstrated improved survival in predialysis patients randomized to sevelamer as compared to those treated with calcium carbonate [157, 158]. A recent meta-analysis comprising 104 studies involving 13,744 adults concluded that sevelamer may lower death (all causes) compared to calcium-based binders in patients on dialysis and incur less treatment-related hypercalcaemia, but demonstrated no clinically important benefits of any phosphate binder on cardiovascular endpoints or fracture risk. Likewise no such beneficial effects could be demonstrated in patients with CKD2–5 [159]. Respective data in children is scant. Still, in view of the key pathophysiological role of phosphate in CKD MBD cardiovascular disease, most children with hyperphosphatemia are treated with phosphate binders, considering the calcium demand of the growing bone and the treatment associated calcium load.

Vitamin D

Oral treatment with ergocalciferol or cholecalciferol is suggested when serum 25(OH)D concentrations are below 30–40 ng/ml in order to prevent muscular weakness, secondary hyperparathyroidism and osteomalacia. At least in early stages of CKD, supplementation of cholecalciferol can prevent or reduce hyperparathyroidism and should be prescribed before calcitriol is considered. In an RCT of 25(OH)D therapy in children with CKD stages 2–4, children on ergocalciferol who achieved 25D levels >75 nMol/L had a significantly longer time to development of secondary hyperparathyroidism compared to those on placebo [34].

No clear consensus exists on the preferable type of native vitamin D supplement (ergo vs cholecalciferol), its dosage, frequency of administration or duration of treatment in healthy children or children with CKD. The KDIGO and ESPN guidelines recommend a loading regimen or intensive replacement period for a variable duration of 4–12 weeks followed by a maintenance regimen [29, 128]. Both guidelines further recommend escalating doses for intensive replacement depending on the baseline 25(OH)D level; cholecalciferol doses range from 2000 to 8000 IU/day for intensive phase replacement and 1000–2000 IU/day for maintenance therapy. The therapeutic window is wide; hypercalcemia, hypercalciuria, and symptomatic toxicity have been reported only when serum 25(OH)D levels exceed 250 nmol/L. Current clinical practice recommendations for children with CKD suggest a target level of 75–120 nmol/L (30–48 ng/ml).

Vitamin D repletion strategies include daily, weekly, monthly and even 3-monthly dosing regimens. Several trials have evaluated the short-term safety, efficacy and tolerability of these protocols. A recent randomized trial compared cholecalciferol dosing regimens for achieving and maintaining 25OHD concentrations ≥30 ng/ml in children with CKD stages 2–4, and showed that cholecalciferol as daily, weekly or monthly administration yielded similar 25OHD concentrations without toxicity [160]. Children with glomerular disease required higher doses of cholecalciferol compared to those with non-glomerular disease.

Active Vitamin D Sterols

Recent ESPN guidelines have addressed treatment with active vitamin D sterols [161]. These are indicated in pediatric CKD patients whose serum PTH levels remain elevated despite normal serum 25D and P levels. Alfacalcidol (1-α hydroxyvitamin D_2) or calcitriol (1,25-dihydroxyvitamin D_3) are most commonly used. The dose of calcitriol depends on initial PTH, Ca and phosphate values. An initial dose of 5–10 ng/kg*d is effective and safe in most children with CKD [128, 161]. Alfacalcidiol is often used in infants and small children since a liquid formula is available, and the dose can be carefully titrated. Higher doses relative to calcitriol may be required due to the requisite hepatic metabolism to 1,25 vitamin D. The frequency of calcium, phosphate and PTH monitoring should be adapted to the dose of vitamin D administered. All vitamin D analogues can get adsorbed to plastic tubing to varying degrees, and therefore should not be administered via feeding tubes.

Calcitriol efficiently controls secondary hyperparathyroidism. With prolonged treatment a substantial number of children, however, develop adynamic bone disease, which has been associated with a reduced growth rate and frequent episodes of hypercalcemia [58, 63].

A second consequence to consider with active vitamin D treatment is the increase not only of intestinal absorption of calcium [162] but also of phosphate. Calcitriol increases intestinal phosphate absorption from around 60% to 90% [163] (Fig. 60.13). Calcitriol use often is limited by aggravated hyperphosphatemia and hypercalcemia. Calcitriol, hypercalcemia and hyperphosphatemia contribute to extraosseus tissue calcifications and decreased survival in children with end stage renal disease [67, 68]. Deterioration of renal function is not accelerated by calcitriol [164], at least if administered at moderate doses not resulting in increased serum calcium and phosphate concentrations.

All activated vitamin D analogs increase FGF23 secretion. Although the consequences of these increased FGF23 levels in dialyzed patients remain to be completely determined, current evidence suggests that excessive circulating FGF23 is associated with increased mortality rates and cardiovascular disease [41]. Two randomized trials have demonstrated that vitamin D analogs do not improve cardiac function for patients with CKD stages 3–5 during one year of treatment [165, 166].

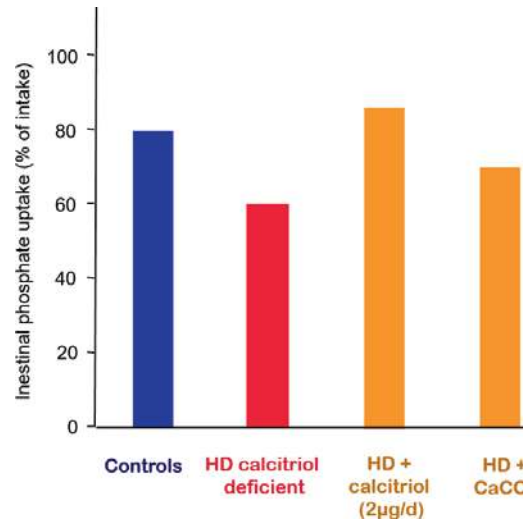

Fig. 60.13 Effect of calcitriol and oral phosphate binder on intestinal phosphate uptake. Calcitriol significantly increases phosphate uptake in hemodialysis patients (HD). $CaCO_3$, given at a dose of 75 mEq prior to a standardized meal, reduces phosphate uptake but increases oral calcium intake by 1.4 g compared to placebo, the intestinal calcium uptake increases to 28% of the ingested amount as compared to 14% with placebo

The Mode of Calcitriol Administration

The mode of calcitriol administration is of minor importance. Some studies including bone biopsies suggested a strong effect of calcitriol on bone metabolism with intermittent oral, intravenous and intraperitoneal administration. These studies, however, were not controlled against a daily administration mode [127]. Prospective randomized studies comparing daily versus intermittent oral calcitriol in healthy children and children with CKD stages 2–4 revealed no differences in PTH suppression, intestinal calcium absorption, the incidence of hypercalcemia and hyperphosphatemia or longitudinal growth rates [167, 168]. Overall, the response to calcitriol depends less on the mode of administration than on the degree of secondary hyperparathyroidism, hyperphosphatemia and the degree of parathyroid gland autonomy.

Synthetic Vitamin D Analogues

Synthetic vitamin D analogues have been developed to reduce intestinal calcium and phosphorus absorption at equipotent PTH suppressive action. Three sterols have been approved, i.e. 22-oxacalcitriol, 19-nor-1,25 dihydroxy vitamin D2 (paricalcitol) and 1α-hydroxyvitamin D2 (doxercalciferol). In adult hemodialysis patients, paricalcitol treatment is associated with a more rapid achievement of PTH control and fewer episodes of sustained hypercalcemia and increased Ca × P product than calcitriol therapy In children with CKD 3–5 and on hemodialysis paricalcitol appears to be efficacious and safe [169, 170]. The still limited evidence for a superior efficacy-safety profile of the synthetic vitamin D analogues must be balanced against their high costs.

There is also observational evidence for a survival benefit associated with the use of vitamin D sterols in general, which awaits confirmation in prospective trials. It may reflect a plethora of beneficial immunological and cardiovascular effects of active vitamin D compounds. In summary, active vitamin D sterols are indispensable therapeutic agents in the management of CKD but should always be used with caution and in awareness of their limited therapeutic window, beyond which major untoward effects to the bone, cardiovascular and potentially other systems may occur.

Calcimimetic Agents

Calcimimetics are a new class of compounds that bind to the parathyroid calcium sensing receptor, increase its sensitivity to ionized calcium by allosteric modification, and dose dependently decrease plasma PTH levels by up to 80%. The effect is largely independent of baseline PTH and phosphate levels and thus allows for control of parathyroid gland function even in patients with otherwise refractory hyperparathyroidism. **Cinacalcet** is the only currently approved calcimimetic agent. Cinacalcet also reduces serum calcium and phosphate levels, possibly via increased mineral deposition into bone. A randomized clinical trial in adult dialysis patients suggested attenuation of vascular and cardiac valve calcification with cinacalcet plus low-dose vitamin D sterols [171]. However, in a large placebo-controlled trial only a non-significant 7% reduction in death or first cardiovascular event was observed. A nominally significant 12% reduction in the risk of death was observed after adjustment for the unevenly distributed baseline characteristics [172].

Observational studies, industry sponsored phase 2 and 3 randomized studies, and one single arm extension study in pediatric dialysis patients demonstrated good efficiency of cinacalcet, i.e. suppression of PTH, and good tolerability [173]. Cinacalcet has therefore been licensed for children on dialysis above 3 years of age. According to a recent position statement of the respective European Working groups, the starting dose of cinacalcet should be about 0.2 mg/kg/day based on dry weight [174]. The dose may be increased at 4-week intervals by about 0.2 mg/kg/day to a maximum daily dose of 2.5 mg/kg (and not exceeding 180 mg), based on serum PTH levels and albumin corrected calcium levels >2.2 mmol/L. The cina-

calcet dose should be decreased in case of PTH levels between 100 and 150 pg/ml, and lower serum calcium, and withheld if PTH levels fall <100 pg/ml and if albumin-corrected serum calcium levels are <2 mmol/L and/or ionized calcium levels <1.0 mmol/L. About 10–20% of the children on cinacalcet developed episodes of hypocalcemia in clinical trials. A fatality reported together with diarrhea-associated hypocalcemia and long QT time demonstrates the need of careful serum monitoring, i.e. within one week after initiation of cinacalcet and up-titration, and monthly with stable cinacalcet dose. Next to hypocalcemia, the main side effects of cinacalcet are nausea and vomiting, which can be attenuated by evening administration. Prior to start of cinacalcet a normal corrected QT (cQT) time should be verified by ECG. Concomitant medications with the potential to increase the QTc or to interfere with cinacalcet metabolism need to be carefully considered [174]. At present, it is recommended to initiate cinacalcet in children in whom SHPT cannot adequately be controlled with standard of care therapy. Whether cinacalcet is useful earlier in the course of treatment, i.e. to prevent high dose active vit. D analogues is yet unknown. The calcium sensing receptor is expressed on epiphyseal chondrocytes and calcimimetics have been shown to reduce serum testosterone levels in HD patients by 30%. Hence, an impact on longitudinal growth and pubertal development cannot be ruled out. Reassuringly, animal studies and the clinical data obtained thus far do not suggest an impact of cinacalcet on pubertal development and longitudinal growth [174, 175].

Etelcalcetide is a novel calcium receptor sensitizing, calcimimetic compound with a prolonged half-life, administered intravenously. It has been licensed for adult patients on hemodialysis and has the potential to improve treatment adherence since it is administered during hemodialysis sessions. A recent phase 1, single dose clinical trial in 11 children on hemodialysis demonstrated similar pharmacokinetics and pharmacodynamics as in adults [176]. A pediatric single arm study over 6 months is currently under way. Whether the different pharmacodynamics with three-times-weekly i.v. dosing as compared to daily oral cinacalcet administration is of clinical relevance is currently unclear.

Parathyroidectomy

Despite intensive vitamin D and phosphate binder treatment, some children with CKD may develop refractory hyperparathyroidism, in particular if renal transplantation cannot be performed for an extended period of time. In young adult CKD patients, the incidence of parathyroidectomy is more than 2 per 100 patient years. In our experience the incidence of parathyroidectomy in children has been at least similar to adults. Indications for parathyroidectomy are persistent and recurrent hypercalcemia and hyperphosphatemia despite optimized dietary efforts and medication including cinacalcet, progressive and debilitating bone disease and progressive extraosseus calcifications. Latest findings in adults indicate a more than 50% reduction in the incidence of unremitting hyperparathyroidism and thus the need of parathyroidectomy, possibly related to the use of cinacalcet [177].

Total parathyroidectomy and autotransplantation of tissue fragments in the abdominal subcutaneous layer is preferred to subtotal parathyroidectomy, since the remaining tissue tends to grow again and the transplant is easier to access for diagnostic and curative purposes. Total parathyroidectomy without autotransplantation may result in difficulties to control calcium homeostasis, in particular after kidney transplantation. Ablation of parathyroid tissue by ethanol injection has been suggested as an alternative to surgery; published pediatric experience is limited to a 15 year boy successfully treated 2 months after renal transplantation [178]. Some centers give active vitamin D 72 h prior to surgery to lessen postoperative hypocalcemia; this, however, is not indicated if calcium and phosphate values are markedly increased.

Postoperatively serum ionized calcium needs to be monitored closely, because most of the children develop “hungry bone” syndrome. Due to

the rapid decline of PTH, skeletal calcium uptake increases markedly. In most of the children calcium infusions are required within few hours after operation, especially in children with high preoperative PTH and alkaline phosphatase levels. Calcium infusion can be started at a rate of 0.05 mmol/kg*h (2 mg/kg*h) of elemental calcium, but must continuously be adapted according to the changes in serum ionized calcium levels. Calcium infusion is often required for several days, subsequent oral calcium administration for many weeks. In addition, patients should be given high doses of calcitriol (up to 2 μg/d) and dialyzed with a high dialysate calcium concentration. Serum phosphate levels also decline; however, phosphorus supplementation may aggravate hypocalcemia and should only be initiated in case of significant hypophosphatemia.

Success rate is high in children. Parathyroid tissue fragments autotransplanted subcutaneously, usually start to secret PTH soon. Symptomatic hypocalcemia may develop in children not adhering to the medication; irreversible recurrent nerve palsy is rare. PTH and calcium x phosphate product remain within in the target range for many years [179].

Treatment After Renal Transplantation

Children receiving a renal allograft are at risk for multifactorial, progressive bone disease. Persistent hyperparathyroidism, hypophosphatemia and glucocorticoid use require close monitoring of bone metabolism, even if transplant function is normal. Serum calcium, phosphate and bicarbonate should be measured weekly within the first 2 months and at least monthly during the following months. Subsequent determinations also have to be adapted to renal function. PTH should be checked initially and 1 month post transplantation. If plasma PTH remains elevated further controls and therapeutic measures are required. If renal function is reduced, the frequency of determinations should be according to the stage of CKD (Table 60.2) and subsequent treatment strategies follow the guidelines for CKD (Table 60.3). Hypophosphatemia should be corrected. Glucocorticoids should be given at the lowest dose possible and may be withdrawn in patients with stable transplant function. Calcium and active vitamin D therapy lessen glucocorticoid induced bone loss in adults and have been recommended, however no pediatric data have been provided to date. Likewise, next to a case report only one observational study on the off-label use of cinacalcet in 20 children after kidney transplantation has been published [180]. It suggests efficient PTH suppression, and acceptable tolerability despite relatively high cinacalcet doses. The effect of cinacalcet on urine calcium excretion and allograft function is uncertain; some adult studies highlighted the potential risk of nephrocalcinosis. In case cinacalcet is used in children after transplantation, e.g. in order to circumvent parathyroidectomy, careful monitoring is required.

Future Therapeutic Perspectives

Bisphosphonates, selectively blocking osteoclasts and thus bone resorption, appear to be safe at all stages of CKD. Dose reduction is probably required in CKD stage 5, at least based on pharmacokinetic studies. These agents have been recommended for high-risk adult transplant patients on glucocorticoid treatment, although according to a recent meta-analysis the evidence for a reduction in fracture risk and bone pain is low [153]. Important concerns regard their long-lasting action and the risk of hypocalcemia associated with overdosing. Likewise, the effects of bisphosphonates on vascular calcifications need further study, because low bone turnover might exacerbate vascular calcifications. Even if bisphosphonates prove safe, their efficacy in CKD is uncertain. More clinical research data are mandatory before their use in growing children with CKD or glucocorticoid-induced osteoporosis can be considered.

BMP-7 has been used successfully in animal models of renal osteodystrophy and vascular calcification. Human data are currently limited to orthopedic interventions, where it has been employed successfully to induce healing of pathologic fractures. **Blockade of** the Wnt pathway inhibitor **sclerostin** is currently investigated in patients with osteoporosis. Whether sclerostin is a simple marker or an active mediator of bone and cardiovascular disease in CKD and whether sclerostin blockade mitigates these essential comorbidities is currently unknown.

Prognosis

Despite an increasing number of therapeutic options, the management of mineral metabolism remains difficult. More than one third of adults with pediatric-onset CKD have clinical symptoms of bone disease and almost one out of five patients is disabled by bone disease [8]. The CKD-associated alterations of bone mineral metabolism disorders and their treatment during childhood years substantially contribute to the development of uremic arteriopathy, and probably to the excessive cardiovascular mortality in early adult life [10]. Barriers to success are limited patient compliance, the limited efficacy of prescribed measures, the prohibitive costs of novel, more efficacious therapies, and the progressive development of parathyroid autonomy.

Still, there is hope that prevention of mineral dysregulation with early and appropriate use of phosphate binders, prevention of 25(OH)D deficiency, new vitamin D analogues and calcimimetics may reduce the prevalence of mineral disorder and prevent bone disease and vasculopathy in children with CKD. Moreover, the attention to the various clinical practice guidelines available now for pediatric patients with CKD, the establishment of patient registries monitoring achievement of targets and prospective, randomized trials evaluating clinical outcome parameters should result in more favorable long-term outcomes of children with CKD.

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Cardiovascular Disease in Pediatric Chronic Kidney Disease

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Anke Doyon and Mark Mitsnefes

Epidemiology

Cardiovascular Mortality: Leading Cause of Death in Children with CKD

In 1998, the National Kidney Foundation Task Force on cardiovascular disease (CVD) declared an epidemic of cardiac disease in end-stage kidney disease (ESKD) patients [1]. This report however provided no information on CVD in children. Over the next two decades, there have been important studies around the world addressing CVD in children with ESKD.

In 2002, as a follow up on a Task Force, Parekh et al. [2], using the United States Renal Data System (USRDS) database, performed the first analysis to evaluate the risk of a cardiac death in children and young adults (age 0–30 years) and to identify factors potentially associated with CVD mortality. A total of 1380 deaths between 1990 and 1996 were analyzed. There were 311 cardiac deaths (23% of the total). These data were in great contrast to the general pediatric population where CVD mortality was low and accounted for less than 3% of all causes of death [3]. The analysis also showed that the rates of cardiac deaths in this age group were approximately 1000 times higher than in the general pediatric population. Subsequent reports from international registries over the next few years have confirmed that CVD is the leading cause of death in children with ESKD and in adults with childhood onset of CKD. The Australia and New Zealand Dialysis and Transplant (ANZDATA) [4], Dutch national cohort study (LERIC) [5] and a large German study [6] report that 40–50% of all deaths are from cardiovascular or cerebrovascular causes. Cross-sectional studies of young adults with childhood-onset ESKD have found a cardiac death rate of 35% in U.S. children transplanted at age 0–19 years [7]; 23% in 150 German patients transplanted as children between 1970 and 1993 (almost ten times higher than the occurrence of malignancies [8]), and 32% in another series of dialyzed or transplanted young adults from Germany [9].

Foster et al. analyzed the long-term survival of 18,911 patients who received a first kidney transplant during childhood (at <21 years old, 1983–2006) and confirmed that the majority of deaths

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were from cardiovascular causes especially after graft failure (45%) versus those with a functioning graft (25%) [10]. The hazard ratios (HR) for cardiovascular mortality associated with dialysis after graft failure was 7.8 as compared to those with functioning graft. As other studies, this study also observed an improvement in cardiovascular mortality in transplant recipients. However, even in these patients, the mortality rates were still significantly higher (approximately 10 times) than in the general pediatric population. Mitsnefes et al. [11] analyzed data from more than 20,000 pediatric ESKD patients followed over two decades, with follow-up to 2010. The authors demonstrated that mortality rates for children and adolescents being treated with dialysis have improved dramatically between 1990 and 2010.

While most published studies analyzing mortality in young adults have focused on patients who developed ESKD during childhood, there have been very few studies examining cardiovascular morbidity and mortality specifically in patients who developed ESKD during young adulthood (21–29 years). Using USRDS data collected in 2003–2013, Modi et al. [12] showed that the risk for cardiovascular mortality in these young adults was 143–500 times higher compared to age-matched young adults in the general population. During the study follow-up period, 16.2% of the cohort died, and CVD accounted for 38% of all deaths. The risk of death among those with onset of disease during young adulthood was similar to that of young adults with childhood-onset ESKD (1–11 years or 12–21 years). Presence of diabetes, coronary artery disease, and heart failure were notable risk factors for cardiovascular-related mortality.

Modi et al. also compared the risk of cardiovascular-related hospitalizations. Not surprisingly, the rates of cardiovascular hospitalization during the five-year follow-up period were highest for those 22–29 years and lowest for those 1–11 years, with risk remaining stable in children but increasing over time in the adolescent group until reaching the hospitalization rate of young adults. As expected, patients receiving hemodialysis and peritoneal dialysis were at higher risk for cardiovascular-related hospitalizations compared to patients receiving preemptive transplantation.

Ku et al. [13] compared trends over time in mortality from CVD-related causes of death among more than 80,000 children and young adults <30 years of age starting dialysis in 1995–2015. Overall, 40% of deaths were cardiovascular-related causes. The risk of cardiovascular-related death was stable initially, began improving after the early 2000s, and became significantly lower after 2006 in those starting dialysis as either children or young adults (Fig. 61.1).

Taken together, these data show that despite significant improvement in patient survival, mostly due to decrease in cardiovascular (especially in those after kidney transplantation) and infectious disease mortality, CVD remains the world-wide biggest obstacle to long-term survival of children and adolescents with ESKD, especially those on maintenance dialysis [14] (Fig. 61.2). Not surprisingly, the American Heart Association guidelines for cardiovascular risk reduction in high-risk pediatric patients continue to stratify pediatric CKD patients in the “highest risk” category for the development of CVD [15, 16].

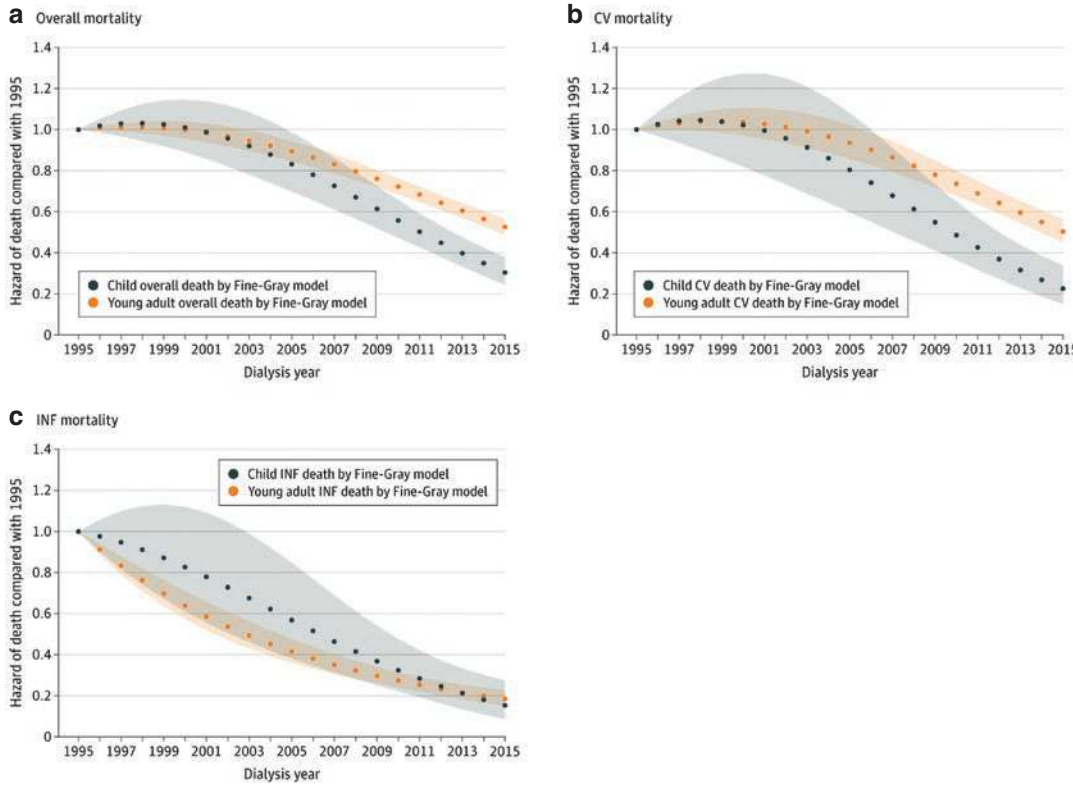

Fig. 61.1 Trend in cause-specific deaths among children and young adults by calendar year of dialysis initiation using fine-Gray models. Shaded areas represent 95%CIs for the point estimates in young adults and children. *CV* indicates cardiovascular, *INF* infection. From Ku et al. [13]

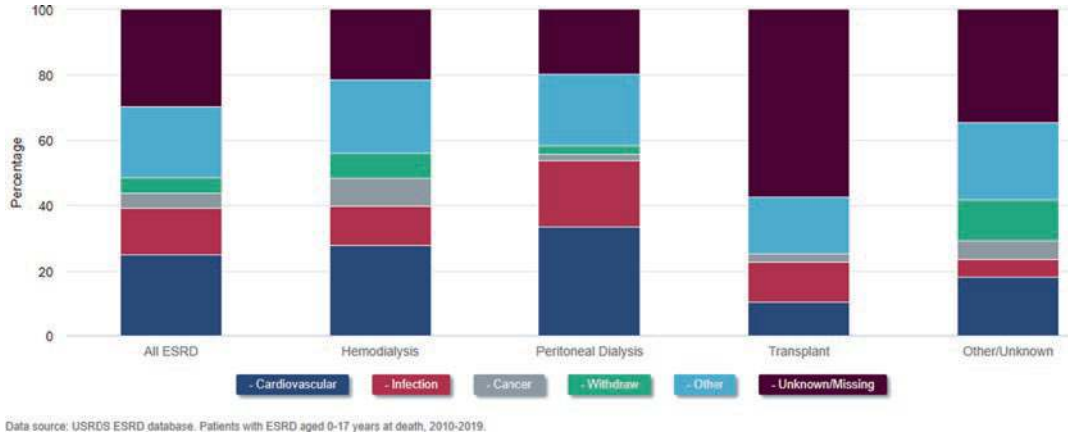

Fig. 61.2 Cause of death in children with ESKD, by treatment modality, 2010–2019. The most common cause of death in children with ESRD was a cardiovascular cause, followed by an infectious cause, but the causes of death differed by treatment modality. For example, children receiving PD died more commonly of infections than children receiving HD. A large proportion of the causes of death in children were of unknown cause (30%), and the cause was particularly likely to be unknown for children with a kidney transplant. *US Renal Data System: USRDS 2020 Annual Data Report: ESRD among Children and Adolescents.* https://adr.usrds.org/2020/end-stage-renal-disease/7-%20esrd-among-children-and-adolescents

Specific Causes of Cardiac Death

The term "cardiac death" may include several diagnostic categories, such as cardiac arrest, arrhythmia, myocardial infarction, valvular heart disease, cardiomyopathy, etc. (Table 61.1).

Although it is common to group these diagnoses together, this may obscure the issue; the diagnosis of arrhythmia could be due to hyperkalemia (possibly due to noncompliance, dietary mistakes, acidosis, insufficient dialysis, etc.) or due to coronary artery disease or other causes. Therefore, diagnostic categories do not necessarily permit conclusions regarding the pathogenesis of CVD events.

In the initial USRDS study almost 20 years ago of Parekh et al., cardiac arrest was the most common cause in each of the age groups, followed by arrhythmia and cardiomyopathy [2]. The incidence of cardiac arrest in the youngest age group (0–4 years) was 5–10 times higher than in other age groups, perhaps, as noted by the authors, a reflection of the difficulty of ascertaining the true cause of death in young children. Some of these young children might have died from other co-morbid conditions such as congenital disorders which are not included in the USRDS database.

Table 61.1 Most common cardiovascular risks, abnormalities and cardiac causes of death

Cardiovascular risks and abnormalities[a]	Cardiac causes of death
CV Risks	*Dialysis*
Hypertension	Cardiac arrest/ arrhythmias
Dyslipidemia	Cerebrovascular disease
Hyperphosphatemia	Congestive heart failure/pulmonary edema
Increased Ca × P product	Cardiomyopathy
Anemia	Acute myocardial infarction
Early CV abnormalities	Pericarditis
Left Ventricular hypertrophy	*Transplant*
LV Systolic and diastolic dysfunction	Cardiac arrhythmias
Increased cIMT	Cerebrovascular disease
Increased PWV	Acute myocardial infarction
Coronary artery calcification	Cardiomyopathy

[a]The frequency of cardiovascular risks and abnormalities increases with advancing of CKD with the greatest prevalence during maintenance dialysis. Despite some improvement, many of these factors persist after transplantation

The most recent study by Ku et al. [13] also using the USRDS indicated that while sudden cardiac death remained the leading cause of death, its risk has improved steadily but to a greater degree in children versus young adults comparing 2015 versus 1995. In contrast, trends differed for heart failure-related mortality: while the risk of dying from heart failure did not change over time in children, it began to decline in those starting dialysis as young adults after 2008. The risk of dying from a myocardial infarction was significantly lower after 2005 compared to 1995 in young adults, but was not different during most of the follow-up time in children although the event rate was very low. The risk of dying of a stroke began to improve after 2009–2010 in both those starting dialysis as children and young adults.

The high rate of sudden cardiac death in children, especially in infants with ESKD, is poorly understood and warrants further investigation. In adults, sudden cardiac death is often a result of fatal arrhythmias due to acute ischemia of preexisting atherosclerotic disease. It is believed that arrhythmias are also the likely cause of most cases of sudden cardiac death in children. However, the origin of acquired malignant arrhythmias in children is unlikely to be an atherosclerotic lesion. Instead, dilated, especially hypertrophic cardiomyopathies are a leading cause of sudden cardiac death in children [17]. The macroscopic and microscopic structural abnormalities in cardiomyopathies involve fibrosis and cellular hypertrophy that predispose to an electrical instability with resultant arrhythmias. Ischemia of small coronary vessels secondary to medial hypertrophy might result in dispersion of repolarization properties and arrhythmia from reentrant or autonomic mechanisms. As we discuss in more detail below in this chapter, many children with CKD develop left ventricular hypertro-

phy (LVH), which is frequently severe, especially in children on prolonged dialysis therapy [18, 19]. It is currently unknown if LVH in young patients with CKD is characterized by structural abnormalities similar to familial or idiopathic hypertrophic cardiomyopathies associated with sudden cardiac death. To what extent LVH can contribute to an increased risk for sudden cardiac death in children with CKD is also not known. Deadly arrhythmias in children with ESKD could also be evoked by acute changes in the cardiac extra- or intracellular ionic milieu, especially involving abnormalities of sodium- and potassium-based repolarization currents.

Symptomatic ischemic heart disease and myocardial infarction are typical complications of CVD in older patients with CKD and account for more than 50% of death in these patients [20]. In contrast, young patients as a rule have no symptoms of ischemic heart disease such as angina pectoris and no myocardial infarction but remain asymptomatic. Could this imply that ischemic heart disease is silent and therefore underdiagnosed? Silent myocardial ischemia is frequently found in patients with CKD, especially during dialysis [21]. Myocardial infarction may also be disguised, as has been noted in younger women who may have significantly different symptoms at initial presentation [22]. There is a paucity of studies regarding EKG monitoring in children with CKD, but circadian rhythms of blood pressure (BP) and heart rate are altered in children with CKD [23]. There are no systematic autopsy studies in young patients with CKD focusing on vascular and coronary pathology. Therefore, the lack of symptomatic ischemic heart disease in young patients and paucity of cardiac deaths due to confirmed myocardial infarction remains an important observation; however, it is presently unknown whether absence of symptoms truly implies absence of advanced vascular and cardiac disease.

Risk Factors

In the general population, CVD in most patients is the result of atherosclerosis. Atherosclerosis is a multifactorial disease affecting the heart and large and medium-sized arteries, with a focal distribution, and with the characteristic appearance of lipid-rich plaques in the intima of the arterial wall. Clinical manifestations include coronary heart disease, cerebrovascular disease, aortic aneurysm, and peripheral arterial occlusive disease. This disease is slowly progressive over several decades. Atherosclerosis has a multifactorial pathogenesis, but the occurrence of the disease is associated with typical risk factors: dyslipidemia, hypertension, smoking, male gender, diabetes, abdominal obesity, lack of physical activity. In the INTERHEART study, a case-control study of acute myocardial infarction performed in 52 countries more than 15 years ago, these traditional risk factors (in combination with psychosocial factors, consumption of fruits, vegetables, and alcohol) accounted for 90% of the attributable risk in men and 94% in women, indicating their worldwide predictive value irrespective of gender, race, or region [24].

CKD has been appropriately described as a "vasculopathic state" because of the extraordinary risk indicated by the accumulation of numerous risk factors [25]. The occurrence and prevalence of these risk factors have been exhaustively described [26–28]. Traditional risk factors (for atherosclerosis), non-traditional (uremic) risk factors and cardiomyopathy (a risk factor in itself) most likely act synergistically, resulting in altered vascular and cardiac structure and function in CKD [29].

What makes children with CKD so highly predisposed to the development of accelerated atherosclerosis/arteriosclerosis and premature CVD during young adulthood is likely a unique combination of extremely highly prevalent traditional and CKD-related risk factors (Table 61.1).

Traditional Risk Factors

The high prevalence of traditional cardiovascular risk factors is already evident in children with early stages of CKD. Many publications from the Chronic Kidney Disease in Children (CKiD) study, an observational cohort study of more than one thousand children (aged 1–16 years) with CKD stages 2–4 initiated in the early 2000s, provided contemporary data on the prevalence of

cardiovascular risk factors over the last decade. An initial cross-sectional study showed that 54% of participants had **hypertension**; among children receiving antihypertensive medication, 48% still had elevated blood pressures [30]. Ambulatory blood pressure monitoring (ABPM) demonstrated a high frequency of masked hypertension (38%) [31]. During a 4-year follow up, only 46% of hypertensive patients achieved controlled clinic BP [32]. In a study comparing ambulatory BP control over two time periods (2005–2008 and 2010–2013), there was a significantly higher prevalence of masked hypertension and a lower prevalence of normotension in the more recent period [33]. Recent data indicate that unrecognized and untreated hypertension is more prevalent and persistent in younger children [34]. Thus, despite publication of the initial CKiD hypertension data in 2008–2010, plus published recommendations and guidelines for stricter BP control in patients with CKD, hypertension has remained under-recognized and under-treated in children with CKD.

Dyslipidemia was initially found in 43% of the CKiD cohort: 32% had triglycerides levels >130 mg/dL; 21% had HDL-cholesterol levels <40 mg/dL and 21% had total cholesterol >200 mg/dL [35]. Dyslipidemia persisted (51%) 6.5 years later in the CKiD participants [36]. Likewise, the prevalence of dyslipidemia was 61.5% in children with CKD enrolled in KNOW-PedCKD, the KoreaN cohort study for Outcomes in patients With Pediatric Chronic Kidney Disease [37].

Among the CKiD participants, about 30% were either **overweight or obese** [38]. Although diabetes is very rare in children with CKD, hyperinsulinemia and insulin resistance was present in 9% and 19% respectively of the CKiD cohort [38]. Importantly, almost one half of the CKiD cohort had a combination of traditional cardiovascular risk factors [38]. Even lean patients had a high prevalence of multiple traditional cardiovascular risks, with nearly one-quarter having two or three cardiovascular risk factors. Overweight or obese study participants had a very high prevalence of multiple risk factors, similar to rates in severely obese (BMI > 40 kg/m^2) children without kidney disease. This pattern differentiates the population of children with CKD from healthy children, in whom the coexistence of multiple cardiovascular risk factors is extremely infrequent, and restricted to those who are obese (metabolic syndrome). However, classical **metabolic syndrome** is also very frequent in the CKiD cohort with a prevalence of 40% in overweight patients and 60% in the obese children [39]. In this study, those with persistent metabolic syndrome had approximately twice the odds of kidney function decline (>10% per year) compared to those without multiple cardiometabolic risk factors and normal BMI.

The frequency of traditional risk factors is highest in children on maintenance dialysis (Table 61.1). Successful kidney transplantation leads to elimination of many uremia-related risk factors for atherosclerotic CVD. However, transplant recipients are not free from multiple complications and transplantation frequently amplifies many of the traditional risk factors (Table 61.1). One multicenter study determined that 38% of kidney transplant recipients had a coexistence of at least three traditional cardiovascular risk factors [40].

CKD-Related Risk Factors

While the high prevalence of traditional risk factors might explain a higher prevalence of accelerated CAD and premature cardiac death in young adults, it cannot explain the high rates of cardiac death in 0–19 year olds on maintenance dialysis or after transplant. To understand the risk of cardiac disease in these children, the focus has been on evaluation of uremia-related risk factors. As in adults, abnormal mineral metabolism is frequent in children with CKD. The role of mineral and bone disorder (MBD) in development of vascular disease in CKD will be discussed extensively later in this chapter. As with traditional risks, the rates of abnormalities are higher in dialysis patients versus those with pre-ESKD (Table 61.1).

Despite widely spread use of erythropoietin and iron therapy, **anemia** is still frequently poorly controlled, especially in children with advanced CKD [41] or on maintenance dialysis [42]. Data

from the CKiD study indicate that 26% of children with non-glomerular and 43% of children with glomerular CKD had hemoglobin levels less than the fifth percentile using age- and sex-specific norms [43]. Data from the Korean cohort (KNOW-PedCKD) demonstrated 40% prevalence of anemia [44].

One of the largest studies to date from the international Pediatric Peritoneal Dialysis Network (IPPN) (1394 pediatric patients aged 1 month to 20 years from 81 pediatric dialysis centers in 30 countries) showed that 25% of patients had a hemoglobin level < 10 g/dL despite more than 95% of participants received erythropoietin therapy [45]. In an early USRDS study, severe anemia has been linked to overall mortality in these children but not to cardiac death [46]. Similarly, in the IPPN study overall death occurred more frequently in those with hemoglobin <11 g/dL (4.2%) versus those with hemoglobin >11 g/dL (2.6%) [45].

Inflammation is also mainly evident in chronically dialyzed children. However, there have been no studies examining the role of inflammation in cardiac death of children with ESKD. Even though there have been no studies of a direct link between specific traditional and uremia-related risks and CVD mortality, epidemiological data presented above showing remarkable decrease in mortality post-transplant versus dialysis, clearly point out to dialysis vintage as a major cause of cardiac death in children and young adults with childhood onset of ESKD.

In summary, children and adolescents with CKD harbor a multitude of risk factors for CVD that at this age seems without parallel [47]. The overall effect of these risk factors and their relative contribution to morbidity and mortality in young patients is yet unknown. While studies in adult CKD patients predominantly focus on symptomatic cardiovascular events and all-cause mortality to identify risk factors, these end-points fortunately are rare in the pediatric setting. Studies in this age group therefore have to rely on surrogate endpoints such as the identification and progression rate of subtle cardiovascular changes (Table 61.1). The scarce knowledge about the extent of the progression of cardiovascular alterations and promoting factors is often a result of relative small patient numbers in individual pediatric nephrology centers. For this reason, multicenter studies are a logic and reasonable consequence to advance cardiovascular research in children with CKD. In this regard, two large consortiums in Europe and the United States have formed more than a decade ago to monitor and evaluate the children with CKD with a special emphasis on the cardiovascular status. In addition to the CKiD study that was introduced earlier in the chapter [48], the European consortium has initiated the Cardiovascular Comorbidity in Children with Chronic Kidney Disease Study (4C Study) which prospectively follows more than 700 children with more advanced CKD all over Europe [49]. In both studies, yearly exams evaluate the cardiovascular status in these patients by vascular ultrasound, BP measurement, other traditional and CKD related CVD risk factors, echocardiography and assessment of arterial stiffness. It has provided important insight about who is or will be at a particularly high risk to suffer from cardiovascular complications. In this chapter, we describe the current findings from the above multicenter observational studies.

Vascular Disease in Pediatric CKD

Non-invasive Studies of Vascular Abnormalities

Coronary Artery Calcification

CT scans were the earliest imaging modalities to detect vascular abnormalities such as coronary artery calcifications (CAC) in young adults with ESKD. Braun et al. [50] were the first to describe increased calcium scores in young dialysis patients when compared with non-dialysis patients with known or suspected coronary artery disease and a number of studies confirmed this finding for young patients with ESKD starting in childhood or adolescents [6, 51–58]. Also, coronary calcium scores increased significantly in dialysis patients even over short time periods [52, 57]. Two studies reported the occurrence of CAC only starting at the age of 20 or 31 years in their

patient cohorts respectively [53, 57] whereas others detected them already during adolescence [51, 58]. The percentage of patients with proven CAC was highly variable between studies and ranged between 10% and 92%, possibly reflecting the wide age range, differences in cumulative time on dialysis or after transplantation, and variation in medical treatment policies. Recent studies of CAC in children with ESKD have been performed by Srivaths et al. [54–56]. In their initial study, 5 of 16 patients (31%) had CAC. In a follow up study, the CT was repeated 1 year after the initial evaluation. Three patients with initial CAC progressed based on increased Agaston score; one patient developed new and none of the patients resolved CAC.

Although heterogeneous in age, duration and mode of renal replacement therapy, several risk factors for the presence and severity of CAC were consistently identified in the majority of studies. Among them are the patients' age, cumulative time on dialysis, CRP, PTH, FGF23, mean serum phosphorus and calcium-phosphorus product levels, and cumulative exposure to active vitamin D.

Carotid Intima-Media Thickness

In adults, carotid intima-media thickness consensus (cIMT) measurement is considered a valid and reliable assessment of atherosclerotic burden [59–62] that can predict coronary atherosclerosis and its clinical sequelae, such as myocardial infarction and stroke [60, 62]. Measurement of cIMT is recommended by the American Heart Association for risk stratification in adults whose CV risk is not clear or is intermediate [61] and as a standard non-invasive assessment of subclinical atherosclerosis in children and adolescents [61].

Briefly, cIMT is measured at the far wall of an ultrasound image recorded with a high-frequency probe. Normal values were first developed for children between 10 and 20 years based on measurements in 241 healthy children [63] and later on for children between 6 and 18 years in a cohort of 1155 healthy non-obese and non-hypertensive children [64]. The study demonstrated low inter- and intraobserver variation, confirming the suitability of cIMT measurements as a surrogate marker of the vascular health status in multicenter trials.

Ultrasound studies in pediatric predialysis, dialysis and transplanted patients revealed significant alterations of the vessel wall compared to healthy controls, which start well before attainment of ESKD. This was first shown in smaller studies [65, 66] and confirmed in the large observational multicenter studies of CKiD and 4C.

CKD patients of the CKiD study showed increased cIMT which was correlated with hypertension and dyslipidemia [67]. The baseline evaluation of the 4C Study cohort revealed elevated cIMT in 42% of patients (Fig. 61.3) [68]. cIMT was higher in girls and associated with higher systolic blood pressure SDS, BMI SDS, physical activity and serum phosphorus [68].

Oxidative stress has been suspected as a potential factor involved in the pathogenesis of large vessel arteriopathy, but so far, few associations to the clinical vascular status have been reported in pediatric CKD. In 134 predialysis and dialysis patients aged 2–16 years, reduced glutathione levels but not other oxidative stress markers such as malondialdehyde, nitric oxide and homocysteine were found to be associated with cIMT [69].

In children with ESKD receiving **dialysis**, cIMT is more markedly increased than in the predialytic CKD population [58, 65, 69]. In these children cIMT is consistently linked to abnormal mineral metabolism and associated medications, as indicated by associations with serum phosphorus, calcium-phosphorus product, iPTH levels, and calcium based phosphate binder therapy. In addition, a strong association between active vitamin D dosage and cIMT was noted in the largest study of cIMT to date in pediatric dialysis patients [58]. Notably, not only an excess of active vitamin D but also 1.25 Vitamin D deficiency is a risk factor for increased cIMT in dialyzed children, constituting a U-shaped relationship of serum 1.25-vitamin D levels and cIMT [70].

Increased cIMT in pediatric **kidney transplant recipients** has consistently been demon-

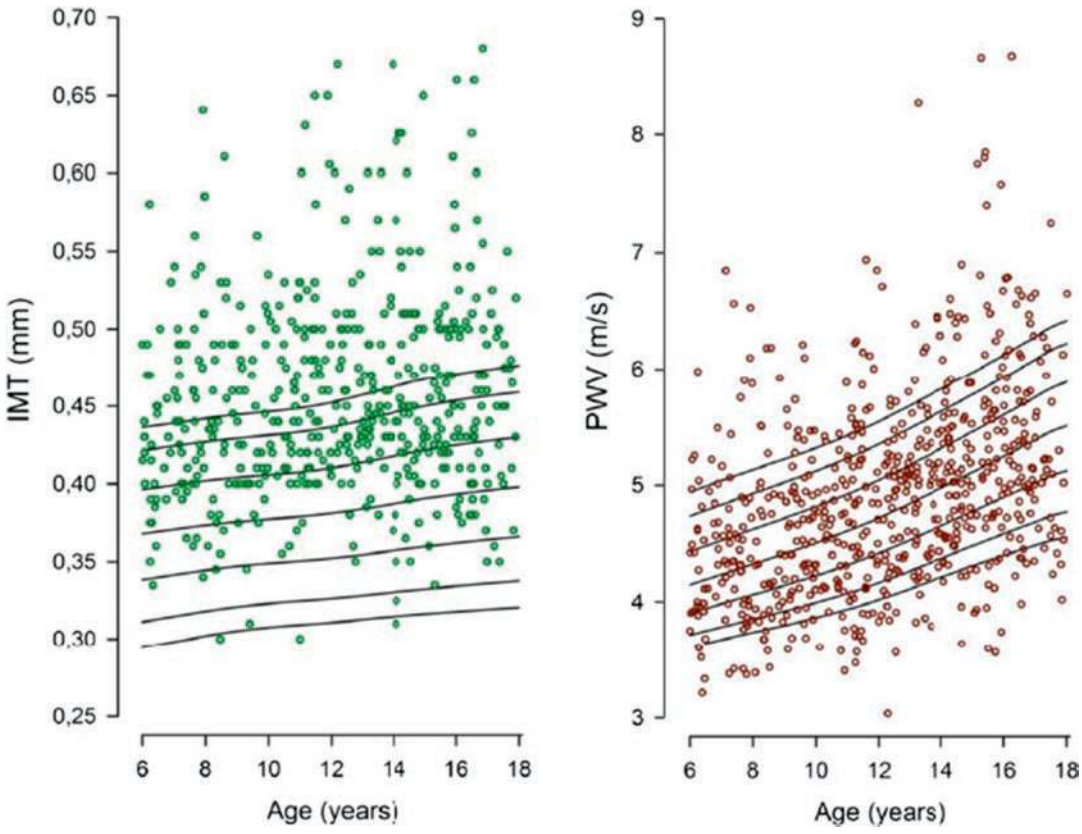

Fig. 61.3 Surrogate marker measurements. Distribution of carotid intima-media thickness (IMT; left panel) and pulse wave velocity (PWV; right panel). Curves represent 5th, 10th, 25th, 50th, 75th, 90th, and 95th reference percentiles. From Schaefer et al. [69]

strated. A meta-analysis of 2016 estimated that cIMT was about 0.05 mm higher in these patients than in healthy controls [71]. It included 9 studies and a total of 259 mostly kidney and few liver transplant patients. In a study of 109 children in three German centers, cIMT was elevated in 43% of patients with a kidney transplant [72].

There is scarce information about the progression of cIMT over time in children with CKD. Time on dialysis is associated with higher cIMT levels, as reported by Chavarria et al. in 60 dialyzed children [73] and by Oh et al. in 39 young adults with childhood-onset ESKD [6]. In a prospective study following 24 children with CKD stage 3–5 and 32 patients on dialysis, cIMT values increased both in the predialysis and the dialysis patients. By contrast, among patients who were transplanted during the observation period, those with elevated cIMT at baseline showed significant regression after transplantation [74]. In children of the 4C cohort who underwent pre-emptive kidney transplantation when reaching ESKD, cIMT did not regress but increased significantly less than in children who started dialysis [75].

Importantly, clinical morphologic measures appear to reflect structural changes even on the molecular level. In patients of the 4C Study, cIMT measured by ultrasound was correlated with arterial calcium content in arterial biopsies and corresponding transcriptional alterations regarding pro-calcifying factors, calcification inhibitors and extracellular matrix components were demonstrated [75].

Pulse Wave Velocity

In recent years, research has focused not only on morphological but also on functional measures of the vascular status. A variety of technologies to non-invasively assess arterial properties *in vivo* have been introduced. Among different techniques, pulse wave velocity (PWV) is one of the most studied in children with CKD. PWV is a measure of arterial stiffness derived from the propagation of the pulse wave along the vessel wall between two defined points of the arterial tree. Similarly to normal values of blood pressure, PWV is strongly age- and height-dependent and considerably lower in children and young adolescents than in adults. Therefore, age- or height normalized values should be used for the interpretation of PWV. The European Heart Association rates PWV as "gold-standard" to assess arterial stiffness and recommends its measurement for the evaluation of cardiovascular risk. In adults, a relationship to mortality independent of blood pressure and other confounding factors was demonstrated in 241 patients with ESKD and 439 patients with CKD stage 2–5 [76, 77]. Savant et al. reported PWV comparable to healthy children in a group of patients from the CKiD study with more than 50% of patients in CKD stage 1 or 2 [78]. The baseline assessment of children with **pre-dialytic CKD** of the 4C study (CKD stage 3–5) showed elevated PWV in 20% of patients (Fig. 61.3) [68]. PWV in these patients was associated with higher systolic blood pressure, albuminuria and iPTH and with lower vitamin D levels [68]. While slightly different methods were used in both studies (applanation tonometry vs. oscillometric recording) a turning point towards increasing arterial stiffness at CKD stage 3 might be implied.

Considerably increased PWV has been reported in several smaller studies in pediatric **dialysis** patients [58, 77, 79]. The 3H study found higher PWV in patients on conventional hemodialysis than in patients receiving hemodiafiltration [80]. PWV decreased over time in both groups.

Increased PWV has also been detected in a cross-sectional analysis of pediatric **transplant recipients** [81]. In a longitudinal analysis of 4C Study patients who started renal replacement therapy, PWV decreased after pre-emptive kidney transplantation but increased after the start of dialysis treatment [75]. The 4C study recently analyzed changes in PWV in children with ESKD who underwent kidney transplantation [82]. In their analysis, PWV significantly increased after transplantation and was significantly higher in girls compared to boys. PWV was positively associated with time on dialysis and diastolic blood pressure in both sexes. Authors concluded that girls with CKD are more susceptible to develop arterial stiffness compared to boys and that this difference persist after transplantation and might contribute to higher mortality rates seen in girls with kidney failure.

Blood pressure has been identified as the most important predictor for PWV in all CKD stages. In addition, associations to markers of CKD-MBD and uremic toxins have been described in pediatric cohorts [83–86]. There is also evidence that the NO pathway is altered in CKD which associates with measurements of arterial structure and function. Shroff et al. showed that HDL derived from pediatric CKD patients inhibited NO production in vitro, which was correlated inversely to measures of PWV and cIMT in these subjects [87].

ABPM and Ambulatory Arterial Stiffness Index (AASI)

Ambulatory Blood Pressure Monitoring (ABPM) does not only characterize BP status, rhythm and daily variability but can also provide information about vascular stiffness. The Ambulatory Arterial Stiffness Index (AASI) is computed as 1 minus the diastolic-on-systolic blood pressure slope of the linear regression line between 24-h ambulatory diastolic and systolic blood pressure readings. It was found highly correlated with PWV and the central and peripheral augmentation index. In children with hypertension, AASI is increased and associated with the duration and origin of hypertension [88]. In a study of 51 kidney transplant recipients, AASI was increased in hypertensive subjects and the main determinants were dipping time, volume overload and time on dialysis, while PWV was increased also in normotensive patients and predicted by a dialysis vintage of more than 1 year. This observation led to the interpretation that PWV might represent

structural long-term changes of the vessels, while AASI rather demonstrates the volume- and pressure-associated arterial stiffness [89]. While further evaluation of AASI as a cardiovascular marker is desirable, it could be a useful tool to monitor volume status and to adjust diuretic therapy [90].

Flow-Mediated Dilation, Postischemic Reactive Hyperemia

Endothelial-dependent flow-mediated dilation (FMD) measures the dilation of an artery after a short occlusion of the subjacent arteries. It depends on the potential of the vessel endothelium to produce nitric oxide (NO) or prostanoids to trigger vasodilatation and thereby characterizes endothelial reactivity and function [91]. Practically, the dilation of the brachial artery is measured by ultrasound at baseline and after the release of a BP cuff which has been placed on the upper arm or the forearm and has been inflated to approximately 50 mmHg above the systolic BP for several minutes. Despite a high interobserver and within-subject variability [92], a multitude of studies in adults have used FMD as surrogate marker for endothelial dysfunction. Several recent studies in adult CKD patients have demonstrated a reduction of FMD in both predialysis and dialysis patients [93]. Moreover, reduced FMD was predictive of fatal and non-fatal cardiovascular events in a prospective study of 304 adult predialysis patients [94]. The applicability of FMD in children is compromised by the limited tolerance to a BP cuff inflated over several minutes, and the unpleasant phase of hyperemia often prevents repeated measurements. Nevertheless a few small studies have been performed in children with CKD, two of which found decreased FMD in 23% and 71% of children with CKD stage 2–4 and 4, respectively [95, 96]. In another study which included predialysis, dialysis and transplanted patients, 60% had impaired FMD, which was significantly associated with cIMT [97]. A study of 44 pediatric patients after kidney transplantation found that treatment including everolimus resulted in reduced FMD compared to standard calcineurin inhibitor treatment [98]. While this study was cross-sectional only, it indicated that implications of different treatment regimens may become apparent by comparing surrogate endpoints, making them a feasible option also for prospective clinical trials.

Mechanisms of Vascular Calcification in CKD

Patients with CKD develop different types of vascular pathology. The predominant alteration consists of progressive calcification of the tunica media (Fig. 61.4). Additionally, calcification of

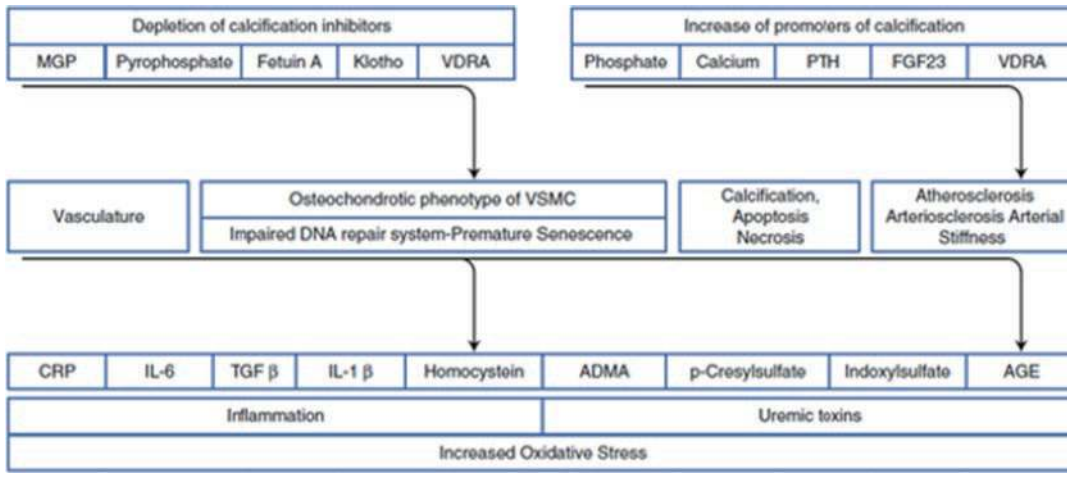

Fig. 61.4 Pathophysiologic mechanisms of vascular calcification. *MGP* Matrix Gla protein, *VDRA* vitamin D receptor agonist, *AGE* advanced glycosylation products, *ADMA* asymmetric dimethylarginin

the intima can occur and further deteriorate the vascular phenotype. Vascular smooth muscle cells (VSMC) play a key role in medial calcification. They are actively involved in calcification processes and undergo alterations including premature senescence and apoptosis, loss of calcification inhibitory capacities, osteogenic transformation and deposition of hydroxyapatite [99]. As a dysregulated mineral metabolism in CKD plays a key role in this process KDIGO explicitly includes "vascular or other soft-tissue calcification" in the definition of CKD-MBD [100]. The so-called kidney-vascular-bone axis reflects the direct and deleterious consequences of an unbalanced mineral metabolism on the vascular system.

The Kidney-Vascular-Bone Axis

In addition to hypertension and dyslipidemia, mineral metabolism plays a crucial role for vascular alterations in CKD. Among the directly or indirectly involved factors are serum levels of calcium, phosphate, vitamin D, PTH, FGF23 and Klotho.

Although serum calcium levels are usually low in untreated CKD patients, medications such as calcitriol and calcium-based phosphate binders can significantly increase serum calcium, especially in the presence of low-bone turnover disease. High calcium uptake by VSMC can induce matrix vesicle calcification similar to processes in the bone [101] and promote apoptosis of the cells leading to release of calcium in the extracellular matrix and formation of calcification "nidus" [102]. High phosphate levels are frequent in advanced CKD and might even occur at milder stages depending on phosphate intake. Phosphate is a key mediator of medial calcification. In vitro experiments have demonstrated that phosphate can induce an osteochondrogenic state in VSMC [103].

Given the outlined consequences of an altered mineral metabolism, a linear association of arterial deterioration and calcification with failing kidney function would seem logical. However, vessels of dialysis patients seem to be substantially more prone to calcification from hypercalcemia and hyperphosphatemia than vessels of healthy individuals, as demonstrated in both in vitro and in vivo models of human vessels. In a model of intact human vessels, Shroff et al. showed that calcium accumulation already started during pre-dialysis CKD while the number of VSMC and vessel architecture was normal. In dialysis vessels however, calcification was significantly accelerated and vessels showed deranged architecture, increased VSMC death and deposition of hydroxyapatite laden vesicles. In addition to high serum calcium levels, it is likely that reduced concentration or activity of calcification inhibitors such as Fetuin-A and MGP contribute to the calcification process [104, 105].

Calcium and phosphate levels are subject to tight regulatory circles. An important mechanism of phosphate homeostasis includes FGF23 and Klotho. FGF23 is induced by high phosphate levels, it increases urinary phosphate excretion and inhibits 1.25 vitamin D synthesis [106]. Klotho is a coreceptor for FGF23 and is involved in the regulation of phosphaturia and 1.25 vitamin D production [107]. There is a free soluble form, but it is also expressed in arteries and VSMC [108]. FGF23 is upregulated and vascular Klotho downregulated early in the course of CKD [109] and several studies have linked increased calcification to reduced vascular Klotho expression in CKD [108, 109]. Reversal of Klotho insufficiency by transgenic overexpression in mice with CKD ameliorated phosphate extretion, preserved renal function and decreased calcification, potentially identifying a new therapeutic option [110]. Increases in FGF23 have been linked to increased mortality independently of phosphate levels [111]. *In vitro*, FGF23 enhances phosphate-induced calcification [112] and *in vivo*, the severity of calcification in patients with a nonzero Agatston score is associated with FGF23 in CKD patients [113]. In children on dialysis with coronary artery calcification, Srivath et al. found significantly increased FGF23 levels compared to those without calcification [55]. In adults however, there are conflicting results about the role of FGF23 for vascular calcification, endothelial dysfunction and arterial elasticity [114, 115].

Regarding phosphate binders, there seem to be different effects on aortic calcification depend-

ing on the type of phosphate binder [116]. As there are no data about the effect of phosphate binders on arterial calcification and Klotho or FGF23 in children, future studies are recommendable to improve therapeutic approaches.

Calcium levels are controlled by vitamin D levels, which themselves play a role in vascular calcification. Besides increasing calcium levels, vitamin D receptor agonists themselves can induce calcification by induction of an osteoblast phenotype of VSMC [117]. They also increase the secretion of alkaline phosphatase, which can worsen the calcification inhibitory capacity of VSMC by degrading pyrophosphate, a potent calcification inhibitor [118]. However, there is also evidence from a mouse model that vitamin D receptor agonists induce Klotho and lower FGF23, thereby exerting a beneficial effect on medial calcification [119]. These contradictory observations may help to explain the bimodal relation between vitamin D levels and calcification in clinical studies [70].

Calcification Inhibitors

Evidently, additional or permissive factors contribute to the disposition of a vessel for calcification particularly in dialysis. In fact, healthy individuals benefit from strong inhibitors of calcification such as pyrophosphate, Fetuin A and MGP. These inhibitors are loaded in vesicles of VSMCs and are released in the presence of increased serum calcium scores. Under normal conditions these vesicles can prevent calcification, but if the inhibitory contents are compromised or if their capacity is overrun by extreme dysbalance of mineral homeostasis, hydroxyapatite crystals can form from these calcium-rich vesicles. Uptake of hydroxyapatite crystals in turn can lead to apoptosis and necrosis of VSMCs, further diminishing the calcification inhibitory capacity of the vessel [99]. This is the initiation of a vicious cycle as a consequence of the disturbed mineral metabolism in CKD and aggravated by abnormalities of mechanisms protective of calcification. Inhibitors of calcification have been studied extensively in adults and to a lesser extent in children with CKD.

The active form of **MGP** is y-carboxylated and antagonizes BMP signaling, which is involved in endothelial dysfunction, formation of vascular lesions calcification and neoangiogenesis [120]. Vitamin K deficiency, as it occurs e.g. with warfarin treatment or in CKD, therefore leads to reduced levels of active MGP and a disposition to increased calcification. But serum levels of MGP alone have no protective effect on calcification, instead MGP therefore has to be expressed in VSMC [121]. Accordingly, no associations of serum levels of undercarboxylated or y-carboxylated MGP to vascular measures have been identified in two studies with dialyzed and transplanted children [85, 122]. Nevertheless, histologically, pediatric dialysis patients showed deposition of undercarboxylated MGP in calcified vessels [104].

Fetuin A is a circulating calcification inhibitor and also a content of VSMC vesicles. In the presence of fetuin A, calcium and phosphate form soluble calciprotein particles instead of hydroxyapatite crystals and thereby prevent ectopic calcification [123]. In children, there are conflicting results of fetuin A levels in dialysis patients. One study found reduced fetuin levels in pediatric dialysis patients compared to healthy controls and children with mild CKD or after transplantation [124]. They were positively correlated with 25OHD dosage and calcium, but not with 25OHD serum levels. Another study found higher fetuin A serum levels in dialysis patients and an inverse correlation with time on dialysis. Vascular calcification was associated with lower fetuin A levels and vascular measures were inversely correlated to aoPWV and augmentation index [85]. Histologically, increased fetuin-A staining was demonstrated in calcified vessels of dialysis patients [105].

Promoters of Calcification and Premature Aging in CKD

There is evidence that the balance of vascular homeostasis shifts towards calcification not only by the loss of inhibitors but also by promoting factors. Uremic toxins induce oxidative stress which in turn hampers the ability of VSMC to

regenerate. Instead, a disabled DNA damage repair system leads to senescence of the cells. An important marker in this process is prelaminin A. Nuclear laminas are of essential significance for the nuclear integrity of cells, and prelamin A normally is converted to laminin A over several steps including farnesylation and subsequental cleavage by the metalloproteinase FACE1/Zmpste24. Oxidative stress induces a down-regulation of FACE1/Zmpste24 and thereby inhibits the completion of functional laminin A [125]. Other than being a biomarker for premature cell senescence, farnesylated prelamin A is possibly actively involved in cell damage, specifically to the nuclear structure. In vessels of pediatric dialysis patients, there was a high prelamin A deposition in calcified arteries which provoked a osteogenic differentiation of VSMC and a senescence-associated secretory phenotype with increased expression of bone morphogenetic protein 2, osteoprotegerin and interleukin 6 [126]. These factors in turn can induce osteogenic differentiation in endothelial and mesenchymal progenitor cells, hereby involving also the vessel endothelium and initiating yet another vicious cycle in the calcification process [127].

Uremic Toxins and Endothelial Dysfunction

Another important pathway of vascular damage is endothelial dysfunction, triggered by the uremic milieu and accumulating toxins. Uremic toxins can be either water-soluble or protein-bound, accumulating either by reduced excretion or alterations of metabolic pathways in uremia. Besides well-studied factors such as homocystein, recently discovered toxins include *p*-cresylsulfate, indoxylsulfate, aysmmetric dimethylarginin (ADMA), advanced glycation endproducts and many more.

Hyperhomocysteinemia is a well-known risk factor for ischemic heart disease and stroke [128]. Homocysteine impairs endothelial function and inhibits endothelium-dependent vasodilatation. It also inhibits angiogenesis and endothelial repair [129]. Plenty of studies have linked homocysteine levels to increased mortality in adults. The highest homocysteine levels in this regard are found in patients with mutations in enzymes or cofactors of the homocysteine metabolism. Also in dialysis patients, homocystein is significantly increased compared to the normal population and high values are associated with an elevated risk for vascular complications [130]. According to a meta-analysis of Wald et al. [131] the risk for ischaemic heart disease, pulmonary embolism and stroke increases with homocysteine levels as identified by comparing individuals with genetically caused higher homocysteine levels to controls without the mutation. However, several controlled clinical trials including trials with CKD patients have failed to show a beneficial outcome when homocystein levels were reduced by substituting folic acid together with either vitamin B6, vitamin B12 or both [132–134]. Several explanations have been proposed to explain this, for example the fact that most studies treated patients with only mildly elevated homocysteine, which might not lead to a measurable effect. Also, homocysteine might be not the real culprit but only a measurable effect of yet another toxic agent. Finally, folic acid, the treatment for hyperhomocysteinemia, increases levels of S-adenosylmethionin, which in turn can increase ADMA levels by providing a methyl donor group to N-methyltransferases. By increasing another uremic toxin, this might partly explain the failure of folic acid to prevent cardiovascular disease by reducing homocysteine [135]. Data about homocysteine and the vascular status in children with CKD is scarce; one small study showed no associations of homocysteine to the cardiovascular status as assessed by flow-mediated dilation of the brachial artery and cIMT [97].

ADMA is a methylated L-arginine derivative which accumulates in chronic kidney disease [136]. It is protein-bound and therefore poorly dialzed. *In vitro* ADMA production is increased by activation of the NADPH oxidase and *in vivo* by angiotensin II infusion [137]. ADMA inhibits NO production, which leads to an increase of systemic vascular resistance, blood pressure and sodium retention [138]. Furthermore, it promotes an inflammatory phenotype and premature senescence of endothelial cells [129]. In children with

CKD stage 1 to 4, increased systolic BP and non-dipping nocturnal BP in 24 h BP measurements were found associated with increased ADMA relative to arginine and symmetric dimethylarginine levels in the urine [139].

***p*-cresylsulfate** and **indoxylsulfate** are produced by intestinal bacterial from *p*-cresol and tryptophan respectively. The EUTox working group analyzed *p*-cresylsulfate and indoxylsulfate in 139 adult patients and both were inversely correlated with renal function and mortality [140, 141]. *p*-Cresyl sulfate was also positively correlated with vascular calcification and mortality [140]. Indoxyl sulfate was associated with vascular calcification and pulse wave velocity [141]. In 609 children with CKD from the 4C Study cohort, serum levels of indoxyl sulfate and *p*-cresylsulfate at the baseline visit were inversely correlated with eGFR. Serum indoxyl sulfate levels were associated with higher cIMT and progression of PWV within 12 months of follow-up [86]. Indoxyl sulfate, but not *p*-cresyl sulfate levels were also independently associated with the prospective loss of kidney function in the same study cohort [142].

The Heart in Pediatric CKD

Cardiac Remodeling

Cardiac remodeling is a chronic and progressive process resulting in genome expression, molecular, cellular, and interstitial transformations that manifest as changes in the size, shape, and function of the heart after cardiac injury [143]. The triggers that initiate cardiac injury are diverse. In case of CKD, mechanical or hemodynamic overload is the initial stimulus, although hormones and cytokines may play an important role in its maintenance. The first response to imposed pressure or volume overload is the hypertrophy of cardiomyocytes (Fig. 61.5). The patterns of sarcomere formation induced by pressure or volume overload are distinct. Pressure-induced hypertrophy, concentric LVH, is characterized by a parallel addition of sarcomeres resulting in the increase of cross-sectional area and diameter of the myocytes. Concentric LVH is closely associated with systolic or pulse pressure. From a physiological view, increased systolic BP and pulse pressure due to increased peripheral resistance and arterial stiffness are the principal factors opposing LV ejection and leading to increased LV workload. With volume-induced hypertrophy, eccentric LVH, addition of sarcomeres occurs in series resulting in longitudinal cell growth with secondary addition of new sarcomeres in parallel. Both myocyte diameter and length are proportionally increased, resulting in a balanced increase in overall wall thickness and LV volume. In the transition to maladaptive LVH, LV dilatation becomes disproportional to wall thickness with myocytes elongated without an increase in diameter.

In patients with CKD, the principal features of volume-induced LV enlargement include volume and sodium retention, anemia, and arteriovenous shunt.

Experimental models of cardiac hypertrophy developed over the last two decades support the theory that mechanical stress due to either pressure or volume overload is a trigger for activation of multiple other mechanisms leading to myocardial remodeling [144, 145]. These factors include metabolic defects in fuel utilization and efficiency, inflammatory responses, lipotoxicity, pathological growth of myocytes and loss of cytoprotective signaling [145]. In patients with CKD, these mechanisms might be activated independently of hemodynamic overload since uremia *per se* is associated with alteration in multiple factors [146–148].

Studies over the last decade have identified fibroblast growth factor 23 (**FGF23**), a bone-derived circulating peptide, as a novel CKD-related risk factor in the development of LVH. Plasma concentrations of FGF23 increase early and progressively in both children [149] and adults [150] with advancing CKD. High FGF23 is associated with premature death [111, 151], cardiovascular events [152], and LVH [153, 154], with experimental data favoring a direct pathophysiologic role of FGF23 in promoting LVH via an FGF receptor 4-dependent pathway [155, 156]. The CKiD study determined that among children and adolescents with GFR

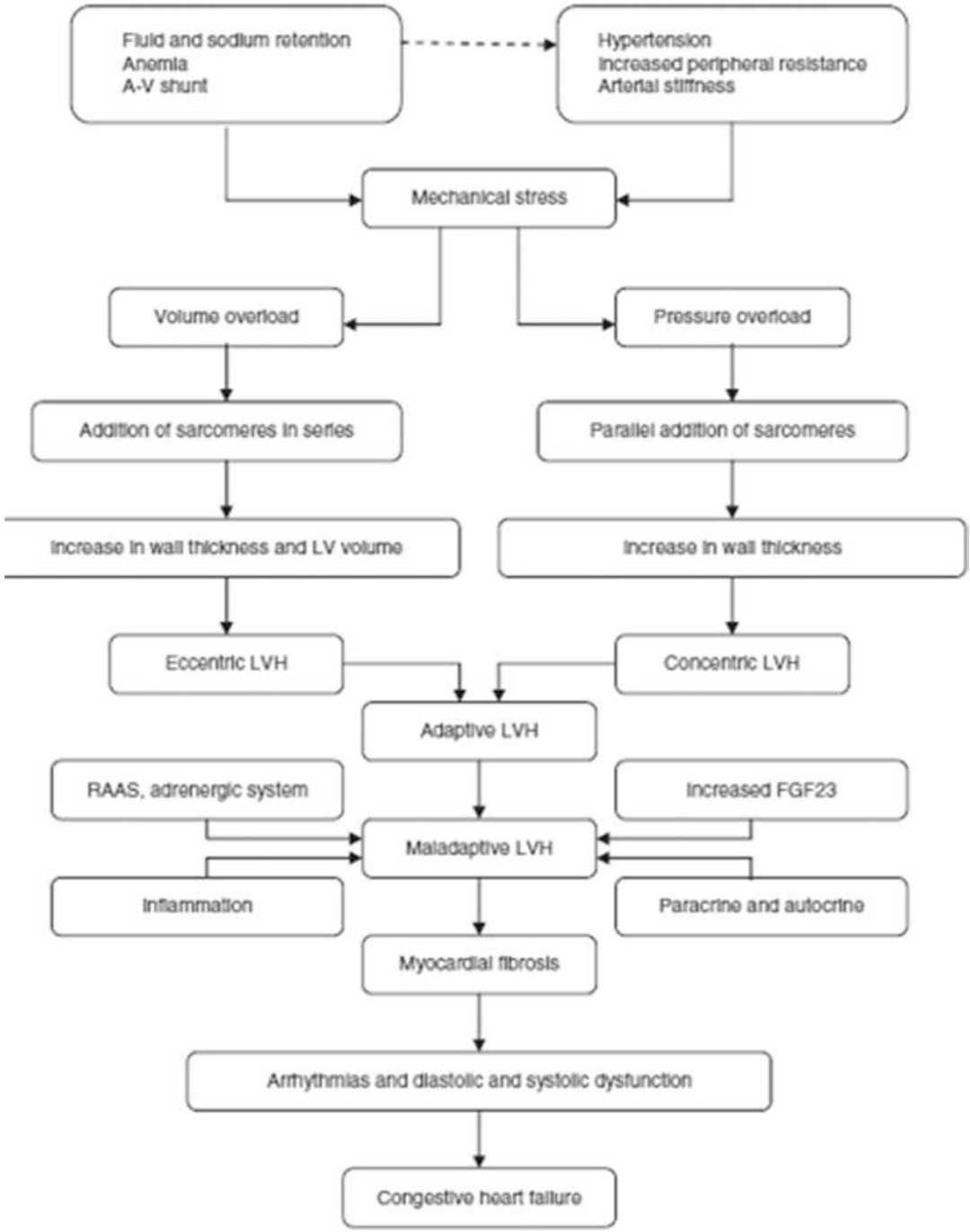

Fig. 61.5 Pathophysiologic mechanisms of LVH. Myocyte hypertrophy is likely an adaptive mechanism designed to improve pump function by expanding the number of contractile units in the myocardium while simultaneously reducing wall stress by increasing wall thickness. The transduction of mechanical stress occurs through the integrin proteins, transmembrane receptors that couple extra cellular matrix components directly to the intracellular cytoskeleton and nucleus. A signal for hypertrophy is mediated by a complex cascade of signaling systems within cardiomyocytes resulting in gene reprogramming. Activated hypertrophy-related genes induce the synthesis of new contractile proteins that are organized into new sarcomeres

≥45 mL/min per 1.73 m^2, higher plasma FGF23 concentrations were independently associated with a higher prevalence of LVH [157]. This association was strongest in participants with FGF23 levels ≥170 RU/mL in whom the odds of LVH was three times higher than in those with FGF23 levels <100 RU/mL.

One of the possible mechanisms of LVH and LV dysfunction is abnormal sphingolipid metabolism leading to **cardiac lipotoxicity**. This condition leading to dilated cardiomyopathy has been described in congenitally obese Zucker diabetic fatty (ZDF) rats [158] and in adults with obesity and diabetes [159]. This is especially relevant to CKD patients, a population with a high prevalence of obesity, metabolic syndrome, and diabetes. In adult incident hemodialysis patients, Mitsnefes et al. [160] demonstrated that high plasma glucosylceramides were consistently and independently associated with CVD outcomes including uncontrolled hypertension, LVH, and decreased ejection fraction. The authors also reported that higher levels of glucosylceramide, C16GC, were associated with increased risk of CVD and all-cause mortality independent of diabetes, comorbidity index, and lipid levels.

A recent study demonstrated that mice with CKD had **T cells** infiltrating the heart and T cell depletion significantly improved both diastolic function and myocardial strain [161]. In the same study, children with CKD had increasing frequency of T cells bearing activation markers PD-1 and/or CD57 that was associated with worsening diastolic function on echocardiogram.

The association of different biomarkers with abnormal cardiac structure and function was examined in the Chronic Renal Insufficiency Cohort (CRIC) Study [162]. The researchers found that the novel biomarker growth differentiation factor 15 (**GDF-15**), a marker of inflammation and tissue injury, and clinical biomarkers N-terminal pro-B-type natriuretic peptide (**NT-proBNP**) and high-sensitivity troponin T (**hsTnT**), were associated with increased LVM and systolic dysfunction.

Initially, LVH is beneficial. It optimizes ejection performance by normalizing systolic wall stress and reducing tension among greater number of sarcomeres. Maladaptive cardiac hypertrophy which develops secondary to prolonged and proportionally increased pressure and volume overload (see above) with declined renal function is a proposed mechanism of congestive heart failure. This in turn results in excessive cardiac myocyte work relative to the supply of oxygen. As a consequence, myocyte death and fibrosis develops, with chamber dilatation and systolic dysfunction. However, the specific mechanisms of *cardiac myocyte death* are not well-determined. Unlike in direct cardiac injury (e.g. acute ischemia) where both necrosis and apoptosis produce cell death, chronic remodeling and transition to overt heart failure have been associated primarily with an elevated degree of apoptosis. However, it is not clear whether apoptosis is a cause or consequence of heart failure. With time, capillary density, coronary reserve and subendocardial perfusion decrease resulting in myocardial fibrosis [163]. *Intermyocardiocyte fibrosis* is a unique feature of uremic heart disease. Experimental uremic models showed selective increase in cardiac interstitial cells and nuclear volume but not in endothelial cell volume [164]. During this phase, patients present with arrhythmias and diastolic and systolic dysfunction, ultimately transitioning to overt heart failure.

Early Markers of Cardiomyopathy

Since symptomatic CVD is very rare in children, the focus of research has been on identifying in children with CKD early markers or intermediate CV outcomes. Over the last two decades, echocardiographically detected abnormalities of the LV such as LVH and LV dysfunction have been accepted as early markers of cardiomyopathy. In adults, the CRIC study demonstrated that the prevalence of LVH was 32%, 48%, 57%, and 75% for eGFR categories ≥60, 45–59, 30–44, and <30 mL/min per 1.73 m^2, respectively [165]. These abnormalities historically have been considered as strong independent predictors of coronary artery disease, heart failure and cardiac mortality both in the general population and in adults with hypertension and CKD. As early as in 1989, Silberberg et al. [166] showed that adult

patients on maintenance dialysis who were diagnosed with LVH had a 52% higher 5-year mortality rate than patients without LVH. A few years later, Foley et al. [167] determined that LV dilatation and systolic dysfunction at start of dialysis were independently associated with mortality. The results of these studies triggered an investigation of cardiac structure and function in pediatric patients with CKD. Studies over last two decades have proven that abnormalities of LV are also present in children with CKD.

Evaluation of LVM in Children

M mode echocardiographic measurement is currently the most commonly clinically used imaging modality to assess LVM. The LVM is calculated according to recommendations from the American Society of Echocardiography [168]. This method applies measurements of LV end-diastolic diameter (LVED) and septal (IVS) and posterior wall (PW) thicknesses and accurately predicts LVM through the equation: $0.8[1.04\{[LVED + PW + IVS]^3 - LVED^3\}.] + 0.6$. Adjusting the calculated LVM to account for differences in age, height and weight is the next step to establish uniform reference values and criteria for LVH. Unfortunately, there is no uniform definition of LVH in children. The most ideal *indexing* parameter is lean body mass (LBM), but this is difficult to measure. Indexing LVM by patient height raised to approximately cubic exponential power has been shown to produce the greatest reduction in LVM variability in normal subjects, most correctly to detect differences between normal and obese subjects [169], and, importantly, correlates most closely to indexing by lean body mass [170]. However, in children, dispersion of residual variation of LVM/height$^{2.7}$ increases with either increasing height or age suggesting that other variables effect ventricular growth in children. Though further investigation is needed to determine the most ideal indexing parameter, dividing LVM by height$^{2.7}$ (g/m$^{2.7}$) seems to work well for older children and adolescents [171]. For children older than 9 years, the value of LVM index 95th percentile is relatively stable and is 38–42 g/m$^{2.7}$. The most recent American Academy of Pediatrics Clinical Practice Guidelines (CPG) published in 2017 for screening and management of high BP in children and adolescents formally recommend LVM index value of 51 g/m$^{2.7}$ as a conservative cut point to define LVH in children >8 years of age [172]. This value is above 99th percentile for children and adolescents and associated with up to a fourfold increase in cardiovascular morbidity in adults with hypertension [171]. The use of this value to define LVH in young children is problematic since the normative values for LVM index (g/m$^{2.7}$) are significantly higher than in older children as can be seen in study by Khoury et who published pediatric reference values for LVM index (g/m$^{2.7}$) based on the analysis of 2273 non-obese, healthy children and adolescents (0–18 years) [173] (Table 61.2. The data indicate that indexing of LVM to a power of height$^{2.7}$ is age and gender specific. For example, the 95th percentile for LVM index varies from 40 to 45 g/m$^{2.7}$ in adolescent girls and boys to about 70 g/m$^{2.7}$ in one-year old children; boys have a slightly larger LVM for a given height throughout childhood and adolescence (Fig. 61.6).

One of the potential pitfalls in using age-dependent LVM indexing in children with CKD is related to short stature. Foster et al. [174] demonstrated that LVMI (g/m$^{2.7}$) varies not only according to age but also according to absolute height with higher values in children with shorter height, especially in those with height < 110 cm. Given that the CKD patients have significantly reduced height relative to age, poor growth may result in some miss-classification of diagnosis of LVH in very young children. To account for short stature, Borzych et al. [175] suggested substituting chronological age by height age (*i.e.*, the age of a child of the same height growing at the 50th height percentile) using Khoury et al. [173] references. Chinali et al. studied 400 healthy children and proposed to use a single value of 45 g/m$^{2.16}$ across the whole pediatric age range to diagnose LVH, arguing that the allometric exponent 2.16 fully normalizes the LVMI in children of all ages and both sexes and removes the need to calculate specific percentiles for height and sex (Fig. 61.7) [176]. This alternative method of indexing LVM is promising and potentially, if validated in larger

Table 61.2 LVM (g) and LVMI (g/m$^{2.7}$) percentile values

				Percentile							
Age	Gender	N	Variable	10th	25th	50th	75th	90th	95th	Minimum	Maximum
<6 months	Boys	62	LVM	7.22	9.04	10.94	14.16	16.28	17.6	6.27	21.18
			LVMI	40.19	46.92	56.44	66.41	75.72	80.1	32.41	83
	Girls	43	LVM	7.59	9.27	11.15	13.76	16.05	16.5	5.49	28.74
			LVMI	39.05	48.62	55.38	65.98	73.47	85.6	21.22	109.2
6 months ≤2 years	Boys	73	LVM	16.95	20.25	23.88	27.84	32.47	33.7	9.43	36.32
			LVMI	36.17	40.66	44.95	53.29	61.27	68.6	26.71	74.75
	Girls	53	LVM	15.39	17.45	22.25	26.46	31.98	34.6	12.22	35.98
			LVMI	32.91	38.67	42.04	49.85	52.86	57.1	24.18	61.06
2 ≤4 years	Boys	124	LVM	24.37	28.52	33.31	38.79	45.48	48.4	13.27	58.13
			LVMI	28.44	33.88	39.5	45.19	48.74	52.4	21.25	77.07
	Girls	84	LVM	24.7	28.4	33.34	38.15	43.88	46.1	17.9	50.98
			LVMI	28.87	31.85	37.88	43.11	47.65	55.3	20.63	66.58
4 ≤6 years	Boys	133	LVM	34.36	39.13	45.49	52.62	59.26	63.2	22.92	83.51
			LVMI	27.68	30.68	36.96	40.2	45.12	48.1	18.76	57.25
	Girls	111	LVM	29.24	34.57	39.67	46.59	50.38	57.3	17.68	76.64
			LVMI	25.85	28.06	32.29	36.43	43.47	44.3	18.17	59.25
6 ≤8 years	Boys	117	LVM	40.23	45.14	51.73	62.06	70.48	77.4	25.95	97.29
			LVMI	24.47	28.56	31.79	36.28	40.18	44.6	20.27	59.47
	Girls	110	LVM	36.88	40.6	48.38	55.84	65.54	72.1	25.29	89.3
			LVMI	23.15	25.77	29.71	33.15	37.73	43.5	20.11	54.76
8 ≤10 years	Boys	111	LVM	45.32	51.49	62.09	73.42	84.61	91.1	32.35	122
			LVMI	22.45	24.85	29.11	34.57	38.25	41	15.24	53.19
	Girls	99	LVM	39.22	48.08	54.76	70.87	75.49	83.6	31.6	91.82
			LVMI	19.07	22.12	26.63	30.37	34.3	36	13.46	44.35

LVM left ventricular mass, *LVMI* left ventricular mass index (with permission of Elsevier from Khoury et al. [173])

studies, could replace currently utilized normative data.

Cardiac MRI is considered to be the most accurate imaging modality to measure LVM. While the normative pediatric percentile values have been developed [177], this modality has limited availability and still remains too expansive to recommend it for routine use. In addition to measurement of LVM, M mode echocardiography can be used to define *LV geometric pattern* (Fig. 61.8).

LV geometry is evaluated based on the 95th percentiles for LVM index and relative wall thickness. Relative wall thickness (RWT) is calculated from measurements made at end diastole as the ratio of the PW thickness plus IVS thickness over the LVED. Normal geometry is defined as LVM index and RWT below the 95th percentile. Concentric remodeling is defined as LVM index below the 95th percentile with RWT greater than the 95th percentile. Eccentric LVH is defined as LVM index greater than the 95th percentile and RWT below the 95th percentile. Concentric LVH is defined as both LVM index and RWT greater than the 95th percentile. As in case of LVM index, the 95th percentile values for RWT are not uniform and vary from 0.375 to 0.41. Thus, the frequencies of different geometric patterns may differ depending on the cut off points used in the study.

Studies of LVH in Children with CKD

As in adults, LVH develops when **CKD** is mild or moderate in children and progresses as kidney function deteriorates. The baseline data on LVH from the CKiD cohort indicated a prevalence of 17% [31]. In this study, in addition to confirmed hypertension (elevated casual and ambulatory BP) and lower hemoglobin, masked hypertension (normal casual BP and elevated ambulatory BP) and female gender were independently associated with LVH. The likelihood of having LVH was four times higher in children having masked hypertension compared to children with normal clinic and ambulatory BP. The follow up study determined that over 4 years, the prevalence of LVH diminished from 16% to 11% [178]. Decrease in BP corresponded to regression of LVH in these children. As in the cross-sectional baseline analysis, the likelihood of developing of LVH was four times higher in females. The 4C study reported an increase in the prevalence of LVH from 10.6% in CKD stage 3a to 48% in CKD stage 5 [68].

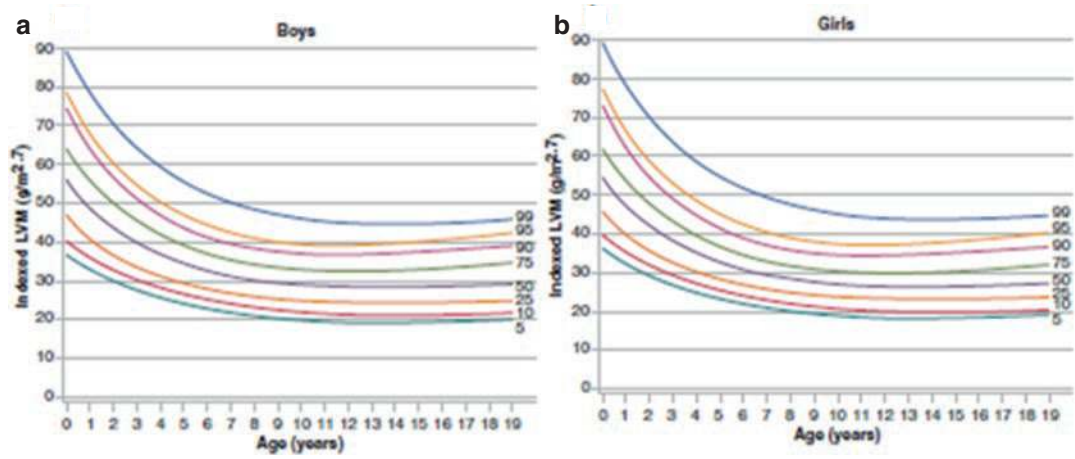

Fig. 61.6 The estimates of the 5th, 10th, 25th, 50th, 75th, 90th, and 95th quantiles of LVM indexed to height$^{2.7}$ for ages 0 through 18 years. Values displayed separately for boys (**a**) and girls (**b**) (used with permission of Elsevier from Khoury et al [173])

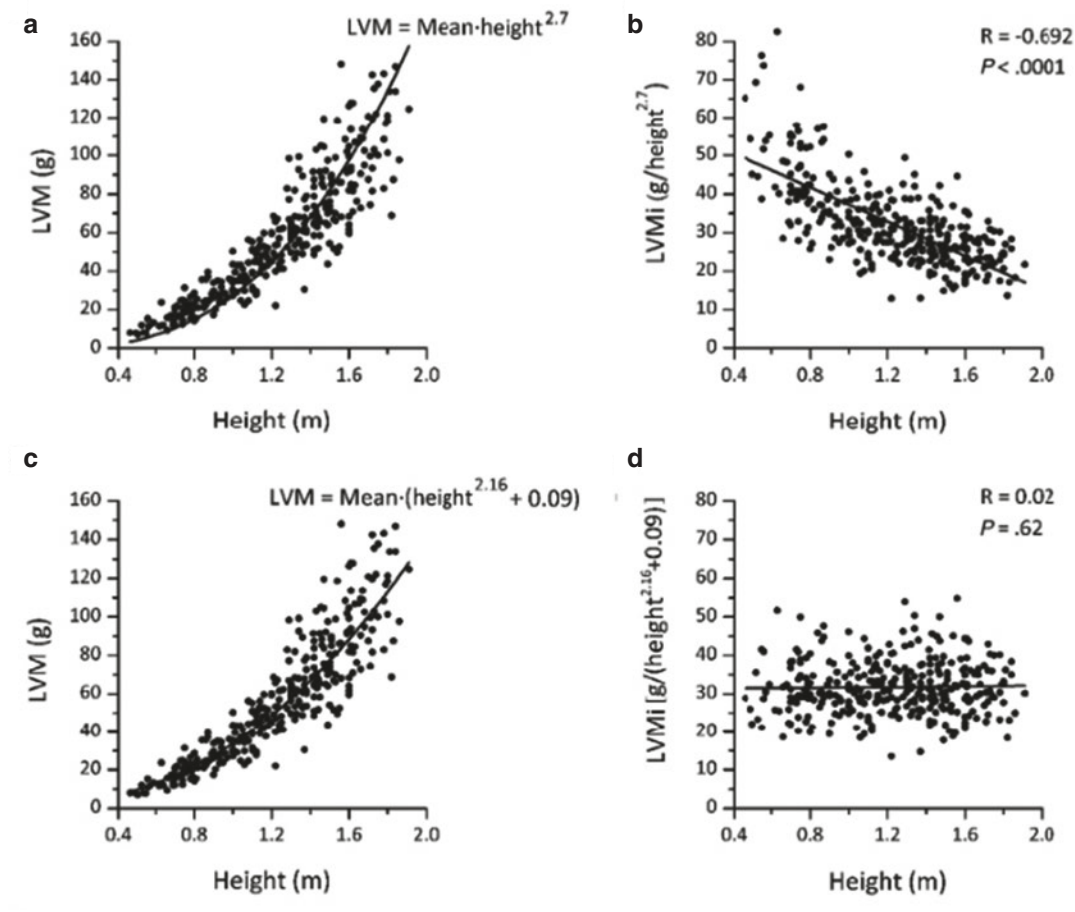

Fig. 61.7 (**a**) Regression of LVM with height 2.7; (**b**) distribution of residuals for height 2.7; (**c**) regression of LVM with height2.16; (**d**) distribution of residuals for height2.16; and. *LVMi* LVM index (adapted and used with permission of Elsevier from Chinali et al [176])

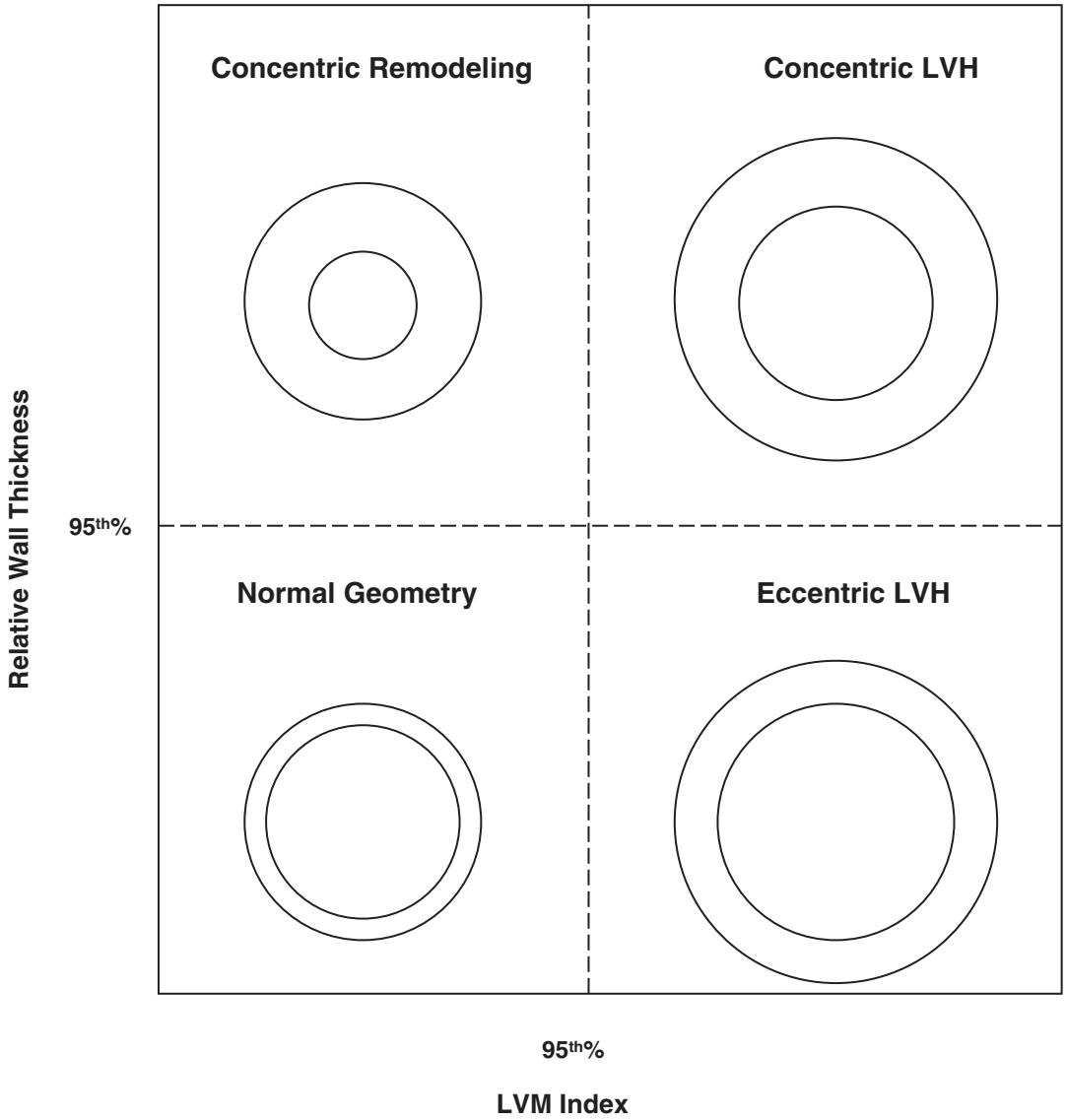

Fig. 61.8 LV geometry

Since the early 2000s there have been many studies around the world evaluating LVH in patients on maintenance **dialysis** and all indicated a much higher prevalence of LVH (40–80%) [18, 179–185] than in children prior to dialysis or after kidney transplantation. Small retrospective studies also suggest that with a better BP and volume control LVH regression might be achieved in young patients on dialysis [179, 180, 186]. The largest retrospective analysis from the International Pediatric Peritoneal Dialysis Network (IPPN), using height-age adjusted normative values for LVMI [175], demonstrated an overall LVH prevalence of 48.1% [187]. In the prospective analysis, the incidence of LVH developing *de novo* in patients with normal baseline LVM was 29%, and the incidence of regression from LVH to normal LVM 40% per year on PD. Transformation to and regression from concentric LV geometry occurred in 36% and 28% of the patients, respectively. Hypertension, high body mass index, use of continuous ambulatory peritoneal dialysis, renal disease other than hypo/dyspla-

sia, and hyperparathyroidism were identified as independent predictors of LVH.

As in children prior to transplantation, most single-center pediatric studies indicate that LVH remains common (30–50%) **post-transplant** and relates to not adequately controlled blood pressure [181, 188–193]. A study from the Midwest Pediatric Nephrology Consortium (MWPNC) showed that LVH was significantly more common in those with metabolic syndrome (55%) than in those without (32%) [40]. In contrast to the above studies, some studies showed a significantly lower frequency of LVH. Englund et al. [194] reported the results of a longitudinal analysis of children who received renal transplants 10–20 years ago. Of 53 children who received a renal transplant between 1981 and 1991, 47 survived and were observed for 10 to 20 years. Before primary transplant, 51% of the 53 children were prescribed antihypertensive treatment. At the 10-year follow-up, echocardiography showed minor LVH in only two children with hypertension. No child without hypertension at 10 years showed LVH. Progressive aortic insufficiency was discovered in two children, one of whom had a supravalvular aortic aneurysm requiring surgical repair 10 years after transplant. Low prevalence of LVH post-transplant (4.5%) attributed to well-controlled ambulatory blood pressure was observed in a study by Balzano et al. [195].

There are have been few small studies assessing LVH status in children with CKD using cardiac MRI. In a study by Schaefer et al. [196], LVH has been demonstrated in 12 of 15 (80%) patients with CKD, while the prevalence of LVH was seven of 18 (39%) in the transplant patient group. Avendt et al. [197] found only 3 of 20 patients on maintenance dialysis and 1 of 18 patients post kidney transplant to have LVH. In the largest study to date (n = 120) utilizing cardiac MRI, Cheang et al. [198] demonstrated increased LV mass to volume ratio (MVR) in children with severe CKD suggesting LV concentric remodeling.

Left Ventricular Function and Pediatric CKD

In contrast to adults, in whom *systolic dysfunction* is frequently associated with early cardiac failure and decreased survival, in children with CKD systolic LV function is usually preserved [181, 199, 200]. The majority of pediatric studies have examined LV systolic performance using indices of performance which are dependent on loading conditions. This presents a major problem in patients with CKD or on dialysis because they may have substantial alteration of preload and afterload. A load independent index of contractility can be determined based on the relation between heart rate-corrected velocity of circumferential fiber shortening (VCF) and end-systolic wall stress (WS) by calculation of the difference between measured and predicted VCF for the calculated WS [201]. Using this index, Mitsnefes et al. [202] showed that children with CKD or on maintenance dialysis had increased LV contractility at rest. However, patients on dialysis had decreased contractile reserve during exercise, which might herald the development of a maladaptive stage of LVH. The mechanism of increased contractility in pediatric patients with CKD is not clear. The combination of increased heart rate, cardiac output and hypertension in these patients is consistent with a hyperdynamic circulation and suggests the possible role of sympathetic overactivity. Similar results were demonstrated in a more recent study by Gu et al. [203].

A study from the ESCAPE trial argues that subclinical systolic dysfunction is already present in predialysis children [204]. The authors analyzed LV shortening at the midwall level (midwall shortening [mS]), which more accurately reflects the contractile force independent of pathologic changes in LV geometry [205, 206]. Using this index, the study determined that the prevalence of subclinical systolic dysfunction as defined by impaired mS was more than five-fold higher in patients with CKD compared with control subjects (24.6 *versus* 4.5%; $P < 0.001$). Systolic dysfunction was most common (48%) in

patients with concentric hypertrophy and associated with lower hemoglobin levels. The authors concluded that the combination of concentric LV geometry with midwall dysfunction might represent a cardiac phenotype designating an increased risk for development of overt CVD.

Additional, more novel and sensitive, assessments of cardiac systolic function, including strain and strain rate, have been shown to detect differences in function prior to ejection or shortening fraction in a variety of populations, such as Duchenne muscular dystrophy [207], sickle cell disease [208] and survivors of childhood acute leukemia [209]. An analysis of the 4C study showed a high prevalence of subclinical systolic dysfunction in children with CKD characterized by lower radial and circumferential LV strain paired with a mild cardiac systolic dyssynchrony [210].

Relatively few studies have assessed *LV diastolic function* in pediatric patients with CKD [181, 200, 211–214]. Doppler measurement of mitral inflow velocity has been the most widely used method to assess LV diastolic function. Unfortunately, the transmitral Doppler velocities and, therefore the E/A ratio, are affected by several factors, including left atrial pressure and preload. This is particularly important for patients with advanced CKD, since many of them are hypervolemic. Tissue Doppler Imaging (TDI) is a more reliable method to assess diastolic function in CKD patients since it is less load dependent and provides a more accurate measure of diastolic function. Studies employing TDI determined that children with CKD have abnormal diastolic function [211, 212]. In these studies, children on maintenance dialysis had significantly worse diastolic function than children with mild-to-moderate CKD or post-transplant. Poor diastolic function in patients on dialysis was associated with anemia, hyperphosphatemia, increased calcium-phosphorus ion product and LVH. Data from the 4C study demonstrated an independent association of worse E/e', a marker of LV compliance, with higher systolic BP and a better E/e' z-score with RAS inhibition [214]. Recent data from the CKID study showed that 15% of participants had abnormally high E/e' ratio [215]. In adjusted analysis, a higher E/e′ ratio was independently associated with ambulatory (sustained) hypertension, higher LVM index *Z* score, increased BMI *Z* score, lower hemoglobin, higher phosphorus level, and younger age. Casual blood pressure was not significantly associated with higher E/e′. These data indicated that ambulatory blood pressure might better identify children with CKD at risk for subclinical cardiac dysfunction than clinic blood pressure alone. Long-term significance of diastolic dysfunction in pediatric patients with CKD is not known. Longitudinal studies are necessary to determine if abnormal diastolic function predicts the development of systolic dysfunction and congestive heart failure in these patients.

Hothi et al. [216]. measured myocardial stunning as a marker of cardiac function in children on maintanence hemodialysis. Eleven of 12 patients developed myocardial stunning with varying degrees of compensatory hyperkinesis in unaffected segments, maintaining left ventricular ejection fraction throughout treatment. Recently, these authors evaluated regional LV function and mechanical synchronicity echographically by two-dimensional segmental longitudinal, circumferential and radial myocardial strain [217]. All patients were assessed pre-dialysis and at the end of dialysis. Radial strain was lower in uremic patients and increased during HD. Circumferential strain was preserved in uremic patients and fell during HD. Intrasegmental deformation synchronicity was progressively worse pre-dialysis and end of dialysis compared with controls. Some observations suggest that cardiac MRI and MR Spectroscopy could play a potentially useful role in the early detection and monitoring of cardiac dysfunction in children with CKD. Malatesta-Munter et al. [218] studied the biomarkers of cardiac function that included peak LV myocardial circumferential strain (Ecc) to assess regional LV function, T2 relaxation time to quantify myo-

cardial structural composition, and phosphocreatinine/ATP (PCr/ATP) ratio from phosphorus-31 Magnetic Resonance Spectroscopy (^{31}P MRS) to assess muscle energy metabolism. This report demonstrated decreased Ecc (45%), decreased energy metabolism and abnormal myocardial micro-composition. These abnormalities were detected despite uniformly normal ejection fractions.

Clinical Approach to CVD in Children with CKD

The primary goal to prevent and minimize development of CVD in children with CKD is avoidance of long-term dialysis and preemptive transplantation if feasible. For those children who must have long-term dialysis, the strategy is directly linked to achievement of adequate dialysis outcomes, including aggressive monitoring and management of hypertension, dyslipidemia, mineral metabolism, anemia, nutrition, inflammation, and other dialysis complications. Unfortunately, achieving recommended Kt/V urea does not necessarily lead to control of the above problems. Epidemiologic data on cardiac death in both peritoneal dialysis and hemodialysis clearly indicate that current dialysis adequacy recommendations based on Kt/V are not adequate to decrease CVD morbidity in children with ESKD. Unlike in adults, there have been no randomized pediatric studies examining the role of more frequent or nocturnal hemodialysis in cardiac outcomes. However, small single-center studies of frequent or nocturnal dialysis indicate clinically important improvement in children' health including growth, hypertension, cardiac hypertrophy and function [219–222]. Hemodiafiltration (HDF) is also advocated as a preferred dialysis modality that might improve cardiovascular health in children with ESKD. Results from the HDF, Heart and Height (3H) study, a nonrandomized observational study comparing outcomes on conventional hemodialysis (HD) versus postdilution online HDF in children showed that HDF was associated with a lack of progression in vascular measures versus progression with HD, as well as an increase in height not seen in the HD cohort [223]. The study also indicated improved patient-related outcomes among children on HDF correlating with improved BP control and clearances. Taking the potential for a longer and possibly more productive life, the benefits of HDF, more frequent and longer dialysis treatment might be more far reaching in children than in older adults [224].

Otherwise, management strategies should be specific to the stage of CKD (predialysis, dialysis, or transplant) as each has a unique subset of common risk factors. KDOQI and/or KDIGO recommendations for the management of most common individual CV risk factors are relatively recent but overall these guidelines are not based on strong evidence, especially when applied to children. For these reasons, the recommendations should always be tailored to the specific patient. Typically, separate recommendations are provided for advanced and end-stage kidney disease.

As in the general pediatric population, traditional risk factors like hypertension, dyslipidemia, and obesity should be a primary focus in CV risk reduction in children with CKD. In addition, evaluation and treatment of mineral metabolism abnormalities should be a prioritized focus of management.

Hypertension

The most recent 2012 KDIGO recommendations on BP control are restricted to non-dialysis (CKD and transplant) patients only [225]. These guidelines do not focus on methodological or diagnostic issues or lifestyle modification options to treat hypertension and are restricted to pharmacological treatment only. The recommendations are based on renal outcomes; however, because of the association of hypertension with LVH and increased cIMT, they can be reasonably applied to prevent and minimize CV outcomes. The BP targets refer to manual auscultatory BP. However, some of the recommendations are based on the results of studies

utilizing ambulatory blood pressure monitoring. Since the publication of the 2012 KDIGO recommendations, there have been updated guidelines on management of BP in children from USA, Europe and Canada. While these updated guidelines are generally consistent with KDIGO recommendations, they are not identical. There are three main recommendations. First, KDIGO recommends initiation of BP lowering treatment when BP is consistently above the 90th percentile for age, sex, and height (1C level of evidence). This recommendation is consistent with the Canadian guidelines [226] but not with that of the 2017 AAP [172] or 2016 European Society of Hypertension (ESH) guidelines [227], which recommend starting BP medications if BP is consistently above the 95th percentile in the general pediatric population. The AAP 2017 also recommends using adult guidelines for initiating treatment (BP is >130/80 mmHg) for children aged 13 years and older. However, some of these patients might have small stature and may require a lower BP threshold to initiate treatment.

The second recommendation is to lower manually obtained clinic BP below 50th percentile for age, sex, and height, particularly for those children who have proteinuria (2D level of evidence). However, this recommendation is based on the results of the ESCAPE study even though the ESCAPE trial used mean arterial pressure from ABPM to classify BP [228]. A 2017 KDIGO controversy conference report on BP in kidney disease advised to revise this recommendation accordingly [229]. However, because ABPM is not widely available, the conference also recommended using oscillometric devices as an alternative.

The third recommendation is the use of ACEI or ARB irrespective of the level of proteinuria. Despite only 2D level of evidence, this recommendation is relatively well accepted by the pediatric nephrology community. The KDIGO guidelines are slightly different from the 2016 ESH guidelines [227]. Based largely on the ESCAPE trial results, ESH guidelines recommend that in children with CKD, BP targets should be below the 50th percentile in the presence of proteinuria (the same as KDIGO) and below the 75th percentile (versus 90th percentile KDIGO) in the absence of proteinuria.

In the child on dialysis the presence of hypertension is mostly related to fluid overload, and attainment of dry weight will result in lowering of BP in the majority of patients. Dry weight and dialysis prescription need to be frequently adapted to avoid fluid overload induced hypertension. Thus, more frequent or prolonged dialysis sessions are the primary intervention to control hypertension. In addition, supportive measures aiming for a low extracellular volume as dietary salt restriction, low dialysate sodium content and restriction of fluid intake are important to complement an adequate dialysis prescription. The BP targets for dialysis patients are not as strict as for non-dialysis patients. The KDOQI guidelines recommend the BP target to be below the 95th percentile for age, sex, and height [230]. The KDIGO 2019 Controversy Conference on BP and volume management in dialysis addresses four major topics including BP measurement and targets, pharmacologic management, dialysis prescriptions as they relate to BP and volume and extracellular volume assessment and management with a focus on technology-based solutions; and volume-related patient symptoms and experiences [231].

Dyslipidemia

The latest guidelines for lipid screening and treatment in children with CKD by KDIGO published in 2013 recommend evaluation with a lipid profile (total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides) in newly diagnosed CKD (including those treated with chronic dialysis or kidney transplantation) [232]. Management of dyslipidemia should include therapeutic lifestyle changes including dietary modification, weight reduction, increased physical activity, reducing alcohol intake, and treatment of hyperglycemia (if present), especially in overweight/obese children. In case of significant hypertriglyceridemia, dietary changes include a very

low-fat diet (15% total calories), medium-chain triglycerides, and fish oils to replace some long-chain triglycerides. The initiation of statin therapy is based on a risk assessment and LDL-cholesterol level. Because children with CKD belong to a high level risk factor group, the statins are recommended at LDL-cholesterol level > 160 mg/dL. However, if there are additional moderate risks (e.g., hypertension, obesity, nephrotic syndrome as a cause of CKD), statins should be started at LDL-cholesterol >130 mg/dL. Given the lack of evidence for the benefit and safety of combination therapy with bile acid resins, colestipol and ezetimibe in pediatric CKD populations, the KDIGO Work Group does not recommend the use of such multi-drug regimens even in children with severely elevated LDL-C. Pharmacological treatment of hypertriglyceridemia with fibrates is not recommended in children with CKD because of concerns of developing pancreatitis.

Obesity and Physical Inactivity

Evidence from adult maintenance dialysis populations suggests a survival advantage conferred by higher BMI [233]; however, this appears to be limited to those with low body fat and high muscle mass [234]. Per KDOQI, "the safety and efficacy of weight loss in the overweight dialysis patient is unknown. Therefore, weight loss in the dialysis patients should be approached with close monitoring by a registered dietician and physician" [230]. However, increased levels of physical activity are encouraged to improve exercise capacity [235]. It is unknown whether obesity in children with ESKD confers survival benefits while on maintenance dialysis. For children with CKD prior to dialysis or post-transplant, treatment of obesity and maintenance of normal body habitus (BMI between fifth and 85th percentile for age, sex) is advisable. This recommendation is based on the evidence that obesity is associated with worse long-term outcomes. For example, it has been long known that severe childhood obesity is a significant risk factor for CKD in children and adults [237–239]. After pediatric renal transplantation obesity is associated with higher rates of graft dysfunction and graft loss [239, 240]. Recommendations on management of obesity in CKD children should be individualized according to their age, physical tolerance, CKD stage, dialysis and co-morbidities and, as in the general pediatric population, include dietary modification, behavior modification and physical activity. Older adolescents with CKD, extreme obesity (BMI $\geq$ 40) and other comorbidities associated with long-term risks who failed to respond to dietary or behavior modifications may consider weight loss surgery.

Left Ventricular Hypertrophy

Overall, based on data that LVH is more frequent in children diagnosed with hypertension, it seems appropriate to perform echocardiography in children with CKD and in renal transplant recipients who have elevated casual BP and/or masked hypertension on ABPM. Echocardiography also should be considered in children with severe anemia. If LVH is diagnosed, periodic follow-up echocardiographic monitoring is suggested. Although not perfect, we suggest using the 2017 AAP CPG recommendations to diagnose LVH. Specifically, one can use the references of Khoury et al. [173] for children $\leq$8 years of age and a single cut off point of 51 g/m$^{2.7}$ for older children. If the child has a short stature, height age adjustment is warranted as described by Borzych et al. [175]. As we mentioned above in this chapter, alternative approach using a single cut off point of 45 g/m$^{2.16}$ to define LVH has recently been introduced and used in Europe [176].

Mineral and Bone Disorder

In clinical practice, the focus should be on maintaining serum phosphorus and calcium levels at age-appropriate normal range and on prevention of significant hyperparathyroidism and low vitamin D levels. The most recent KDIGO Clinical Practice Guideline Update for the Diagnosis,

Evaluation, Prevention, and Treatment of Chronic Kidney Disease–Mineral and Bone Disorder (CKD-MBD) [241] provides specific recommendations on timing of assessment and treatment options if abnormal markers of MBD are found (discussed in details in chap. 58). The dietary management of calcium and phosphate in children with CKD stages 2–5 and on dialysis is updated by recommendations from the Pediatric Renal Nutrition Taskforce [242].

Future

Since the first description of increased cardiovascular risk in children with CKD more than two decades ago, significant improvements have been achieved and guidelines have been developed to address individual cardiovascular risk factors. However, many recommendations are still based on opinion rather than on clear clinical evidence. Current and future longitudinal multicenter studies have the potential not only to monitor the implementation of treatment goals, but also to analyze the success in improving cardiovascular outcome in pediatric patients with CKD. Insights from these studies will also pose new questions which have to be brought from bedside to bench and vice versa, ultimately helping to continuously improve recommendations for each individual patient.

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Ethical Issues in End Stage Kidney Disease

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Introduction

Since the development of pediatric nephrology as a discipline in the twentieth century, we have experienced a progressive expansion of our capabilities in caring for children with significant kidney disease. As treatments improved, patients and their families have been asked to choose from a wider array of treatments, and decisions to pursue or forgo those interventions have acquired greater ethical weight. Moreover, as care of children with kidney disease is provided within the context of health care systems and policies on a regional, national, and international level, the ethics of medical care must be considered with a broader lens than simply doing "what is best" for our individual patients. In this chapter we will review both the structures of contemporary bioethics as it applies to decisions made by patients and their families, as well as some of the current areas of ethical concern within pediatric nephrology.

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Contemporary Bioethics and the Limits of Parental Decision-Making

When providing care to a competent adult, there is a strong presumption that the decisions made by the patient should direct care. While it is true that factors such as the clinician's assessment of what might be beneficial or harmful to the patient, as well as broader system issues regarding resource availability may also play a role, the wishes of the patient are generally paramount. This framework of medical decision-making is derived from an understanding that individual adults have both a right to dictate the care that they receive based on a fundamental respect for bodily integrity and are in the best position to assess how a given treatment course will support their own goals and values [1]. In contrast, by nature of the fact that they are continuing to grow and develop, children are assumed to lack a long-standing system of values or the appropriate understanding of the implications of a given treatment decision. As such, there is a long-standing tradition of deference to parental decisions in pediatric care.

Although parents are generally considered the primary decision-makers in the pediatric setting, their authority is not absolute. Both the child, as well as society more broadly, have an interest in the child's well-being. This is reflected across laws in numerous countries that place limits on

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parental activities such as corporal punishment, and mandate that parents provide for children's basic needs, including nutrition, education, and health care [2, 3]. While the details of these laws are shaped by local custom and other social aspects, they reflect an acknowledgement that the community has an obligation to provide for children given their vulnerable state. Within the context of healthcare, this raises the possibility of significant conflict when a decision made by a patient and their family is incompatible with what the clinician thinks would be most beneficial to the patient.

Historically, when these conflicts have arisen, they have been resolved by an assessment of what would broadly be considered in the child's best interest [4]. Within bioethics, the best interest standard requires a decision-maker to consider the child's present and future self-regarding interests alone and select the treatment that maximizes benefit and minimizes harms [4]. While the best interests standard is well suited to conflicts when the risks and benefits of each treatment course are clearly disproportionate (such as a decision to forgo antibiotic therapy for bacterial pyelonephritis), there are other instances in which the determination of what is "best" is dependent on highly personalized assessment of the relative value of different factors. In a more complicated case, such as dialysis for a preterm infant, it is entirely possible that parents and clinicians may consider the same risks and benefits of treatment and reach different conclusions on what is "best." Additionally, children have a variety of physical, intellectual, social and emotional interests and a precise determining what actions "best" support these interests may not be possible. Finally, the best interest standard fails to acknowledge that we do not typically require that parents do what is absolutely "best" for children in all circumstances. Given the varied obligations that parents have to themselves, their children, and their community this would be impossible.

Instead of seeking to overrule parental authority for decisions that are not in the best interests of the child, Diekema argues such actions should be reserved for parental choices, generally refusals, that place a child at clear risk of significant harm [5]. The harm principle allows for the differing values that parents may place on benefits and burdens of treatment options when compared to the opinions of medical professionals, but does not allow for parents to refuse a treatment in which serious, imminent harm is expected to occur and where there is a reasonable intervention that could avoid that harm [5]. The best interest standard and harm principle were further operationalized through the concept of the zone of parental discretion, which sought to highlight the range of choices parents may make so long as they do not exceed a harm threshold [6]. Although the concept of "best-interest" is frequently referenced in the clinical setting, there is broad consensus within the field of pediatric ethics that in complex medical decision-making, such as the care of children with chronic kidney disease (CKD), the best interests standard is better considered aspirational to guide decision-making and the harm principle is better reserved for situations where clinicians must consider overruling parental authority [7].

Ethical Issues in Dialysis and ESRD

Withholding, Withdrawal, and Forgoing Dialysis Treatment

There is no universally accepted criterion for forgoing life-sustaining treatments such as dialysis. Decisions concerning withholding or withdrawing dialysis remain a source of controversy and distress for children, their families, and nephrologists. Withdrawal of dialysis is common among adults on dialysis, accounting for approximately one quarter of deaths among dialysis patients in the United States. Withdrawal of life-sustaining treatments is also a leading cause of death in neonatal and pediatric intensive care units [8, 9]. Less is known about withholding or withdrawal of dialysis treatment for children, although one analysis of French-speaking pediatric nephrology centers from 1995–2001 found 50 cases where dialysis was withheld or withdrawn among

440 children with end-stage kidney disease (11.5%) [10]. The most common reasons for withdrawal included concerns of subsequent quality of life, severe neurological handicap, and consequences of the disease on the family [10].

Decisions made to forgo dialysis should be individualized, shared, and consistent with the interests of the child and the benefits and burdens resulting from renal replacement therapy or compassionate conservative treatment [11]. Choices should reflect the patient and family's goals of care that are achievable and be centered upon the patient's quality of life [11–13]. Four concepts that may influence decisions to forgo dialysis treatment include: perception of moral difference between withholding and withdrawing treatment, the technological imperative, futility, and time-limited trials of therapy.

Withholding, Withdrawal, Equivalence

Withholding dialysis is defined as foregoing dialysis in a patient for whom dialysis has yet to be initiated (i.e. never starting). Withdrawal of dialysis means the discontinuation and forgoing of ongoing dialysis therapy (i.e. stopping after dialysis has been started or attempted). Both situations are similar in that life-sustaining treatments are possible but are not provided.

A series of surveys of pediatric specialists, intensivists, neonatologists, general practitioners, intensivists, and palliative care specialists have shown that many clinicians do not feel that decisions to withhold and withdraw life-sustaining treatments are morally the same [14–18]. Clinicians may sense that withdrawing dialysis or other life sustaining-treatments feels more distressing than never starting. This may reflect a perception of greater moral agency, responsibility, and culpability on the part of the healthcare provider for a patient's death associated with withdrawal of treatment (commission) vs. never initiating treatment (omission). There is a tendency to describe a situation in which treatment has begun as "the train has left the station" and cannot be stopped [19]. Other clinicians may sense it is more problematic to withhold rather than to withdraw a life-sustaining treatment like dialysis. For example, Ladin and colleagues found that many adult nephrologists do not routinely discuss withholding dialysis treatment with elderly patients for fear of missing out on potentially beneficial therapy, being perceived as abandoning the patient or not providing care, and concern that withholding dialysis did not represent an "active" option [20]. Others have argued that withholding a life-sustaining treatment like dialysis precludes the possibility of an unexpected recovery and denies an opportunity to learn more about the benefits and burdens of therapy and the patient's prognosis. If dialysis is initiated and later withdrawn, the treatment is forgone only after its lack of utility has been confirmed [21].

Nephrologists' perceptions of a moral difference between withholding and withdrawing dialysis may result in negative consequences for patients. Implicit belief that withholding is preferable to withdrawing can create an "up front barrier" to appropriate treatment which may result in both inappropriate under treatment (reticence to begin therapy due to concerns of being trapped by biomedical technology that once begun cannot be stopped) and overtreatment (failure to withdraw harmful treatment once started) [22, 23]. Overtreatment may result in waste of limited medical and financial resources by insisting on a therapy that is no longer beneficial or desirable for the patient [22–24]. Others who feel withholding dialysis is more problematic than withdrawal may require all patients to undergo dialysis treatment, as withholding precludes a dying patient of a chance, even if extremely limited, of benefitting from dialysis treatment. While this approach offers the opportunity for unlikely patients to benefit, it would result in suffering for the majority who will not benefit and significant waste of resources by providing treatment unlikely to be beneficial [21, 25, 26].

It is generally accepted that there is no ethical distinction between withholding and withdraw-

ing life-sustaining treatments, even if there may be an emotional distinction for patient, family and the medical team [1, 11, 19, 23]. The equivalence thesis is an important concept in understanding these difficult cases. The equivalence thesis holds that all other things being equal, if it is permissible to withhold a medical treatment for a patient, it is also permissible to withdraw the same treatment and vice versa [27, 28]. Both not initiating and stopping life-sustaining therapy can be justified, depending upon the circumstances. Both can be instances of allowing to die, and both can be instances of killing [27, 28]. In situations where the physician has a clear duty to treat, omission of treatment by withholding or withdrawing violates that duty. Conversely, if there is no a clear duty to treat, then both withholding and withdrawing could be considered to be permissible [27, 29]. Given the consequences that arise from implicit beliefs of moral differences between withholding and withdrawing, nephrologists would be better served to combine the concepts into a single term, foregoing.

Technological Imperative

The technological imperative may influence pediatric nephrologists reluctance to forgo dialysis treatment [30]. The technological imperative has been suggested as something which is imprinted on physicians early in training: the drive to use the best, most modern and most high-tech interventions because they are available [31]. It may be best understood at its core as "That which is possible to do must be done" [32]. Pediatric nephrologists are well trained in the technical aspects of dialysis, but are less well trained in holding back, declining to offer therapy when the burdens outweigh the benefits or when a patient or family does not want the treatment. Recognizing that the technological imperative that is a natural part of pediatric nephrology may help clinicians when faced with a patient or family who desire to forgo medically available treatment. The technological imperative should not be allowed to stand in the way of shared decision-making, as it sometimes is just as powerful to leave a tool unused.

Futility

When considering life-sustaining treatments such as dialysis, nephrologists may be tempted to claim that treatment should not be provide because it is futile. Futility is a tool used by clinicians to make a unilateral decision to forgo a treatment because the treatment cannot produce the benefit that the patient or family seek [33]. In this sense a claim of futility is extremely powerful because if a treatment is futile not only is there no duty for clinicians to perform it, but there may also be a duty not to perform it. In spite of this, futility has important limitations that generally preclude its use in pediatric nephrology. The greatest limitation of futility is that it has many different meanings to clinicians, families, patients, and society [33]. Generally, three definitions of futility are most often utilized [34]. First, physiologic futility is a claim that the treatment cannot produce the benefit sought. A classic example is treating a viral infection with antibiotics. Antibiotics cannot accomplish the goal of treating viral infection. In contrast the goal of dialysis treatment is to improve fluid and metabolic balance. If access can be obtained and BP is sufficient, dialysis will almost always improve fluid and metabolic balance. Therefore, dialysis is unlikely to be physiologically futile. Second, quantitative futility is a claim that, while it is possible the treatment may produce the benefit sought, it is so unlikely as to not be worth it. In proposing this definition, Schneiderman and colleagues ask clinicians to reflect on the last 100 similar patients or the published literature [35]. If chance of failure is >99%, then the treatment is futile. For dialysis, if access can be obtained and blood pressure managed this threshold is very rarely achieved. Third, qualitative futility is a judgement that while a treatment has a reasonable prospect of producing the benefit sought it isn't worthwhile all things considered. This judgement does not reflect clinical expertise or medical judgement and instead is a cost/benefit analysis informed by the goals of care which are typically determined by the patient or their parents with the input of clinicians rather by clinicians alone.

The purpose of this discussion is not to claim that in every instance dialysis should be pursued; rather, that futility is an inappropriate reason not to do so. The assessment of benefit in such cases goes beyond whether dialysis will provide metabolic clearance and ultrafiltration to more global questions focused on quality of life for the patient. These considerations are inherently value-based and should be determined by the child's informed parents except when there is reason to believe that the parents are not acting as appropriate decision makers for the child [12, 33, 36].

Time-Limited Trial of Therapy

In the setting of uncertainty or discordance between a treating team or between treating team and family, a time-limited trial is sometimes considered as a third option in addition to initiating or forgoing life-sustaining treatments like dialysis [24, 37, 38]. A time-limited trial is an agreement between care providers and surrogate decision-makers to provide a medical therapy over a defined period of time to determine if the patient improves or deteriorates according to agreed-upon objective clinical outcomes [37]. Time-limited trials offer potential benefits of alleviating the burden of decision-making in the setting of uncertainty, avoiding interprofessional conflict, and providing support for patients, their families, and clinicians. A trial of dialysis therapy may allow for further information to be gathered about the benefits and burdens of dialysis without committing the child to a lifetime of renal replacement therapy [11, 38, 39]. Yet, when considering life-sustaining treatments such as dialysis unlikely to meet a physiologic definition of futility, time-limited trials have important limitations. These include the arbitrary duration of a trial, use of value-laden considerations as "objective" outcomes, and concern that parents may have a different understanding of a "trial" than clinicians. With the exception of some neonates, most children with chronic kidney failure will require some form of renal replacement therapy for the remainder of their lives. As the treatment is life-long, any time duration of a trial is arbitrary. Seemingly objective outcome often considered in a time-limited trial of dialysis include factors such as dialysis catheter failure, peritonitis, extubation, or neural imaging findings. Catheter failure and peritonitis are relatively common and treatable complications of dialysis treatment and thus do not seem to describe failure of dialysis treatment. Rather than being objective, outcomes such as extubation or neuroimaging findings instead reflect implicit assessments of the quality of a life on chronic mechanical ventilation or with a neurodevelopmental disability. This is an example of a technical criteria fallacy in which inherently value based decisions are medicalized without engagement in the key ethical arguments that would justify their use [40]. Finally, parents may have a different conception of a trial of a life-sustaining treatment like dialysis. Parents have described a "trial" of therapy as treatment until a complication arose where it was clear that the burdens of continued treatment (generally pain) outweighed the benefits of continued treatment; otherwise, treatment continues indefinitely [24, 41]. This is a reasonable assessment, but is a description of initiating treatment, not a trial. Treating teams and families should continually reassess whether the benefits of any treatment outweigh its burdens and whether the treatment continues to support the patient's goals of care. For an efficacious life-sustaining treatment like dialysis, nephrologists are better suited to avoid discussion of time-limited trials and instead focus on initiation or forgoing treatment with the understanding that such a decision should be continually revisited in light of the benefits and burdens experienced by the child [10, 24].

Palliative Care

Discussion about initiation or forgoing life-sustaining treatments like dialysis and transplant can feel dichotomous in nature. It is important to recognize that relationships between patients, families, and clinicians are not. This means duties to care and support patients, families, and colleagues persist regardless of any decision made

about life-sustaining treatments and intensification of palliative treatments should occur in conjunction with any decision to forgo life-sustaining treatments [42]. This highlights the importance of continued care including palliative care.

Pediatric palliative care strives to (1) relieve physical, psychological, social, practical, and existential suffering, (2) improve quality of life, (3) facilitate decision-making, and (4) assist with care coordination for children with life-threatening or life-shortening conditions [43–47]. Even in resource-limited health system settings, the Worldwide Palliative Care Alliance identifies early integration of palliative care as a human right for children [48]. While initially limited to patients with terminal illnesses such as children with kidney failure where renal replacement treatments were forgone, pediatric societies increasingly recommend PPC for all children with a "life-threatening" condition [43, 44, 45, 47, 49] This encompasses children with life-limiting conditions, where cure is not possible, and children with life-threatening conditions, where cure is possible and disease-directed treatments are ongoing [43–45, 47, 49] Importantly, most, if not all, children with advanced CKD fall into one of those two groups [47, 50].

CKD is an incurable, life-long and life-shortening condition which imposes significant burdens on children and their families. Children with kidney failure receiving dialysis treatment or who received a kidney transplant experience poorer health-related quality of life (HRQoL) compared to healthy peers and peers with other chronic illnesses [51–57]. CKD negatively affects mental health and well-being including high rates of depression and anxiety [58–60]. Further, pain needs of children with CKD may go under recognized. For example a multicenter study utilizing the Patient Reported Outcomes Measurement Information System (PROMIS) demonstrated that about half of children with CKD, regardless of stage or transplant history, experienced pain within the last week interfering with daily activities [61]. Children with CKD also experience impaired sleep, with studies showing high rates of sleep disturbances in children with non-dialysis dependent (37%) and dialysis dependent CKD (86%) [62, 63]. The importance of recognizing and developing strategies to address these burdens was highlighted by a recent qualitative study in which adolescents with CKD shared their desire that outcomes such as fatigue, lifestyle restrictions, and physical activity be prioritized [64].

To date, utilization of pediatric palliative care for children with CKD and their families has been limited [27, 65, 66]. This calls for future clinical and research efforts to integrate palliative care into the care of children with CKD similar to the evolution in practice in adult nephrology and other pediatric chronic diseases [47, 67, 68–70].

Ethical Issues in Kidney Transplantation

Consideration of Quality of Life in Transplant Decisions

Some have argued that transplant listing decisions should consider degree of improvement in quality of life that can be expected from transplant rather than expected graft and patient survival [71]. The net effect would be to prioritize children without intellectual disabilities as transplant candidates. Indeed, survey data suggests many pediatric kidney transplant programs do consider cognition as criterion for kidney transplant and may utilize different considerations for potential candidates with intellectual disabilities [72, 73]. These approaches appear to lack empiric support. Graft and recipient survival following kidney transplant do not appear to be notably different between recipients with and without intellectual disability [74]. Further, a successful kidney transplant improves the quality of life for children with kidney failure regardless of level of cognition [75, 76]. Rather than focusing on relative degree of improvement in quality of life that can be expected from a transplant, nephrologists are better suited to reflect on whether of whether the potential recipient would benefit sufficiently relative to the burdens that transplantation would pose.

Assessment of Adherence

Transplant organs remain an inherently scarce resource. As such, within the transplant system there is a strong emphasis on avoiding circumstances expected to result in premature graft loss. Assessment of a potential recipient's adherence to treatment recommendations is part of that process and referral to transplantation may be delayed or denied due to concerns of non-adherence [77]. There is evidence that non-adherence is common in the pediatric kidney disease population, particularly among adolescents [78–80]. Potentially as a result of this phenomenon, older adolescents and young adults demonstrate a markedly increased rate of graft failure as compared to both younger children and older adults [81].

While adherence is a critical for a transplant recipient to be successful, there are important limitations in applying concerns about non-adherence to clinical practice. Physicians' ability to predict the future adherence of their patients are quite limited [82, 83]. Further, patients who are members of minority population are more likely to be judged to be non-adherent [84, 85]. This is of particular significance, as many of the studies that have sought to describe factors that might predict non-adherence rely, at least in part, on healthcare provider's assessment of non-adherence [80, 86]. These same studies have frequently identified psychosocial risk factors as predictive of non-adherence, including gender, ethnic background, being a member of a single parent household, and socio-economic status [86, 87]. Given the subjectivity of many assessments of non-adherence, particularly in disease states where more 'objective' markers of adherence such as drug levels are not routinely followed, it becomes readily apparent how this can represent a vicious cycle. Patients who are socially disadvantaged by these psycho-social criteria may be more likely to experience delays or denial in transplant due to concerns regarding adherence or be judged to be non-adherent following transplant. This in turn has the potential to reinforce existing biases and disparities in care.

Acknowledgement of these concerns is not meant to be an argument against the inclusion of assessments of adherence and social support within the transplant process. Instead, nephrologists should be aware of the limitations of their ability to assess these factors and incorporate this uncertainty into the relative weight placed on these factors in decisions.

Assessments of Alcohol and Other Substance Use

A recent study of pediatric transplant programs suggest that substance use by potential pediatric transplant recipients remains a concern for clinicians and in some cases sobriety or cessation therapy is a requirement for listing a patient for organ transplantation [88]. Unfortunately, substance use is very common among adolescents with chronic illness. In a recent study of youth in the US with a variety of chronic illnesses, 36.5% of high school students reported alcohol use and 20% reported marijuana use [89]. Similar to studies of the broader adolescent population in the US [90]. Population level studies in a variety of other countries demonstrate prevalent adolescent substance use as well [91, 92].

In pediatric populations, studies suggest a correlation between substance use behaviors and non-adherence to medical regiments [89, 93]. Studies assessing the influence of alcohol and other substance use on post-transplant outcomes among adults have been mixed, but are broadly indicative of worse outcomes, at least among heavy substance users [94–98]. As such, consideration of substance use among potential pediatric transplant recipients is a valid concern in considering post-transplant outcomes. Unfortunately, many pediatric transplant programs lack clear policies regarding substance use in transplant referral and listing decisions [88]. In a recent study of transplant center practices, the majority of programs did not routinely or universally use urine or blood toxicology screening to assess for substance use, instead utilizing this testing only in circumstances of clinician suspi-

cion of substance use or in the setting of patient self-report [88]. This approach is not reliable due to the limitations of self-report of substance use by adolescents in the clinical setting [99, 100] and pediatric clinician's limited ability to assess substance use by clinical impression alone [100].

From an ethical standpoint, the use of screening approaches that are neither standardized nor universal increases the risk of clinician bias influencing the selection of patients that require more intensive screening. While research suggests that substance use in pediatrics may increase the risk of a poor transplant outcome as compared to a control population, it is important to acknowledge that many transplant candidates may be at greater risk of poorer transplant outcome than their peers due to a variety of factors such as their underlying disease [101], concurrent illnesses [81], and age at transplantation [81]. Undue emphasis on "risk" arising from substance use (as opposed to other categories) may perpetuate existing racial, ethnic and psychosocial biases in transplant care.

Justice and Broader Social Concerns in Pediatric Kidney Care

Caregiver Burden

Pediatric CKD affects not only the child, but their family and community. Caregiver burden is a multidimensional construct reflecting an individual's perception of overload during the caregiving process in one or more of four perspectives: physical, psychological, social, and financial [102]. While caregiver burdens may be greatest for younger children and those with more advanced kidney disease, they are experienced caring for all children at all levels of CKD [64, 103–108, 109]. At its most significant, caregivers (particularly mothers) experience extraordinary burdens which, in some circumstances exceed their available physical, financial, or psychological resources so they are unable to meet their duties to themselves or to others for whom they are responsible [64, 103–105, 110, 111]. This experience has been reported by caregivers throughout the world and is influenced by the available social resources within each country [105]. Patients also express concern over caregiver burden. In a recent study, adolescents and young adults with kidney disease expressed guilt over the stress and burdens that their treatment imposed upon their families [64].

In light of the severity of the burdens experienced by at least some caregivers and the concern for caregiving demands voiced by adolescents and young adults, it seems cruel and unjust for nephrologists not to discuss expected burdens with parents. This raises challenging ethical questions, including how clinicians and parents could or should incorporate considerations of caregiver burden into medical decision-making for a child with kidney disease. Where caregiving burden is significant, it is possible a treatment choice may be in the best interest of a child, but not in the best interest of other children in the family, the family as a whole, or the community. Yet, the dominant frameworks of pediatric medical decision-making utilize the best interest standard, which focuses exclusively on the self-regarding interests of the child when weighing treatment options. Such frameworks seek to highlight the primacy of the needs of the child, protect the child from exploitation by those more powerful, and hold that a child's basic needs must always be met [5, 112, 113]. While intuitively appealing, such a stance overlooks that parents, siblings, and other caregivers are also moral agents with finite resources and pragmatically that even in the wealthiest countries public resources may be insufficient to meet the needs of children with kidney disease and their families [105]. Yet it seems unfair for societal failures to support children and families to fall exclusively on the vulnerable child with kidney disease. The ethical tension between the needs of the child, other members of the family, and community in a setting of constrained resources does not offer a ready solution. It highlights a gap in the current ethical framework of pediatric medical decision-making. Recognition of caregiver burden calls for advocacy from the pediatric nephrology community to appeal for greater supports for children and their families, but also for nephrologists to recognize the situation their patient and their patient's families find themselves.

Racism, Disparities and Structural Injustices

Social determinants play a dramatic role in shaping health outcomes for children with CKD and their families [114]. Healthy People 2020 organizes social determinants of health around 5 key domains: (1) economic stability, (2) education, (3) neighborhood and built environment, (4) social and community context, and (5) health and healthcare [115]. These are influenced by societal problems of structural racism, sexism, ableism, classism and the biases associated with them which are experienced differently by particular individuals and groups within populations [116–120]. In addition, adverse environmental conditions and differences in access to healthcare, housing, clean water, healthy food, and opportunities for exercise experienced by particular groups can influence both the emergence and the progression of kidney disease [116, 119]. This insight supports the need for critical reflection in nephrology clinical practice and research. For example, the use of race as a prognostic factor or association with an outcome of interest may overlook the impact of racism rather than race in influencing clinical outcomes [121–124]. The intersection between kidney disease and racial, ethnic, gender, and socioeconomic disparities further highlights how particular groups are disadvantaged [125, 126]. One notable example is the intersection between race, socioeconomic deprivation and access to kidney transplant [127–130]. Nephrologists should seek to become aware of the impact of social determinants of health, systemic inequities, and implicit biases and work to dismantle structural causes of inequity that impact the health and well-being of patients, families, and colleagues [118, 119, 131]. Additionally, nephrologists should seek to engage with the communities they serve to promote partnerships and build trust and be trustworthy to the underserved populations that historically have been victimized by acts of injustice [119, 131–133].

Further, it is incumbent upon nephrologists to recognize how historic injustices, including colonialism and racism, contribute to the inequities resulting in high rates of acquired kidney disease in children in low- and middle-income countries. These impacts include high prevalence of bacterial, viral, and parasitic infections, limited access to reproductive care, low birth weight, malnutrition, food insecurity, poor sanitation, unsafe water supply, and pollution, all of which contribute to the development of kidney disease [116, 134–137]. These injustices highlight the need for increased and sustained efforts to support children with kidney disease and those caring for them throughout the world as an integral part of pediatric nephrologists' roles as advocates and global citizens.

Ethical Care in the Setting of Resource Limitations

It is important to acknowledge that all health care environments have limited resources. In the setting of national or other public health systems, these limitations may be dictated by realities of budgeting and health care prioritization. In the setting of private insurance, these limitations may be enacted by the policies of individual insurance programs. Institutions may vary in their ability to provide ancillary support services to the populations they serve, and families may be limited in the financial or psychosocial burdens that they can assume when caring for a child with kidney disease.

Given this reality, it is evident that there are inequities in the healthcare provided and received in different clinical settings. This is a challenge to the notion of fairness, which suggests that differential treatment of individuals is only justified if there is a morally significant difference between them [1]. Considerations such as wealth are not thought to be morally significant. Yet, resource limitations in a given practice environment may force nephrologists to deviate from what they consider optimal kidney care [138–140]. This in turn induces moral distress, a term that describes the distress that occurs not because of uncertainty regarding the most ethical course of action, but because the most appropriate ethical course is known but cannot be pursued [138, 139, 141].

Resource constraints and the lack of equity in resources for pediatric kidney care are likely beyond the efforts of individual clinicians to address. Given these constraints, however, one can consider three general guidelines in the provision of care that is limited by resource availability:

1. *In the face of resource limitations, ethically optimal care is contextual*: Different practice environments may have different prevailing standards of nephrologic care. While it is incumbent upon healthcare providers to provide the optimal care their treatment environment allows, they cannot be considered ethically required to provide care for which there is insufficient resource support. If better care could be provided in another practically accessible care setting (for example, transfer to another institution with increased resource availability or placement of the child in a different home environment to allow the provision of outpatient dialysis services), these interventions should be considered. However, it is important to recognize that these transitions may themselves be restricted due to a lack of resources and may be associated with other significant burdens that make this approach untenable.
2. *In the face of resource limitations, ethically optimal care is holistic*: It is important to recognize that a given treatment course may result in benefits and burdens beyond simple medical efficacy. The impact of treatment recommendations on patients and their family should be assessed with this in mind. For example, if the medial costs associated with the "standard of care" in a given setting might be financially ruinous to a family, deviation from this standard of care may be ethically permissible or even necessary. If the harm experienced by the child and their family from a treatment course exceeds the value of the health benefits gained, an alternative treatment course should be considered.

   It is important to note that this concept may be applied more broadly on a systems level. As an example, while the overall benefits of the use of phosphate binders as a whole are at times called into question [142], meta-analyses have suggested that the use of sevelamer results in a reduction in all-cause mortality as compared to calcium containing phosphate binders [143]. However, sevelamer based phosphate binders are substantially more expensive than calcium-containing phosphate binders and as such wholesale transition away from calcium containing binders may place a substantially increased burden on patients, dialysis units and healthcare systems [144–146]. The relative utility of making changes of this type depend on the resources available and the alternative use to which those resources might be applied.
3. *In the face of resource limitations, ethically optimal care requires advocacy*: While it is true that resource constraints may be beyond the ability for individual medical practitioners to control, our obligations to our patients require us to serve as an advocate to ensure that their health care needs can be addressed appropriately. At its core, advocacy involves attempting to address the factors that constrain healthcare providers from providing the highest quality care possible. On an individual patient level, advocacy often takes the form of seeking to secure resources that have, in the view of the healthcare provider, been misallocated or circumstances in which the patient may experience a disproportionate benefit from receiving those resources. This may be as simple as obtaining additional financial support for patients facing significant transportation barriers, or as complex as petitioning private or national formularies to consider an exception to provide a therapy that is typically not available. More expansively, advocacy on the local, regional, national and international level seeks to alleviate those resource limitations that adversely and unjustly affect the populations that we care for and about.

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Introduction

The psychosocial functioning of children and adolescents with chronic kidney disease (CKD) typically receives little attention in the busy pediatric nephrology clinic, but there is a growing literature to suggest that it should play a critical role in the overall care of this population. It is important for the quality of care received by the child and their family, the impact that psychiatric difficulties can exert on that quality of care, the interaction of such problems with other neurodevelopmental functions such as cognitive functioning and social development, and the economics of care as related to poor adherence to and the risk of adverse events. Psychosocial issues have been shown to have an adverse impact on disease progression and health care costs [1–4].

This chapter provides a comprehensive overview of what is known about the broad area of psychosocial issues in children with CKD going back approximately three decades. Couched within the context of the epidemiology of childhood psychiatric disorders, this chapter discusses many of the available studies addressing (1) psychiatric disorders and symptoms, (2) social functioning, (3) quality of life, and (4) long-term functional outcomes. Additionally, guidance for measuring psychosocial issues in the clinical setting and associated management strategies will be reviewed. The chapter concludes with a brief discussion of gaps in the literature and future clinical research suggestions for the future.

Psychiatric and Psychosocial Challenges

In general, the psychiatric and psychosocial challenges faced by all children and adolescents is staggering. Based on data from the National Survey of Children's Health [5], approximately 7.7 million children and adolescents in the United States have at least one mental health disorder. These disorders included depression, anxiety, attention deficit-hyperactivity disorder, and a variety of other conditions. With rates of identification ranging from 7.6% to over 27%, this equates to about 1 in 7 children and teenagers (14.3%) who have a condition that could be treated; however, nearly half of these individuals do not receive treatment to address their mental health needs. Left untreated, these conditions can impact neurodevelopment, general health, and wellbeing. These rates coincide with rates of psychiatric disorders

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from large epidemiological studies in the United States (e.g., The Smokey Mountain Study = 13.3% [6]), although some have been alarmingly higher (e.g., National Comorbidity Survey = 40.3%) [7], and with rates across the globe (13.4%) [8]. These rates are lower than those for children with various disabilities, such as cerebral palsy, with rates as high as 57% [9]. Additionally, these rates do not include those children and adolescents who may be experiencing significant psychological stress/distress or those who may have subthreshold psychiatric symptoms.

Further, studies of children with pediatric chronic illnesses have clearly demonstrated the consequences of co-morbid psychiatric disease on the primary medical disease, with higher health care utilization and costs [10], worse medical adherence [11], and worse control of the underlying medical condition [10, 11]. These outcomes are less rigorously studied in children and adolescents with CKD, but a study of pediatric hemodialysis (HD) patients and a study that included renal transplant patients identified a relationship between adherence and depressive and/or anxiety symptoms [12, 13]. Accordingly, a complete understanding of the psychiatric and psychosocial challenges of CKD is critically important to optimal clinical management and care across the lifespan.

Psychiatric Disorders and Symptoms

Life with CKD is difficult, and as a child the multiple adversities related to living with such a complex disease can predispose to psychiatric complications (see Fig. 63.1). Children with CKD experience higher rates of psychiatric illness when compared to their healthy peers. Although there are many reports on the prevalence of psychiatric disease, estimates vary depending on the specific population evaluated and the tools used for diagnosis. The difficulties surrounding psychiatric complications in pediatric CKD exist universally, with studies documenting the morbidity of psychiatric complications extending from North and South America to Asia (see Table 63.1).

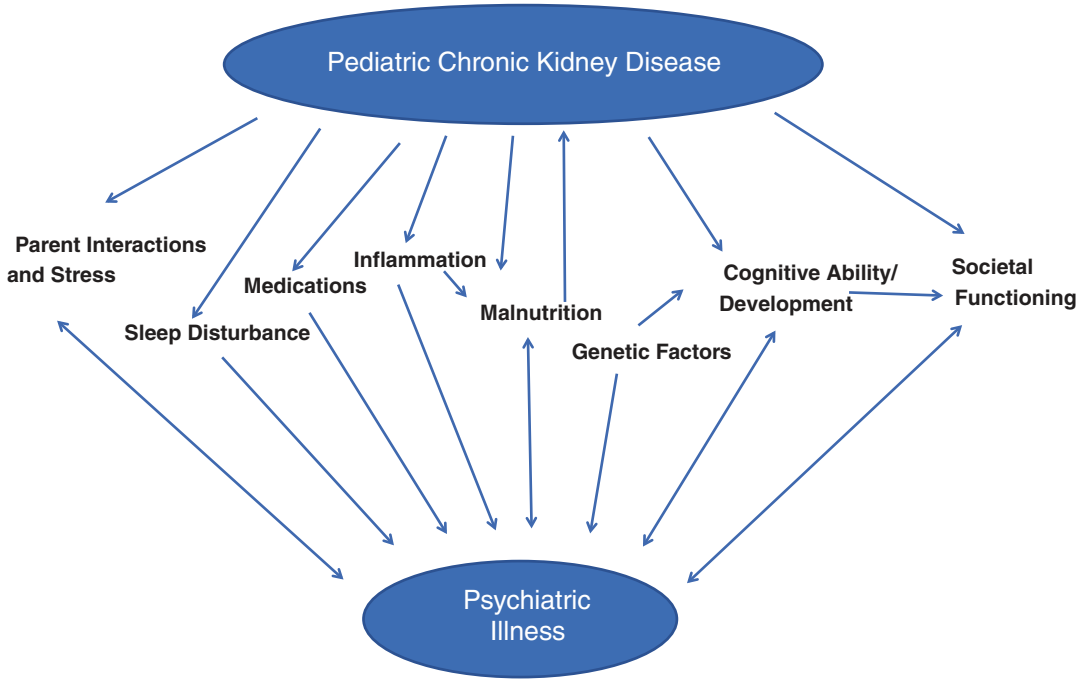

Fig. 63.1 The multiple factors that contribute to psychiatric illness and symptoms in pediatric patients with chronic kidney disease

Table 63.1 Studies examining the psychiatric and social functioning and quality of life functioning in pediatric chronic kidney disease

Study	Sample	Measures	Findings
Psychiatric disorders and symptoms			
Garralda et al. 1988 [14]	22 children on dialysis, 22 children with CKD not on dialysis; 31 controls matched to age and sex with dialysis patients; 2–18 years	Patients: Full psychiatric interview with a doctor, The Birleson Depression inventory, Lipsitt self-concept scale for self-esteem, General health questionnaire if they left school, Denver developmental screening test Teachers: Rutter Child Scale Parents: Modified psychiatric interview with parents, Rutter parental screening, Behavior check list, Highlands Dependency questionnaire or Vinelands Social Maturity scale, Modified social stress and supports interview, General health Questionnaire to assess mental distress in parents	Excess psychiatric adjustment noted in ill children with marked disturbance more common in dialysis group. By composite report, a definite psychiatric disorder noted in 32% dialysis, 23% CKD and 21% of controls. Marked worrying/ anxiety identified in 30% of dialysis, 40% of CKD and 14% of control participants
Eisenhauer et al. 1988 [23]	15 children with CKD: 8 ESKD and 7 mild CKD 8–16 years old	DICA	Depression was most common psychiatric disorder diagnosed in those with ESKD at 63% and anxiety the most common disorder diagnosed in those with mild disease at 57%
Reynolds et al. 1991 [32]	29 functioning renal transplant recipients, 22 on HD, 22 CKD and 31 healthy controls 1–18 years of age	Rutter A and B, Birleson and Lipsitt questionnaires	92% of parents reported improvements in their child's health after transplant and none of parents felt that their child had serious behavioral or emotional difficulty as compared to 1/3 of those with CKD and dialysis. Parents also described their child as happier and less irritable than before transplant. Behavioral problems in the Rutter A questionnaire were more than twice as common in the group who had transplants than in the HD or healthy group. Behavior at school of transplanted patients as assessed by Rutter B did not differ from controls but showed worse scores than HD
Brownbridge et al. 1991 [33]	73 ESKD, 32 HD, 28 CAPD, 13 transplant recipients; 2–21 years of age	Study specific questionnaires completed by patients and parents for measures of health status; Structured family interviews; CDI STAIC Rutter A scale Leeds scale for the self-assessment of anxiety and depression	Transplant recipients had less functional impairment and less social impairment, but groups did not differ on levels of anxiety, depression or behavioral disturbance. Patients on CAPD had less social impairment, lower depression scores, and less behavioral disturbance than HD patients. Parents of children on CAPD had less depression and anxiety then the parents with children on HD

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Table 63.1 (continued)

Study	Sample	Measures	Findings
Fukunishi et al. 1993 [27]	23 children on CAPD, 23 healthy controls; 4–18 years if age	DICA obtained monthly for 3 months followed by 9 months psychiatric interview	69.6% of dialysis patients had an anxiety disorder vs 8.7% of controls. 65.2% of dialysis patients experienced separation anxiety
Fukunishi et al. 1995 [28]	26 patients on CAPD, 27 transplant recipients, 27 controls; 6–15 years old	DICA –administered at least 3 times over 3 months Interviewed parents regarding school maladjustment	65% of dialysis patients had separation anxiety which associated with the family psychological environment. After receiving a transplant, school maladjustment remained and adjustment disorder was in 27% which was related to poor relationship with peers. For those on CAPD, 68% had separation anxiety, along with 18.5% of transplant recipients and 3.7% of controls
Brownbridge et al. 1999 [90]	60 dialysis patients 2–21 years of age	Structured family interview	Low adherence associated with self-ratings of anxiety and depression
Fielding et al. 1999 [90]	60 patients on dialysis; 2–21 years of age	Patient: CDI, STAIC Parents: Rutter A scale, Leeds Scale for the self-assessment of anxiety and depression	Poorer socioeconomic status associated with more behavioral disturbances in children and increased depression and anxiety in parents. Children who suffered greater functional impairment as a result of illness were more likely to be depressed, anxious, and show behavioral disturbance. Children's scores on social impairment scale correlated with parents' depression and anxiety scores
Soliday et al. 2000	41 parents of children with kidney disease:15 with SSNS, 12 with CKD, 14 transplant recipients compared to 34 controls; 1–18 years	CBCL PSI-SF FES	Mean scores on CBCL and parenting stress scores were within normal limits. 28.6% of transplant patients had clinically significant levels of internalizing symptoms and 20% of children with SSNS had clinically significant externalizing symptoms
Penkower et al. 2003 [91]	22 renal transplant recipients; 13–18 years	Face to face interviews BDI state anxiety, state-trait anxiety and state anger subscales of Spielberger	36.4% demonstrated symptoms of depression, 36.4% anxiety and 18.2% excessive state anger. Adolescents with excessive anger were at greatest risk for missing medications
Wallace et al. 2004 [30]	64 renal transplants recipients; 6–21 years old	FEATS CDI Davidson Trauma Scale	36% had results consistent with depression and/or PTSD; FEATS identified a subset of patients who were not identified using the self-report measures. Davidson scores correlated with hospital days and FEATS correlated with height Z score and donor type. Depression wad identified by CDI in 19% and PTSD by Davidson scores in 42%

Table 63.1 (continued)

Study	Sample	Measures	Findings
Bakr et al. 2007 [16]	9–15 years, 19 children on HD, 19 children CKD	Semi-structured clinical interview for children and adolescents on structured observation and self-report	Prevalence of psychiatric disorders 52.6%: 68.4% HD, 36.8% CKD. Adjustment disorder 18.4%, Depression 10.3%, NC 7.7%, Anxiety 5.1%
Berney-Martinet et al. 2009 [29]	Matched 20 CKD, 40 transplant recipients and 40 controls; 12–18 years	K-SADS-PL CBCL	Lifetime depressive disorder in of 35% transplant recipients, 35% CKD, and 15.2% of controls. Anxiety disorders seen in 22.5% of transplant recipients, 35% of CKD, and 5% of controls. Behavioral disorder seen in 30% transplant recipients, 15% of CKD, 20% of controls
Amr et al. 2009 [34]	19 patients on HD, 19 CKD and19 controls	CBCL SCICA	Mean internalizing score was significantly higher in dialysis than in CKD and controls. No difference in externalizing scales. Mean scores of SCICA observed problems and total self-reports higher in the control group than CKD groups. Positive correlation between SCICA self-report and CBCL
Dobbels et al. 2010 [26]	23 kidney transplant recipients; 10–18 years old	BDI KIDSCREEN-27	Depressive symptoms occurred in 17.4% and 75% of those with depressive symptoms were non-adherent to medications. Parents rated their adolescents to be significantly lower on psychological well-being, autonomy, and school functioning
Hernandez et al. 2011 [36]	67 patients on dialysis: 43 PD, 24 HD; 4–18 years old	Birleson Scale	10% with high occurrence of depressive symptoms and 42% without any symptoms-all of those with high depressive symptoms were female and none had a friend to confide in. For those on PD, depressive symptoms associated with lower Kt/v
Kogon et al. 2013 [25]	44 children: 20 pre-ESKD, 15 dialysis, 9 transplant recipients; 9–18 years	CDI-II	30% of cohort had depression: 25% of those pre-ESKD, 13% of those on dialysis, 67% of the transplant recipients
Selewski et al. 2014 [92]	233 children with CKD III-V including ESKD; 8–17 years old	PROMIS	Recent hospitalizations and edema predicted worse depression and anxiety scores. Those with 2 or more co-existing medical conditions had worse score in depression, anxiety, social-peer relationships
Moreira et al. 2015 [93]	28 pre-ESKD CKD, 28 controls; 9–18 years old	CDI self-report for childhood anxiety related disorder scales	Higher clinically significant depressive symptoms and higher scores for separation anxiety in those with CKD. Less resilience in those with depression

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Study	Sample	Measures	Findings
Yousefichaijan et al. 2016 [31]	80 children stage 1–3 CKD and 80 controls; 7–17 years old	Obsessive compulsive inventory-child	Mean scores for doubting/checking, ordering, and total scores significantly higher in CKD
Kogon et al. 2016 [38]	420 children CKD; Median age 11.45 years	BASC	Genomic disorders associated with more internalizing problems
Kogon et al. 2016 [24]	344 CKD subjects; 6–17 years	CDI	7% diagnosed with depression. Higher scores associated with less maternal education and lower HRQOL
Kogon et al. 2019 [37]	71 CKD stage II & higher (including ESKD) and 64 controls; 8–25 years old	CDI-II or BDI	Depression in 17% of those with CKD and 12.5% controls. Obesity associated with depression
Cueller et al. 2019 [40]	47 patients with ESKD: 12 PD, 17 HD, 18 renal transplant recipients; 7–18 years old	CDI	64% with symptoms of depression: 61% of transplant recipients, 65% of HD patients, and 66% of those on PD. More depression associated with shorter duration of ESKD and older age. Less depression in transplant recipients from a living donor
Dinc et al. 2019 [15]	66 CKD subjects: 21 transplant recipients, 27 dialysis, 18 CKD and 37 controls	Parent: Parental Attitude Scale, Symptom Checklist-90 by mothers Self: CDI, STAIC, K-SADS	Higher median CDI score for CKD group but no difference in STAIC scores. 41% of children in CKD group diagnosed with current psychiatric disorder vs 16% in control group: 43% of those with transplant, 33% of those on dialysis,50% of those with CKD. A past psychiatric disorder was identified in 52% of those CKD vs 22% of controls: 71% of those with renal transplant, 30% of those on dialysis, 61% of controls. Major depression identified in 9% of CKD overall vs 0% controls: 9.5% of those with a transplant, 15% of those on dialysis, 0% of those with pre-ESKD CKD. No differences found within CKD modalities. No difference in STAIC scores versus controls
Kang et al. 2019 [21]	166 participants with CKD stage 1–4; <18 years old	Korea-CBCL	20.5% had significant mental health problems, 22.3% had psychosocial adjustment problems. 12.7% had clinically significant internalizing problem and 9% had clinically significant externalizing problem. Those with short stature and with multiple comorbidities were more likely to score within the clinical range
Johnson et al. 2020 [20]	845 CKD patients	BASC	On depression scale, higher income and IQ associated with fewer symptoms. Time in study associated with lower depression scores

Table 63.1 (continued)

Study	Sample	Measures	Findings
Social functioning			
Fukunishi et al. 1995 [41]	30 CAPD, 35 transplant recipients, 33 controls; 6–15 years old		No difference between CAPD and transplant patients for school maladjustment with academic problems. CAPD endorsed significantly worse school absenteeism than controls and CAPD and transplant patients had worse and relationships with peers than controls
Qvist et al. 2004 [39]	32 children with renal transplant; under the age of 5	CBCL, CBCL-TRF, HRQOL	Total scores on CBCL did not differ from normative sample. Somatic complains and social problems more frequent in boys. Patients with low scores had more comorbidity and were more likely to attend a special school. Girls had less externalizing problems as rated by teacher than reference. Scores on attention scales were higher in boys and girls as rated by parents and teachers. HRQOL scores differed significantly
Gerson et al. 2004 [94]	13 pediatric renal transplant patients 2–21 years old	BASC Children's Health Questionnaire Young Adolescent Body Image Scale PSI-SF FES	More attention problems associated with worse adherence and better child behavior associated with better medication adherence
Berney-Martinet et al. 2009 [29]	Matched 20 CKD, 40 transplant recipients, 40 controls; 12–18 years old	CBCL	Transplant scored lower than CKD in social competence score; no difference between groups in activities score. Social issues related to psychiatric problems
Hooper et al. 2009 [19]	26 children with CKD: 13 on dialysis, 13 CKD and 33 controls 7–19 years old	BASC	More internalizing problems and behavior symptoms index score in CKD group, although scores fell within the average range. No group differences on adaptive skills or externalizing problems. On parent ratings, CKD participants scored worse on anxiety and depression
Hooper et al. 2016 [22]	124 preschool children mild to moderate CKD	BASC-2, ABAS-II	Parent rating of social-behavioral functioning were in the average range. No problems noted for internalizing, externalizing, or overall behavioral symptoms. On ABAS-II, scores were in the low average to average range in particular for the Practical Composite Score-reflecting capabilities in age-appropriate social functioning. 32–51% of preschoolers were at risk for not developing age-appropriate activities of daily living. No CKD related variables were risk factors

(continued)

Table 63.1 (continued)

Study	Sample	Measures	Findings
Xiao et al. 2019 [35]	318 patients and reference population, CKD stage II or higher including ESKD 13–19 years old	National Youth Risk Behavior Survey	Children with CKD reported significantly lower prevalence of risk behaviors
Johnson et al. 2020 (repeated from above) [20]	845 CKD patients	BASC-2 Parent Rating Scale	Average BASC scores for the group, with ratings improving over time. Male was risk factor for poorer scores. Persistent hypertension was associated with worse scores on the Behavioral Symptoms Index
Quality of life			
Fadrowski et al. 2006 [58]	78 adolescents with CKD, including ESKD; 11–18 years old	CHQ	Increasing height associated with positive change in physical and psychosocial summary score. Increasing age associated with decreasing psychosocial score
Sundaram et al. 2007 [52]	51 liver transplant and 26 kidney transplant recipients; 11–18 years old	CHQ-PF and CF	Caregivers reported lower physical functioning and general health than the normative population, but similar psychological health. All caregivers shared a negative emotional impact on them and their families. Parent-youth concordance was highest for physical functioning, pain, behavior problems, and self-esteem, but lowest for mental health and family cohesion
Falger et al. 2008 [44]	37 children renal transplant recipients; 5–17 years old	Child Quality of Life Questionnaire & CBCL completed by parents and youth.	Parents and youth reported impaired emotional functioning, which was correlated with adverse family relationships and maternal distress. No differences between parent and child quality of life ratings, but ratings were correlated with maternal distress
Gerson et al. 2010 [61]	402 children with mild to moderate CKD 2–16 years old	PedsQL completed by parents and youth	Patients had significantly lower physical, school, emotional and social domain scores than healthy youth. Longer disease duration and older age were associated with higher quality of life. Short stature associated with lower physical functioning scores
Diseth et al. 2011 [53]	28 transplant recipients and 40 cancer survivors and 42 controls; 12–19 years old	Strengths & Difficulties Questionnaire & PedsQL completed by parents and youth; General Health Questionnaire & Quality of Life Scales for parents	PedsQL emotional functioning of the transplant group was lower than the health controls, but similar to that of the cancer group. Parents from both transplant and cancer groups reported similar levels of negative impact of their child's illness on their functioning and on family functioning

Table 63.1 (continued)

Study	Sample	Measures	Findings
Heath et al. 2011 [50]	225 patients with CKD stages 3–5: 47 dialysis, 128 transplant recipients, 49 pre-ESKD; 6–18 years old	Generic Children's Quality of Life Measure Questionnaire completed by child	No significant differences between scores when compared to normative group, and no differences seen across various treatment modalities
Marciano et al. 2011 [48]	136 CKD patients: 39 dialysis, 29 transplant recipients 75 ore-ESKD	Strengths & Difficulties Questionnaire; PedsQL rated by parents & youth	Parents of CKD patients reported the rate of emotional & disruptive disorders to be about 50% higher than in normative group. In contrast, about 1/3 of children reported emotional problems. There was a large negative correlation between the presence of behavior and emotional disorders and the PedsQL total score
Park et al. 2012 [51]	92 children with ESKD: 11 HD, 44 PD = 44, 37 transplant recipients; 2–18 years old	PedsQL ESKD Module	Children with kidney transplant or on dialysis did not differ on perceptions of family and peer interactions, worry, physical appearance, or communication. Parents reported communication problems were worse for children on dialysis than transplant recipients
Neul et al. 2013 [55]	53 dialysis patients and their parents	PedsQL & PedsQL ESKD Module administered at 6-month intervals over a 2-yr. period completed by both patients and parents.	Parent Global PedsQL ratings did not change over time, but ratings were lower in girls and in those with longer dialysis vintage. Emotional and school domains decreased over time. Patient reported scores were higher compared to the parent ratings
Al-Uzri et al. 2013 [57]	483 children with mild to moderate CKD; 2–17 years old	PedsQL completed by parents and youth over two visits.	Significant association between catch-up growth and growth hormone use on parent reports of child physical and social functioning domains. Older children had higher ratings than their parents on all PedsQL scales.
Haavisto et al. 2013 [45]	77 transplant patients: 16 heart, 44 kidney, 14 liver; 6–16 years old	HRQOL & CBCL completed by parents, patients, & teachers.	No differences in HRQOL scores between transplant recipients, but younger participants had lower scores compared to norms. When compared to normative data, the adolescent transplant group reported less distress and more vitality, but more problems attending school and being with friends. Parents and teachers reported more internalizing problems than the normative group, but scores were in the average range. Parents reported more problems with health and physical functioning than patients

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Table 63.1 (continued)

Study	Sample	Measures	Findings
Killis-Pstrusinska et al. 2013 [46]	203 children with CKD: 25 HD, 41 PD and 137 pre-ESKD and 388 parent proxies; 2–18 years old	PedsQL Inventory completed by patients & parents	Lower HRQOL scores in all CKD groups compared to norms, and HD patients had the lowest scores. There was significant discordance between parents and patients on emotional functioning in the PD group, but not in the HD or conservative treatment groups
Baek HS et al. 2017 [54]	376 parents and 305 children; <18 years of age	PedsQL	Lower HRQOL identified in female patients and those with more co-morbidities and by parent report in those with lower kidney function and anemia. Growth and bone mineral density were positively correlated with QOL. There was no relationship between HRQOL and age or duration of disease
Splinter et al. 2018 [47]	192 ESKD patients 8–18 years in Europe	PedsQL	Patients reported significantly lower scores compared to the healthy norms and other chronic health conditions. Those who had undergone a preemptive transplant reported better physical health scores that were similar to other chronic conditions
Francis A et al. 2018 [49]	375 children 6–18 years with CKD stages I-5 including dialysis and transplant recipients	Health Utilities Index (HUI)	The lowest scores were for those on dialysis, followed by transplant recipients, then CKD stages 3–5 with the highest scores seen in those with CKD stages 1–2
Pardede SO et al. 2019 [56]	112 children including hemodialysis; 2–18 years of age	PedsQL	The lowest scores were seen in the school and emotional domains. HRQOL was lowest in those with CKD diagnosis >60 months, female and in middle school.
Carlson et al. 2020 [60]	733 children and adolescents with mild to moderate CKD; Median age 11 years	PedsQL completed by parents and children over multiple visits.	Presence of anemia was associated with significantly lower overall HRQOL and physical functioning as per child ratings. On parent ratings, anemia was associated with lower emotional functioning scores. Caregivers did not observe declines in their children's other PedsQL subscales in the presence of anemia
Díaz-González de Ferris ME et all 2021 [59]	734 children median age of 11 years	PedsQL	Average HRQOL scores were higher in younger children, but were more highly related to number of medications, with lower HRQOL associated with the number of unique medications
Long-term functional outcomes			
Roscoe et al. 1991 [95]	118 adolescents treated between 1966 and 1986	Functional status obtained by structured telephone interview	80% survival after 15 years for transplanted patients. 29% were living on their own or with a spouse, the rest mostly living with a family member.14% were neither enrolled in an educational program nor employed

Table 63.1 (continued)

Study	Sample	Measures	Findings
Mellerio et al. 2014 [96]	374 adult aged patients transplanted as children; Median age 27 and 12.3 years since first transplant	Questionnaire with data compared to French general population using indirect standardization matched for gender, age, period	Compared to the general population 31% versus 52.2% lived with partner, 36% v 21% lived with parents and 19% v 10% were unemployment. Factors predictive of poor outcome were primary disease severity, presence of comorbidities or disabilities, being on dialysis, and female gender
Lewis et al. 2014 [64]	236 young adults with ESKD presenting as children compared to those presenting as adults.	Questionnaires	30% of those with a pediatric and 20% of those with an adult presentation were labeled disabled. There was lower educational attainment in pediatric presentation and those with pediatric presentation less likely to have full or part-time paid work (57% v 76%) and less likely to be living with a partner
Murray et al. 2014 [65]	57 young adults:5 CKD, 8 dialysis, 45 TX; 27 were pediatric presentation	Semi-structured interview	Median age leaving school was 16 year: 17.5% still studying, 75% completed education, 60% employed, and 33% unemployed. For those with pediatric presentation: 7% were in special ed. and 26% earned a general certificate of secondary education. 75% felt that their employment or work was negatively affected by ESKD. 33% unemployed and not in school vs 22% in general population. 8.5% were receiving welfare vs 4% in general population
Enden et al. 2018 [97]	29 young adult men who underwent renal transplantation in childhood and 56 matched survivors of childhood acute lymphoblastic leukemia and 52 controls with other childhood illness	RAND-36 BDI	Compared to leukemia survivors and controls transplant recipients had higher BDI scores and reported more bodily pain and worse general health which associated with older age, longer duration of dialysis, multiple transplantations and lower graft function. Transplant recipients were less likely to be in permanent relationship (40% v 8-% controls and 50% leukemia survivors). Transplant recipients reported lower HRQOL. 10% of transplant recipients had at least one child compared to 21% of leukemia survivors, and 43% of controls. 4% transplant recipients graduated from university compared to 14% of leukemia survivors and 18% of controls. 67% of transplant recipients were employed compared to 90% leukemia survivors and 100% of controls

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Table 63.1 (continued)

Study	Sample	Measures	Findings
Groothoff et al. 2018 [42]	144 patients with onset of ESKD before 14 years of age born before 1979		67.4% of ESKD patients were employed with 19.1% involuntarily unemployed vs 6.4% of general population. 53% had low skill occupation and 10% had high skilled-significantly different than general population. Being on dialysis, motor disabilities and chronic fatigue most important predictors of unemployment. Average income was significantly lower than general population. 21% felt that disease has significant negative influence on professional achievement and career. 34% lived with their parents but 10 years later 67.4% were married or lived with a partner. 31.5% had offspring compared to 74.4%, and 65% of ESKD children scored lower in all domains of developmental milestones than healthy peers
Hamilton et al. 2019 [43]	417 transplant recipients and 173 dialysis patients; 16–30 year old	Study developed survey	Compared to the general population those with ESKD were less likely to live with partner or have children, more likely to live with parents, had poorer quality of life and were twice the likely to have a psychological problem. They were also less likely to have drunk alcohol, 1.6 years older at first alcohol consumption, less likely to have tried cannabis or street drugs, spent money gambling or been in trouble with the law, and twice as likely to have never had sex
Kerklaan et al. 2020 [98]	30 patients diagnosed with non-ESKD CKD during childhood; 18–35 years old	Semi-structured interview	Struggled with daily restrictions, felt defeated hopeless and lagging behind studies and life goals. 20% worked full time, 23% part-time, and 13% unemployed. 43% were students, 26% married or living together, 60% single, 63% living with parents/families, 23% living with partner, and 7% had 1 child

CKD chronic kidney disease, *ESKD* end stage kidney disease, *DICA* diagnostic interview for children and adolescents, *CAPD* continuous ambulatory peritoneal dialysis, *CDI* children's depression inventory, *STAIC* state-trait anxiety inventory for children, *CBCL* child behavior checklist, *PSI-SF* parenting stress index-short form, *FES* family environment scale, *SSNS* steroid-sensitive nephrotic syndrome, *BDI* beck depression index, *FEATS* formal elements of art therapy scale, *PTSD* post-traumatic stress disorder, *K-SADS-PL* Kiddie Schedule for Affective Disorders and Schizophrenia Present and Lifetime Version, *SCICA* semi-structured clinical interview for children and adolescents, *HRQOL* health related quality of life, *PROMIS* The Patient-Reported Outcomes Measurement Information System, *BASC* Behavior Assessment System for Children, *ABAS* Adaptive Behavior Assessment System, *CHQ-PF/CF* Child health questionnaire-Parent form/child form

With respect to general rates of psychiatric disorders in pediatric CKD, Garralda et al. completed modified psychiatric interviews with parents and determined that psychiatric disorders were more common in dialysis patients and CKD patients than in healthy controls [14]. More recently, a study from Turkey also found a high prevalence of psychiatric disorders in children with CKD, including those with a transplant and on dialysis, when compared to the control group. Specifically, they found that 41% of children with CKD were diagnosed with a current psychiatric disorder, compared to 16% in the control group, and that 52% of those with CKD reported a prior psychiatric diagnosis as compared to 7% in the control group [15]. A study from Egypt of children on dialysis and with pre-dialysis CKD evaluated for the presence of a psychiatric disorder using a semi-structured clinical interview. The overall prevalence of psychiatric disorders was 53%, including 68% of patients on HD and 37% of CKD patients without end-stage kidney disease (ESKD) [16].

Emotional-behavioral functioning more broadly has often been assessed through the use of the omnibus ratings scales, such as the Child Behavioral Checklist (CBCL)[17] and Behavior Assessment System for Children (BASC) [18]. A study compared ESKD, CKD, and controls and found that behavior ratings on the BASC by parents and children fell within the average range. Parent ratings showed increased number of internalizing symptoms, but otherwise did not show concerns for social-behavior functioning [19]. Likewise, the Chronic Kidney Disease in Children (CKiD) cohort study investigated emotional-behavioral functioning in children using the BASC-2 completed by parents and found that, on average, children with CKD scored in the typical range, although more children than expected scored >1 standard deviation towards a score of impairment [20]. This was also shown in a study of children with CKD stage I–IV in Korea, which found that based on the parent-proxy CBCL 20.5% and 22.3% of children had scores consistent with clinically significant mental health and psychosocial adjustment problems, respectively [21]. Similar to the findings in older children, this pattern has been demonstrated in preschool children with CKD. The median parent scores on the BASC-2 were consistently within the average range, although 37% of children scored >1 standard deviation above the mean on adaptive behavior problems [22].

In addition to the general prevalence rates of psychiatric disorders and emotional-behavioral functioning in pediatric CKD, there also have been studies that have focused on the prevalence of depression and anxiety. The prevalence of depression or elevated depressive symptoms identified in studies of children with CKD ranges from 7% to 67% (see Fig. 63.2) [23]. The most commonly used instrument to document these findings is the Children's Depression Inventory (CDI). In CKiD, one of the largest studies to date to evaluate depressive symptoms, 7% of 344 children with CKD had a diagnosis of depression or elevated depressive symptoms [24]. This study, which is part of a multi-year, prospective, observational cohort design, identified a lower prevalence than described in other studies, which have primarily been single-center studies. This may be due to selection bias since participants in the CKiD study may be self-selected and have a lower likelihood of psychiatric comorbidity. A single-center study that used the CDI to evaluate for depression in children and adolescents with CKD found that 30% of the participants scored within the clinical range. The most likely to be depressed were the transplant patients (67%) followed by the pre-ESKD patients (25%) and the dialysis patients (13%). This pattern persisted even after adjustment for age and gender [25]. Similarly, Berney-Martinet et al. evaluated CKD patients, healthy controls and transplant recipients for the lifetime prevalence of depression with the Kiddie Schedule for Affective Disorders and Schizophrenia (K-SADS). They found that 35% of transplant recipients and 35% of pre-ESKD recipients have been depressed, compared to 15% of controls. Another study of adolescent transplant patients identified that 17.4% were depressed based on the Beck Depression Inventory [26].

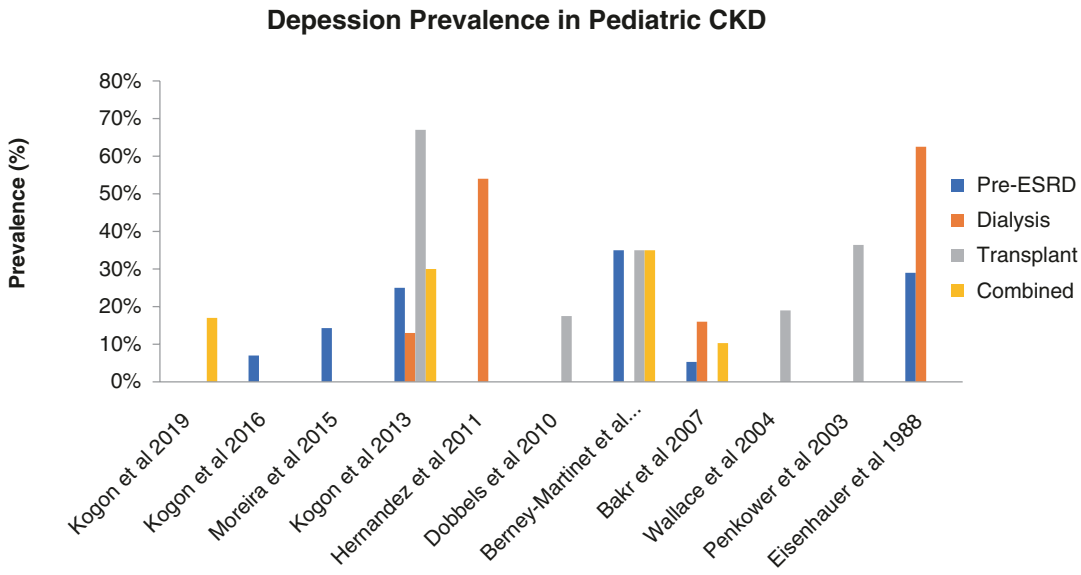

Fig. 63.2 Studies depicting depression prevalence in pediatric chronic kidney disease: prior to end-stage kidney disease (pre-ESRD), dialysis, and transplant

Although less studied than depression, anxiety disorders are also common in children with CKD compared to controls. A study in Japan from 1993 found that 69.6% of children on peritoneal dialysis (PD) experienced an anxiety disorder as compared to only 8.7% of controls, with separation anxiety disorder being the most common in both groups [27]. A subsequent study by the same group also found anxiety to be more common in renal transplant recipients, though not as common as in dialysis patients, than in controls [28]. In another study, the prevalence of an anxiety disorder was 22.5% kidney transplant recipients, 35% in those with pre-ESKD CKD and only 5% in healthy controls [29]. Post-traumatic stress is also of concern, particularly for those with more morbid disease. In a study of renal transplant recipients, 6–21 years of age, 42% of participants had Davidson Trauma Scale scores consistent with post-traumatic stress disorder (PTSD), and the scores correlated with the number of hospital days in the previous year [30] One study from Iran evaluated 80 children with CKD stages 1–3 and 80 healthy children for the presence of obsessive compulsive disorder (OCD) using the Obsessive Compulsive Inventory-Child Version. The mean total scores of children with CKD were significantly higher than for the healthy children, with higher scores being noted particularly in the symptom of doubting/checking and ordering [31].

Renal transplantation remains the optimal therapy for ESKD and is highly advocated for patients on dialysis. Despite receiving a renal transplant, the challenges of living with CKD remain, and as noted above, renal transplant recipients may still suffer from psychiatric and emotional distress. Many studies have compared the burden of psychosocial disease of living with a transplant to pre-ESKD and dialysis. One study clearly demonstrated that families report considerable improvements in a child's physical health. Yet, behavioral problems were twice as common in children who had received a transplant than in the healthy and pre-ESKD groups. Additionally school behavior in the transplant group, as rated by teachers, was similar to the healthy group and overall families reported an improvement in their children's behavior and quality of life [32]. Berney-Martinet et al. likewise did not find that transplantation associated with a lower prevalence of depression when compared to those who were pre-ESKD [29]. Another study also showed similar scores on the CDI and the State-trait anxiety inventory for children (STAIC) between the

transplant and dialysis groups [33]. Other studies even document that the prevalence of psychiatric disorders in renal transplant recipients exceeds those with pre-ESKD CKD [25].

Studies have attempted to identify the risk factors for psychiatric morbidity in children with CKD. Although limited by sample size, most studies have not found disease-related variables to be significantly associated with psychiatric functioning [14, 24, 25, 34, 35], although one study reported higher parent-rated internalizing problems scores in children with non-glomerular disease than glomerular disease [20]. When comparing psychiatric morbidity by dialysis modalities, children undergoing PD appear to fare better than those undergoing in-center HD [33]. A study in Peru showed that 10% of children and adolescents with CKD reported high depressive symptomatology based on the Birleson Scale, and for children on PD the presence of depressive symptoms was significantly associated with Kt/V [36]. Additionally, as in the general population, obesity may play a role in increasing the risk for symptoms of depression [24, 37]. There is also evidence of a genetic role for depression. A CKiD study found that children with CKD who also had a pathogenic genomic copy number variation were more likely to endorse increased internalizing symptoms [38]. An increase in comorbidities, including preterm birth and developmental delay, also likely predisposes an individual with CKD to worse psychiatric functioning [21, 39].

Overall, psychiatric disorders and symptoms appear to be more common in pediatric CKD than in the general pediatric population, and the complications do not fully resolve with transplant. However, longitudinal studies of psychiatric functioning have found that instead of worsening with progression or duration of disease, there are fewer psychiatric complications in those with longer duration of disease and over time [40]. This can be seen in a CKiD study of parent-completed BASC-2 scores that identified improved scores over time [20]. These findings suggest that some children with CKD adapt to their circumstances; however, the examination for the presence of psychiatric disorders and emotional distress remain a critical area.

Social Functioning

The research on social functioning of children and adolescents with CKD is more limited than that of psychiatric functioning (see Table 63.1). Unlike other illnesses, such as childhood cancer, there are no studies directly comparing the classroom and extracurricular behaviors of children with CKD to healthy peers. Much of the available literature is embedded within studies that investigate psychosocial functioning more broadly. For example, the opportunities to make friends is likely impacted by CKD, but there are few studies examining this important social function. One study found that only 30% of children on dialysis, 55% of children with CKD not on dialysis and 83% of control participants reported having a special friend [14]. Similarly, the children with CKD also reported more loneliness than the control participants. Interestingly, in this study, those with CKD not on dialysis reported double the prevalence of being lonely (10%) than those on dialysis. In Japan, a study used the Diagnostic Interviews for Children and Adolescents monthly for 3 months in order to assess school adjustment of children with a renal transplant, children on PD, and control participants [41]. They found the children on dialysis had the most indicators of school maladjustment and that even children in the transplant group indicated significantly more school maladjustment than the control participants.

Evidence for the impact of social functioning and psychosocial development in CKD is also found in studies that compare risky behaviors in adolescents with CKD to control participants. In a multicenter study of North American adolescents with CKD stage II or higher, including ESKD, completed the National Youth Risk Behavior Survey [35]. Compared to a reference group, adolescents with CKD reported significantly fewer high-risk behaviors, including smoking, sexual activity, and unsafe driving, than the reference group. Although it is reassuring that adolescents with CKD appear to be participating in less high-risk behavior than their peers, there is concern that this indicates a delay in developmental milestones since engaging in risky behavior is part of normal adolescent behavior.

Additionally, children with CKD report a delay in the ages of first sexual intercourse and first alcoholic beverage [42, 43]. These delays may indicate a break with typical development that could negatively impact adult outcomes.

Quality of Life

As seen in Table 63.1, quality of life is heavily studied in pediatric CKD. The bulk of these studies have been cross-sectional, with only a few longitudinal studies. Most of the literature has focused on quality of life in patients receiving renal replacement therapies, while other studies have focused on group comparisons across different chronic health conditions and specific CKD groupings. The measure of quality of life usually includes multiple domains (i.e. physical, school, emotional and social) that reveal particular aspects of health related quality of life (HRQOL) that may be affected more than other aspects in particular settings.

There have been a number of studies examining quality of life in kidney transplant recipients. Falger et al. looked at the psychosocial adjustment post-transplant using the Netherlands-based Quality of Life Questionnaire and the CBCL [44]. They found that the dimension measuring emotional functioning was rated as impaired according to both parent and youth report. There were no statistical differences between parent and child ratings with regard to quality of life. Maternal distress showed a negative correlation with most dimensions of the quality of life scale. In Belgium, Dobbels et al. conducted a cross-sectional study with adolescent kidney transplant patients [26]. They assessed HRQOL using the short form of the KIDSCREEN and depression using the BDI. Adolescent transplant patients perceived their physical and psychological functioning about the same as healthy controls. However, parent's ratings of their adolescent's psychological well-being, level of perceived autonomy, and school functioning were significantly lower when compared to healthy controls. Finally, Haavisto et al. examined the quality of life in kidney transplant patients [45]. Data were collected from patients, caregivers and teachers. Using a Finnish HRQOL Questionnaire and U.S. normative date, the adolescent transplant group had significantly less self-reported feelings of distress and experienced more vitality than the controls; but, they described more problems attending school and being with friends. Additionally, parents described the transplant group as experiencing more problems in health and physical functioning than the transplant patients themselves. Taken together, while a transplant clearly contributes to improved health for children and adolescents with CKD, quality of life ratings remain significantly lower.

With respect to renal replacement therapies, a number of studies also have compared quality of life findings across different treatment modalities. Killis-Pstrusinska et al. administered the PedsQL (Pediatric Quality of Life Inventory) to Polish children with CKD and to their parents [46]. The sample included children on HD and PD and pre-ESKD patients. Children on PD reported significantly better quality of life compared to those on HD, and there was significant disagreement between parents and children, with parents reporting worse quality of life. Another study from Europe also demonstrated significantly lower HRQOL across all domains of ESKD (dialysis and transplant) when compared to healthy norms and other chronic conditions, with those who had received a preemptive transplant reporting higher scores on physical health [47]. They also identified that comorbidities was the most important determinant associated with lower HRQOL. Marciano et al. investigated the prevalence of behavior disorders (measured using the Strengths and Difficulties Questionnaire) and the relationship to HRQOL (measured using the PedsQL Global score) in children from Brazil with CKD, including pre-ESKD and dialysis patients and kidney transplant recipients [48]. Parents reported a negative correlation between the presence of behavior and emotional disorders and the HRQOL score. A large study from Australia and New Zealand used the Health Utilities Index (HUI) to show that children on dialysis had the lowest HRQOL, followed by transplant recipients, children with CKD stages 3–5 and children with CKD stages 1–2 [49].

In contrast, several studies have not demonstrated significant concerns for quality of life in children and adolescents with CKD receiving renal replacement therapies. Specifically, Heath et al. evaluated dialysis and kidney transplant patients in the United Kingdom using the Generic Children's Quality of Life Measure (GCQ) and compared their results with normative data [50]. The GCQ questionnaire assesses how the child views his or her life and how they would like it to be. Quality of life is measured as the discrepancy between the two viewpoints. No significant differences between the mean GCQ scores of the participants in various treatment modalities or from the mean of the normative group were observed. Similarly, Park et al. examined the quality of life in Korean children on HD or PD and transplant recipients using the PedsQL End-Stage Renal Disease Module [51]. There were no significant group differences on perceptions regarding family and peer interactions and communication. However, parent reports suggested that communication problems were worse in children on dialysis than in transplant recipients.

A few studies have compared quality of life in children with CKD and other medical conditions. Sundaram et al. evaluated a cohort of liver and kidney transplant patients using the Child Health Questionnaire (CHQ) completed by both parent and child [52]. Caregivers reported lower physical functioning and general health, but similar psychological health to the normative population. Caregivers expressed a negative emotional impact of their child's health on themselves and the family. Diseth et al. evaluated the mental health and quality of life of 28 transplanted youth and compared them to healthy controls and children who had survived acute lymphoblastic leukemia in Norway [53]. In addition to the Strength and Difficulties Questionnaire, the PedsQL was administered to youth and their parents. Similar to previous reports, emotional functioning of the transplant group was poorer than the healthy group, but similar to the cancer group. With regard to maternal mental health and maternal quality of life, parents of the transplant and the cancer groups reported similar degrees of impact of the child's illness on their functioning and on family functioning.

One of the challenges with examining the CKD population is that most studies enroll small numbers, with heterogeneity of age and CKD severity. These challenges likely explain some of the inconsistencies between reports. However, there are several consistent risk factors for lower HRQOL. Repeatedly, females have reported lower HRQOL than males [54–56] and those with short stature suffer lower HRQOL, particularly in the physical functioning domains [54, 57, 58]. Expectedly, maternal distress and adverse family relationships has also repeatedly been identified as associating with poorer HRQOL [44]. Similarly, emotional and behavioral disorders also associate with poorer HRQOL [48]. Other factors identified as associating with HRQOL include number of medications prescribed and the presence of anemia [59, 60]. The relationships between duration of disease and age with HRQOL are more mixed [54, 56, 59, 61].

Lastly, there are only a few longitudinal studies examining quality of life in CKD Neul et al. used a longitudinal case control method to track changes in physical, emotional, social, and educational functioning in dialysis patients and parents over 2 years [55]. The PedsQL and the End-Stage Module of the PedsQL were administered at approximately 6-month intervals. Parent-reported Global PedsQL scores did not change over time; however, global scores assessed by parents were significantly lower in females than males. After adjusting for a number of variables (e.g. dialysis), parents rating of school functioning declined significantly over the 2 years. Further, patients on dialysis had significantly lower emotional functioning. In contrast, patients reported more positive functioning compared to their parent's perceptions. Fadrowski et al. conducted a longitudinal study of physical and psychosocial functioning [58]. In this multicenter study, adolescents and their parents completed the CHQ over 4 years. After adjusting for targeted covariates, the Physical Summary score declined as glomerular filtration rate declined. Additionally, annualized height gains were associated with a improvements in Physical and Psychosocial summary scores, as seen in other studies [49, 57]. Finally, the effect of anemia on

HRQOL using the PedsQL was investigated in 773 children with mild to moderate CKD, with approximately 30% having anemia at the index visit [60]. The presence of anemia was associated with significantly lower overall HRQOL and physical functioning as reported by the children. For the parent ratings, the development of anemia was associated with lower emotional functioning and decreased physical functioning over time compared to those who remained anemia-free. No other findings on the PedsQL were noted.

Long-Term Functional Outcomes

As can be seen in Table 63.1, there is a paucity of scientific exploration into the long-term functional outcomes of childhood CKD. In a study from the United Kingdom that compared 976 young adults with ESKD to the general population, ESKD patients were more likely to live at home with parents and to be unemployed. Further, those who were employed were more likely to participate in low-skill occupations and have significantly lower incomes [43, 62]. Similar findings have been reported in young adults with ESKD in France, the United Kingdom and the United States [63–66].

Fortunately, there is some evidence that socio-professional outcomes are improving for young adults with ESKD. A study from the Netherlands compared the socio-professional achievements of adults with ESKD in 2010 and 2000. In 2010, they were more likely to live with a partner and to have completed a high level educational degree [67]. Clearly, this is an area of interest from a psychosocial perspective, and there is a need for more evidence-based interventions.

Identification and Measurement of Psychosocial Issues in the Clinical Setting

The identification of psychosocial issues may be straight forward. A patient may present with somatic complaints that are not compatible with their medical condition; they may appear withdrawn, with blunted affect; or demonstrate concerning grooming or social habits. A family member or member of the treatment team may raise concern. There are several methods for making a formal diagnosis, including standardized inventories, clinical or structured interviews or direct observation. Interpretation of data gathered from these methods requires consideration of the type of measurement being employed (e.g., is it a rating scale, a clinical interview, an observation?), its psychometric properties (i.e., is it reliable and valid?), who is providing the information (i.e., parents, teachers, the child or adolescent?) including their honesty, forthrightness and insightfulness, and the clinical utility of the obtained information (i.e., how will the information be used and what resources are available to address the chronicity and/or severity of the concerns?).

Clinical interview is a long-held approach to obtaining patient information [68]. When systematic and structured, this approach can provide substantial information about the psychosocial functioning of a child or adolescent. Children can be interviewed alone or with their parent or caregiver. Adolescents typically are interviewed alone. The challenge with clinical interviews is that they tend not to be systematic and there is no assurance that all diagnostic questions will be asked of each patient. This can be addressed by using a structure interview that addresses all necessary questions based on the clinical situation. Examples of structured interviews include the Diagnostic Interview of Children and Adolescents (DICA) [69], the Kiddie Schedule for Affective Disorders and Schizophrenia (K-SADS) [70], the Child and Adolescent Psychiatric Assessment (CAPA) [71], and the Preschool Age Psychiatric Assessment (PAPA) [72], for preschoolers and early elementary age children. The primary advantages of a structured interview is that it increases the probability that necessary questions will be asked, particularly with respect to diagnostic criteria; however, it can be time consuming, limiting its application in a rapid-paced clinical setting. Nonetheless, structured interviews are usually considered the gold standard in clinical research for psychiatric conditions, and

several studies have employed these scales with pediatric CKD.

Another approach to psychosocial assessment is behavioral rating scales. These scales are standardized, normed across a wide age range, typically have strong psychometric properties, and are cost and time efficient, with many now being computer administered and scored. They require some level of literacy on the part of the respondent, but can be completed by a child, adolescent, parent, teacher, or other caregiver. Further, rating scales have been developed to assess a wide variety of psychosocial issues as well as to assess specific concerns. For example, omnibus rating scales that utilize a multi-rater, multi-setting, multi-instrument assessment design include the Child Behavior Checklist (CBCL) [17], Behavior Assessment Scale for Children (BASC) [18], and the Conners' Rating Scale-3 (Conners) [73]. These scales provide a vast amount of information, as informed by the rater, and can assist in identifying psychosocial difficulties as well as screening for psychiatric diagnoses. All of these measures have been used in the assessment of psychosocial functioning in pediatric CKD.

Similarly, rating scales have been developed to identify symptoms and behaviors associated with specific disorders. For example, the Child Depression Inventory-2 (CDI-2) [74] assess for depression or depression symptoms and has been commonly used to identify depression in pediatric CKD. There are also specific rating scales for anxiety (e.g., Multidimensional Anxiety Scale for Children-2 [75]), anger [76], attention deficit hyperactivity disorder (Vanderbilt ADHD Diagnostic Rating Scale [77]), self-esteem (Multidimensional Self-Concept Scale [78]), OCD (Yale-Brown Obsessive-Compulsive Survey [79]), quality of life (PedsQL [80]) and other conditions [81]

While most assessments of psychosocial functioning typically use some combination of clinical interviews and rating scales, the applicability and utility of any of these approaches are determined by the nature of the clinic and the type of services that can be offered. Additionally, the use of multi-rater, multi-setting, multi-instrument assessment models, while providing a wide array of data on the social and emotional functioning of the patient, can present discrepancies in the data across different instruments and different raters. In fact, discrepancies between child and parent perceptions regarding the presence and the severity of psychosocial issues should be expected as they are consistently observed in nearly all of the studies cited in this chapter. For this reason, obtaining information from multiple informants is advisable [82]. Additionally, it is important to note that perceptual discordance is particularly pronounced during adolescence [57] and it is important to consider this discordance in the interpretation of psychosocial measures. Working with a pediatric psychologist or other mental health professional (e.g., social worker, counselor, child psychiatrist) can assist in the interpretation of these assessments.

Management of Psychosocial Issues in the Clinical Setting

Addressing the psychosocial needs of children and adolescents with CKD is important but can present challenges. Nephrology providers manage the medical complexity of CKD and may have limited time to address psychosocial issues, especially given limited knowledge in this area. In addition, families may be reluctant to raise yet another problem that may need to be addressed by adding more providers, appointments, or medications to their already complicated regimens. Moreover, there are no data specific to treating psychosocial issues in the context of pediatric CKD.

To address the psychiatric challenges, a medical social worker is often already part of the team in many pediatric nephrology programs and can be a good resource for families. The social worker can address any background stressors and can provide coping strategies to the patients. Community mental health provides also may be an option, although in the United States access can be limited by insurance constraints and community providers may feel more comfortable partnering with the medical team given the complexity of CKD. Referral to pediatric psycholo-

gists can be helpful for administration of evidence-based therapies, such as cognitive-behavioral therapy, to address psychiatric issues. In the United States, access to pediatric psychologists can also be a barrier. For adolescents, referral to an adolescent medicine specialist can be helpful for strategies to manage living with a chronic condition. Lastly, pharmacotherapy for treatment of anxiety, depression, ADHD, and other psychiatric conditions can be useful. Although not specifically studied in children and adolescents with CKD, the selective serotonin uptake inhibitors have been studied in adults with CKD and ESKD and therefore should be considered first-line agents for managing depression and anxiety. Fluoxetine and citalopram do not require dosage adjustments in CKD, while the maximum dosage of sertraline may need to be reduced [83].

There are additional options to support the social and behavioral health of children and adolescents with CKD. Families can be supported by connecting them with other families of children with CKD, which can include connections through social media or kidney camps. Attending kidney camps has been shown to be beneficial for both patients and their families [84–86]. Pediatric psychologists and medical social workers can provide parenting tools and stress management techniques. In addition, child life providers can help manage the anxiety and psychological trauma that may come from repeated invasive medical procedures and encounters. To reduce stress in the school setting, medical social workers can advocate for a patient's needs and accommodations.

While a discussion of school-related functions is beyond the scope of this chapter, there is a longstanding recognition of the negative impact of physical illness on educational and eventual occupational functioning as these disruptions can affect psychosocial functioning. Educational and occupational problems appear to be major areas of concern for children and their parents; chronic school absenteeism is alarmingly common in children with CKD [87]. Not only do school absences have a direct impact on gaining fundamental academic proficiencies, but missed school also interferes with children acquiring essential social competencies and developing social support networks. Even with the relatively reassuring educational and occupational assessments of children with CKD [88], continued efforts need to be made to allow for school-based accommodations as well as work-based accommodations for absences due to medical appointments and sporadic exacerbations of kidney disease.

Finally, the type of mental health treatment and/or social supports for children and adolescents with CKD may vary by developmental level. For example, adolescents with CKD may face barriers during their transition to early adulthood, particularly with respect to their healthcare. The transition to adulthood is an important period in human development that requires an individual to increase autonomy, find gainful employment, and build social relationships. Pediatric CKD and the medical complications that can be associated with CKD can prevent many adolescents from making this transition and facing these developmental challenges successfully. To date, intervention research geared toward the medical and psychosocial barriers that impede transition to adulthood is emergent and several possibilities exist that might prove instrumental in smoothing the transition for adolescents with CKD. The issue of transition is critical to the psychosocial health of this population, particularly given the medical needs that will continue into adulthood and the concomitant psychosocial burden that many of these youth will experience as they move into the adult world [89].

Gaps in the Literature and Future Directions

It is clear that children and adolescents with CKD experience psychosocial challenges and that these challenges should be addressed. More research is needed to provide evidence-based literature to define appropriate interventions. In addition, more interdisciplinary support, such as therapists, psychologists and psychiatrists, would enable better care for pediatric CKD patients. Research that clearly establishes the relationships

between psychosocial distress and outcomes, such as healthcare costs and utilization and educational and professional attainment, and demonstrates improvement in outcomes with appropriate interventions would be helpful to justify increased psychosocial support.

Conclusions

Children with CKD have many challenges, including short stature, chronic school absenteeism and reduced social opportunities. Consequently, children and adolescents with CKD at all stages of disease may experience mental health and adjustment problems that extend into adulthood and affect quality of life. Although renal transplant is the optimal therapy for ESKD, it is not curative, and children and adolescent renal transplant recipients have psychosocial issues, and some may even be exacerbated. Disease related variables have not consistently been shown to associate with psychiatric functioning, but obesity, genetics, comorbidities and preterm birth may predispose a child with CKD to impaired psychosocial health. There are many tools to measure psychosocial performance to aid in identification of impairment. Although medical social workers, community mental health providers and adolescent medicine doctors may offer support and strategies to struggling patients, more research is needed to define the interventions that will improve the psychosocial health, and ultimately improve the long-term outcomes of children with CKD.

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Part XIII

Kidney Replacement Therapy

64 Initiation of Kidney Replacement Therapy: Strategic Choices and Preparation

Jérôme Harambat and Iona Madden

Introduction

During the past three decades, the management of children with chronic kidney disease (CKD) and end-stage kidney disease (ESKD) has improved dramatically. In high-income regions, though the financial cost is important, provision of kidney replacement therapy (KRT) by dialysis or pre-emptive transplantation is considered standard care for the great majority of children for whom this therapy is indicated. However, in lower income countries, the economic, human, and technical resources required for the treatment of childhood ESKD make it a major health challenge.

Children with CKD and their families/caregivers experience a complex decision on KRT initiation and treatment modality choice for a lifelong condition. The timing and circumstances of KRT initiation, and the choice of KRT modality can significantly impact clinical outcomes as well as the child's and caregivers' mental health and social life. The chosen KRT modality for children should ensure the lowest risk of mortality and morbidity and provide the best quality of life. In this regard, (living donor) pre-emptive kidney transplantation, when feasible, is considered the best choice of KRT in children. However, the majority of pediatric patients with ESKD will require dialysis. Chronic peritoneal dialysis (PD) can offer several advantages over hemodialysis (HD) for children.

This chapter outlines the current distribution of KRT modalities among incident and prevalent pediatric patients worldwide, the main outcomes according to KRT modality, the optimal timing of KRT initiation (dialysis initiation, preemptive kidney transplantation), the dialysis preparation and transplant workup. In addition, the chapter explores the pros and cons of each KRT modality taking into consideration the widely variable access to KRT around the world, and the lifetime perspectives of the choice to be made. Topic reviews that include more comprehensive management, complications and outcomes of the different modalities of KRT are discussed in separate chapters.

Current Distribution of Initial KRT Modalities Among Pediatric Patients Worldwide

The incidence, prevalence, and treatment modalities of pediatric ESKD worldwide are only partially known because of differences in pediatric patient definitions, disease classification, and lack of national registries and health information systems, especially in low- and middle-income

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countries [1, 2]. Most of the epidemiological data on pediatric ESKD requiring KRT (dialysis or kidney transplantation) is derived from high-income countries due to the availability of robust national or international registries mainly in Europe, Australia and New Zealand, and the United States [1].

The incidence of ESKD in children varies widely across different countries but ranges from 5–15 per million children per year among patients under 20 years. The number of children starting KRT has decreased in the United States where the incidence rate was the highest worldwide, remained stable in other high-income countries [3, 4], and may have increased in some lower income countries. The prevalence of ESKD in children varies between 40–60 per million children in Western Europe and Australia, with the prevalence being higher in the US (around 100 per million children) and lower in South America and Eastern Europe. Globally the prevalence of children undergoing KRT (functioning transplant or maintenance dialysis) may be increasing, yet there is considerable variation in access to and practices of initiating KRT worldwide. Several factors may have contributed to the increase: improved survival of ESKD children, broadening pediatric KRT acceptance criteria, and better access to pediatric KRT (especially dialysis) in low- and middle-income countries.

However, major disparities currently exist in access to kidney care services for children, particularly in low- and middle-income countries so that most pediatric patients with ESKD in these countries have no or limited options for KRT and the vast majority of children receiving KRT worldwide reside in high-income countries [1, 5, 6]. A wide variation in the initial KRT modalities exists across the world.

Kidney transplantation is the preferred KRT option in children and adults with CKD, conferring significant survival advantage, improved quality of life, and health system cost savings. According to the Global kidney Health Atlas, kidney transplantation is not available in more than 20% of countries worldwide (>60% of African countries) and the proportion of countries that cannot provide pediatric kidney transplantation is expected to be much higher [7]. In Europe and the United States, approximately 20% of children start KRT with pre-emptive kidney transplantation [3, 4]. The access to pediatric kidney transplantation is influenced by ethnic, economic and geographic disparities [8–10]. For example, across 32 countries in Europe, a strong association was found between country income based on the GDP per capita and the country-specific rate of pediatric kidney transplantation [11]. In some countries, kidney transplantation is not available as an option for initial KRT in children.

Children who do not receive pre-emptive kidney transplantation may receive PD or HD, but the prevalence of the use of each of these modalities varies substantially depending on the center, country, reimbursement system for ESKD care, and public policies [5, 12]. In Europe and the UK, there is an almost equal distribution between HD and PD as initial modality. HD is the most common initial dialysis modality in the US and Brazil, while PD is more frequently chosen as first modality in Australia/New Zealand and Japan (Fig. 64.1). There seems to be trend towards a decrease in PD use versus HD as first KRT modality, particularly in the UK and the US [3, 13]. PD may be the preferred chronic dialysis modality in low-resource settings although HD in adult dialysis facilities is often the only option because of the cost and logistical challenges associated with PD in children [14, 15]. Globally, PD, most commonly provided with an automated cycling device (CCPD), is usually the first dialysis modality for infants and young children, while HD predominates among children above 10 years and adolescents. Home hemodialysis (HHD) is offered to a small number of selected children by a few centers [16].

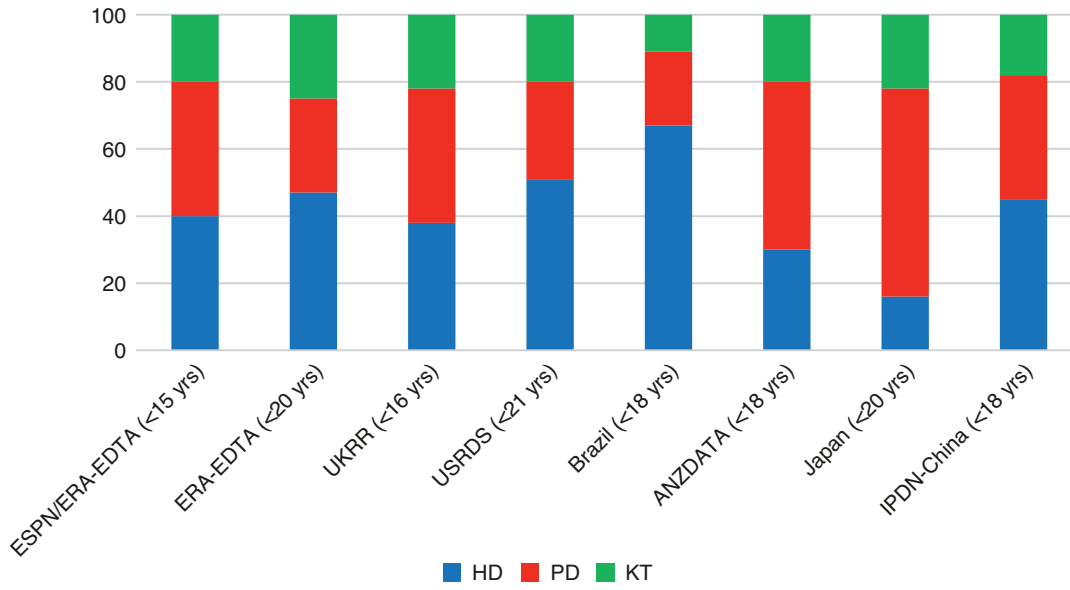


Fig. 64.1 Distribution of initial kidney replacement therapy modality in children according to various registries

ANZDATA Australia and New Zealand Dialysis and Transplant Registry, *ESPN/ERA-EDTA* European Society for Paediatric Nephrology/European Renal Association-European Dialysis and Transplant Association, *IPDN-China* International Pediatric Dialysis Network-China, *UKRR* United Kingdom Renal Registry, *USRDS* United States Renal Data System, *HD* hemodialysis, *PD* peritoneal dialysis, *KT* kidney transplantation

Main Outcomes According to KRT Modality

It has been established that (pre-emptive) kidney transplantation offers better patient survival than dialysis [17–19]. In the US, children treated with HD or PD have a five and two times higher 1-year mortality rate after initiation of KRT than those receiving a preemptive transplantation [3]. In Europe, starting KRT with dialysis is associated with a two-fold higher hazard of death as compared to preemptive kidney transplantation [20]. Children and adolescents <21 years of age initiating KRT with preemptive transplantation have a higher 5-year survival of 96–97% in the US and Europe as compared to ~86% (US) and ~ 90–92% (Europe) with dialysis [3, 21]. Mortality on dialysis is particularly high in infants and children younger than 5 years of age. In the very long-term, both European and US data suggest that children treated with dialysis would have a deficit in their expected remaining lifetime of 40–50 years vs. 12–20 years for transplanted patients [3, 22]. Nonetheless, about 80% of pediatric patients are either started on dialysis to bridge the preparation time needed for transplantation or will require dialysis after graft loss [3, 4].

Survival comparisons by dialysis modality in a randomized clinical trial setting proved extremely difficult [23]. Consequently, survival comparisons rely on registry data [24, 25]. In the pediatric dialysis population, there seems to be a consistent trend showing survival advantage in patients on PD with a 20–30% reduced mortality risk as compared to those who start KRT on HD [24–26] (Fig. 64.2). In the USA, this better survival on PD was only present in children <5 years, whereas in Europe, the effect size was smaller in children <5 years and no difference was observed in infants <12 months [26]. Furthermore, European data show that this PD treatment effect was stronger during the first year of dialysis, in older children, and in children late referred to pediatric nephrology care [25]. However, despite adjusted or stratified analyses, the risk of indica-

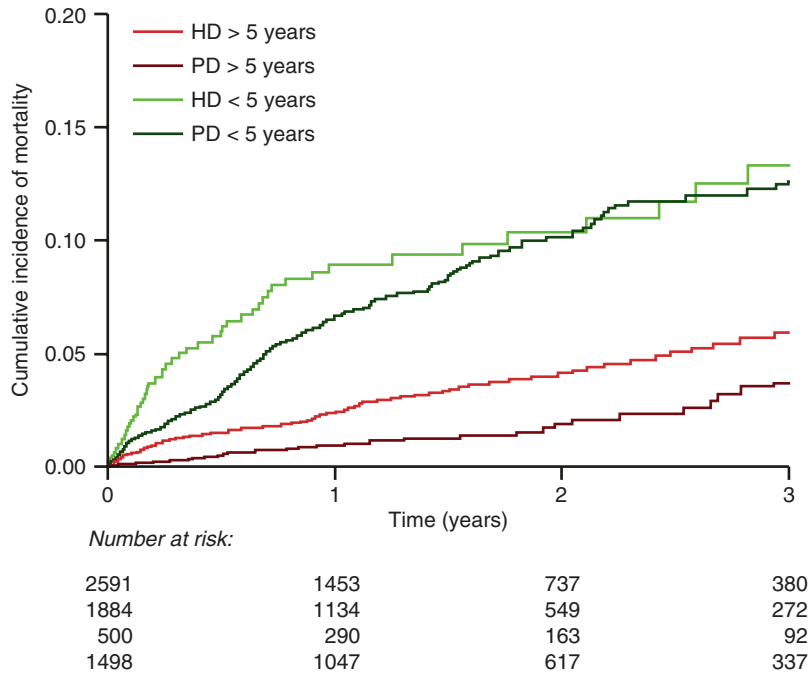


Fig. 64.2 Cumulative incidence of mortality in European children on hemodialysis and peritoneal dialysis at start of kidney replacement therapy (used with permission from [25])

HD hemodialysis, *PD* peritoneal dialysis

tion bias inherent to observational studies remains, as sicker patients are more likely to be started with HD. Dialysis in neonates is challenging but technically feasible. Data on 264 patients from 4 international registries who commenced chronic dialysis in the first month of life showed a 3- and 5-year survival rates of 80 and 76%, respectively [27].

Children and adolescents undergoing dialysis report severely decreased health-related quality of life (QoL) compared to those with a functioning transplant but, surprisingly, most studies did not find significant differences between patients on HD and those on PD [28, 29]. However, PD has been associated with more dietary and fluid freedom, and less disruption in children's schooling and social life compared with HD [30].

Moreover, preemptive kidney transplantation has been associated with improved post-transplant outcomes such as significantly reduced risk of graft loss [19, 31, 32], reduced risk of acute rejection [33], and better health-related QoL in the domain of physical health [34].

Optimal Timing of KRT Initiation

Current Recommendations and Guidelines

In 2020, a position statement from an expert group recommended dialysis initiation when the eGFR drops below 10 mL/min/1.73 m^2 or when the child has uremic symptoms refractory to medication or dietary management [35]. All other recommendations come from adult guidelines. The Kidney Disease Outcomes Quality Initiative (KDOQI) suggested in 2006 that dialysis should be considered when eGFR is <14 mL/min/1.73 m^2 and recommended when eGFR falls to <8 mL/min/1.73 m^2 [36]. The updated 2015 KDOQI (for HD) stated that symptoms and signs associated with CKD complications, rather than a specific eGFR, should guide the decision to initiate dialysis [37]. The KDIGO conference report in 2018 confirmed that there is no specific eGFR threshold for dialysis initiation in the absence of symptoms, and that current data do not support pre-emptive dialysis initiation [38].

Regarding transplantation as an initial KRT modality, the European Renal Best Practice (ERBP) recommended to develop programs for preemptive living donor kidney transplantation [39]. There is no guidance for the timing of preemptive transplantation but a statement that the optimal timing should be to avoid dialysis in a patient who otherwise would need to start it according to current guidelines i.e., shortly or a few months before the need to initiate dialysis [39]. Indeed, in adults, neither patient nor graft survival was influenced by the level of pretransplant eGFR [40]. A recent KDIGO clinical practice guideline also recommended that patients should be referred for evaluation 6–12 months before anticipated dialysis to facilitate preemptive kidney transplantation [41]. The KDIGO guideline specifically recommends preemptive kidney transplantation (living or deceased donor) in children when the eGFR is <15 mL/min/1.73 m^2 or earlier with symptoms [41].

Pre-ESKD Care and Referral for KRT Initiation

Optimal CKD care should include timely referral and frequent visits with a multidisciplinary team of physicians, nurses, dieticians, social workers and pharmacists. This integrated model of CKD care has been associated with improvement in measurable outcomes [42, 43]. Recommendations for "timely referral" to a pediatric nephrologist should allow access to multidisciplinary care for CKD, patient and family education and support, and considering a preemptive kidney transplantation.

Delayed referral, lack of patient preparedness and urgent-start of dialysis have been associated with higher morbidity on dialysis, lower access to kidney transplantation, and worse kidney graft survival [44–46]. Late referral has been defined as starting dialysis between 1 and 3 months after the first appointment with a pediatric nephrologist. Late referral in adults has been associated with limited choice of dialysis modality and worse clinical outcomes, including more hospitalizations and deaths [47]. Similar findings have been reported in children. In the UK, 25% of children with ESKD were referred late (defined as having <3 months between first visit with a pediatric nephrologist and commencement of KRT). These patients were less likely to receive a kidney transplant after 1 year of dialysis (21% vs. 61% for early referral) without difference in mortality [45]. In a study from Poland, children with a late referral had more metabolic and clinical complications, more admissions to ICU, and a lower chance to receive a kidney transplant within 3 years [48]. Of 1527 children in the ESPN/ERA-EDTA registry whose first appointment with a pediatric nephrologist was known, late starters (45%, defined as eGFR <8 mL/min/1.73 m^2 at start of KRT) were significantly more likely to be late referrals [49]. However, the access to kidney transplantation at 1, 2, and 5 years was similar regardless of the timing of referral [49].

Urgent Versus Non-urgent and Planned Versus Unplanned Start of KRT

In 2018, a KDIGO Consensus Conference defined urgent-start dialysis by the need to initiate dialysis imminently or in less than 48 h after presentation in order to correct life-threatening manifestations [38]. An unplanned start is defined as dialysis initiation when access is not ready for use or when dialysis is initiated with a modality that is not the patient and family's choice. HD and PD are possible in both planned or unplanned and urgent or non-urgent start of dialysis. However, children requiring KRT in the setting of hyperkalemia, volume overload or critical illness may not be good candidates for urgent start with PD. The risk factors for urgent and unplanned start of dialysis include late referral, lower socio-economic background, lower health literacy, acute illness, rapid (and often unpredictable) loss of kidney function, and severe symptoms [50–52]. When urgent or unplanned start dialysis limit the initial modality choice, patients and families need to subsequently be provided with education and support to allow them choos-

ing their preferred modality when feasible. Conversely, a planned initiation of dialysis is performed when the modality has been chosen prior to the need for dialysis and the access is ready for use. A preemptive kidney transplantation is by definition a planned modality of KRT.

Outcome According to the Timing of KRT Initiation

There is a large variation around the world in the threshold of eGFR for the initiation of dialysis in children. There is variation in approach both between and within countries. The median eGFR at dialysis initiation in pediatric patients in Europe and the USA is around 8 mL/min/1.73 m^2 but the range is wide, from less than 5 to over 10 mL/min/1.73 m^2 [49, 53, 54]. In the Japanese registry, the median eGFR at KRT initiation (including kidney transplant) was 12 mL/min/1.73 m^2 [55]. There is a trend towards earlier start of dialysis: a USRDS registry study including more than 15,000 children found that the proportion of children starting dialysis with an eGFR >10 mL/min/1.73 m^2 increased from 16% in 1995 to 40% in 2015 [54]. In Canada, there has been a change over the past 20 years with dialysis currently being initiated at an eGFR ≥10.5 mL/min/1.73 m^2 in one-third of children, a higher rate than previously reported [56]. The reasons for this trend are unclear. Possible reasons could be the adoption of changing trends in the clinical practice guidelines, starting from KDOQI 2006 in which the recommendations were to consider dialysis if eGFR was below 14 and start when eGFR was below 8 mL/min/1.73 m^2 [36]. However, updates from KDOQI 2015 and KDIGO 2019 recommend commencement of dialysis when the patient becomes symptomatic [37, 38].

A specific eGFR value for initiating dialysis in the absence of symptomatic kidney failure has not been established in adults. The IDEAL trial could not demonstrate a benefit of commencing dialysis at higher levels of eGFR [57]. Recently, there have been three large pediatric registry studies from USRDS and ESPN/ERA-EDTA examining the association between eGFR at initiation of chronic dialysis and clinical outcomes [49, 53, 54]. Of nearly 10,000 incident dialysis patients aged 1–17 years in the USRDS registry (1995–2016), higher eGFR at dialysis start was associated with a higher mortality risk. Specifically, compared with eGFR of 7 to <9 mL/min/1.73 m^2, eGFR <5 and ≥12 mL/min/1.73 m^2 were associated with lower and higher mortality, with adjusted HR of 0.57 and 1.31, respectively [53]. In stratified analyses, mortality risk was associated with higher eGFR in children ≥6 years, whereas the association was attenuated among those younger than 6 years. Although this study suggests that initiating dialysis at eGFR >12 mL/min/1.73 m^2 is associated with increased mortality, there seems to be a similar outcome across the range of 5–12 mL/min/1.73 m^2 in which the majority of ESKD patients will start KRT [53]. Another study from the USRDS assessed the association between eGFR at dialysis initiation and hazard of death in a total of 15,000 children aged 1–18 years in the period 1995–2015 [54]. Mortality risk was 36% higher among those with higher (> 10 mL/min/1.73 m^2) versus lower (≤ 10 mL/min/1.73 m^2) eGFR at dialysis initiation. The mortality associated with higher eGFR risk was more pronounced among children receiving HD (HR 1.56) while the association with eGFR was no longer significant among those treated by PD [54]. In Europe, a retrospective ESPN/ERA-EDTA Registry study of ~3000 children aged <18 years from 21 countries found no difference in mortality risk and access to transplantation between children who started dialysis at eGFR ≥8 mL/min/1.73 m^2 vs. < 8 mL/min/1.73 m^2 [49] (Fig. 64.3).

Notably, the ESPN/ERA-EDTA Registry study found no significant difference in other outcomes such as anemia, hyperphosphatemia, growth, and access to transplantation between late starters at eGFR <8 mL/min/1.73 m^2 (median 6) and early starters at eGFR ≥8 mL/min/1.73 m^2 (median 10.5) [49]. However, the prevalence of hypertension was higher in late starters (61%) than in early starters (54%) [49]. In a Turkish study of 245 children, patients with early-start dialysis at eGFR >10 mL/min/1.73 m^2 (mean 13.5) and late-starters at eGFR <7 mL/

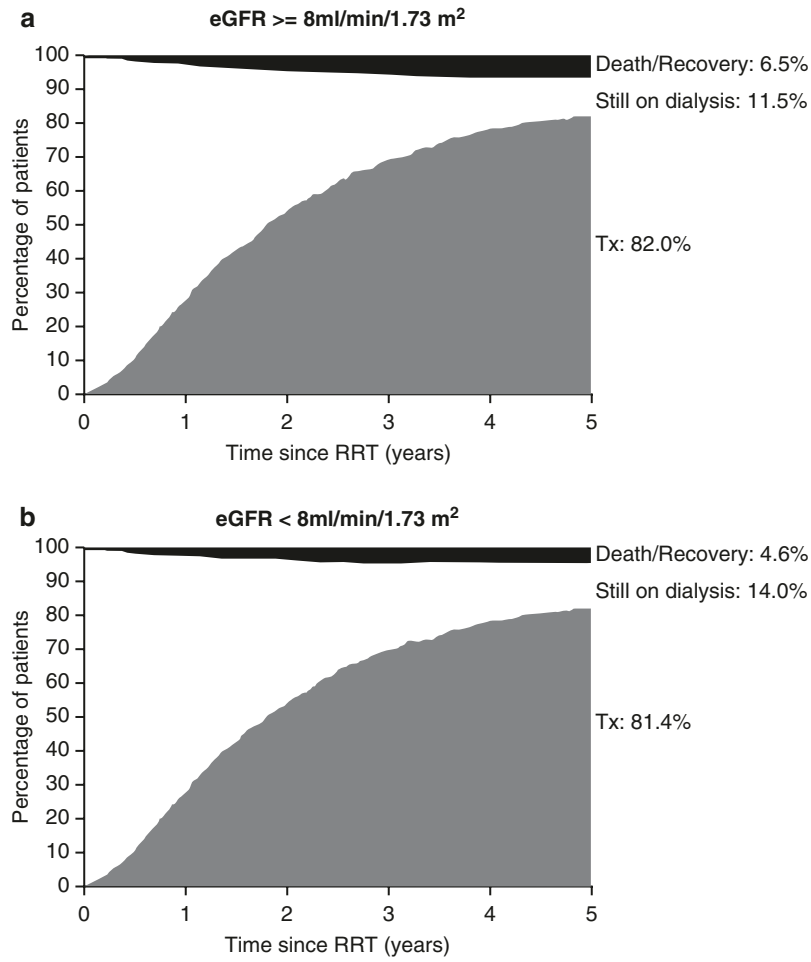


Fig. 64.3 Cumulative incidence of death and kidney transplantation within 5 years of dialysis initiation for European children starting dialysis early (eGFR ≥8 mL/min/1.73 m^2) (A) and children starting dialysis late (eGFR <8 mL/min/1.73 m^2) (B). Used with permission from [49]

Tx kidney transplantation, *eGFR* estimated glomerular filtration rate, *RRT* renal replacement therapy

min/1.73 m^2 (mean 5.5) did not significantly differ in left ventricular mass index (LVMI) and left ventricular hypertrophy (LVH) [58].

Regarding nutrition and growth, the ESPN/ERA-EDTA study showed that height SDS was not different at start of dialysis with an eGFR above or below 8 mL/min/1.73 m^2 (−1.79 vs. −1.76), and the drop in height SDS after 1 year of dialysis was similar in both groups (−0.22 vs. −0.24) [49]. The distribution of BMI was also comparable between the two groups. However, in a cohort study of the International Pediatric Peritoneal Dialysis Network (IPDN) (1000 PD children from 35 countries), an underweight status was twice more common in children starting PD at eGFR <6 mL/min/1.73 m^2 (11%) compared to those starting PD at eGFR 9–12 mL/min/1.73 m^2 (5%) [59].

There is no pediatric data to suggest that patient or graft survival could be influenced by the level of eGFR at time of preemptive kidney transplantation.

KRT Initiation

The timing of KRT initiation is a complex decision that needs to take into account the eGFR and the rate of its loss, the treatment modalities available, and symptoms or signs attributable to kidney failure. Initiation of KRT is usually considered in case of inability to control volume status or blood pressure, deterioration in nutritional status or growth failure, severe biochemical abnormalities such as hyperkalemia, hyperphosphatemia or metabolic acidosis, and

patient reported outcome measures such as fatigue, nausea and loss of appetite, declining cognitive performance, or poor QoL [30, 35].

While the optimal timing for starting KRT is unknown and the reasons for initiating dialysis are highly variable, risk equations may be potentially helpful to predict the time when KRT will be necessary although validation studies are limited in children [60, 61]. This may be combined with, and not replace, clinical judgement. The decision to start KRT should ideally always be reached by discussions between the child when appropriate, caregivers, and the CKD multidisciplinary care team.

Once maintenance dialysis has been initiated in pediatric patients there is a recovery rate of 2% within 2 years after the start of dialysis [62]. There is a particularly high chance of recovery (>10%) in children starting dialysis for vasculitis, ischemic kidney failure, and hemolytic uremic syndrome, which should be considered when planning expedited kidney transplantation [62].

Dialysis Availability and Choice of Modality

Dialysis modalities include in-center and, rarely, home HD, as well as continuous ambulatory and automated PD. Prescription patterns can be categorized as conventional, intensive (short daily or nocturnal), or exceptionally palliative in children [63, 64]. Availability of modalities and prescription patterns usually depends more on local resources and infrastructures, reimbursement policies, center practices and expertise, than patient and family preferences. In most high-income countries, in-center hemodialysis is the predominant modality whereas PD is the first option in some areas. In high-income countries, PD may be more cost effective than HD, yet the opposite might be true in low- and middle-income countries where KRT is offered to children but manufacturing or importing PD fluids could be more expensive [38].

The selection of dialysis modality is based on the patient's age and comorbidity, family support and psychosocial conditions, local resources, modality contraindications, healthcare team expertise, and child and family choice (Table 64.1). The only absolute contraindication for maintenance HD is the absence of possible vascular access or prohibitive cardiovascular instability (Table 64.2). PD is contraindicated if the peritoneal membrane is not functional or PD catheter is not possible (Table 64.2). All other conditions are relative contraindications.

Table 64.1 Issues to be considered in the selection of kidney replacement therapy modality in children

Factors to be considered
Patient and family preference
Patient age and size
Medical/surgical comorbidities, contraindications
Geographic location, distance to the center
Local nephrology/surgical expertise
Family burden
Psychosocial support
School attendance
Growth
Lifelong morbidity and mortality
Healthcare system, out-of-pocket costs

There is no study to suggest that either PD or HD is superior in children with ESKD. PD is often preferred and widely used in infants and small children for whom the creation and maintenance of a suitable vascular access can be challenging. Home dialysis modalities such as automated PD using a cycling device (CAPD) and rarely home HD (HHD) can improve patient autonomy, flexibility and QoL, and facilitate regular school attendance [35, 63]. PD is also preferred over HD in children with cardiovascular instability, and some reports have suggested benefits of HHD in children with cardiac failure [65]. Preserving residual kidney function is important in the choice of KRT modality and should be a goal for pediatric nephrologists. Although PD may be associated with a slower decline in residual kidney function than HD, the evidence regarding the effect of initial dialysis modality and therapeutic strategy is limited in children [66, 67].

In addition to medical factors, the proximity to a pediatric HD center and the heavy burden of home dialysis care for families should be considered when choosing a dialysis modality. In some countries, it has been reported that children with

Table 64.2 Contraindications to the different kidney replacement therapy modalities in children

	Preemptive kidney transplantation	Peritoneal dialysis	Hemodialysis
Absolute contraindications	• Active infection • Severe irreversible multisystem organ failure • Severe pulmonary or cardiac dysfunction in a child not suitable for multiorgan transplant • Life-threatening extrarenal disorder not correctable by kidney transplant • Uncontrolled malignancy	• Abdominal wall defects • Diaphragmatic hernia • Bladder exstrophy • Obliterated peritoneal cavity • Peritoneal membrane failure	• No vascular access • Severe cardiovascular instability • Unavailability of facilities
Relative contraindications	• Active systemic disease (lupus, vasculitis, anti-glomerular basement membrane disease) • Unstable psychiatric disorder • Ongoing, health-compromising nonadherent behavior	• Ileo/colostomies • Recent major abdominal surgery • Infant with organomegaly • Inadequate living situation for home dialysis, lack of caregiver support	• Difficult vascular access • Coagulopathy • Unstable psychiatric disorder

social disadvantage tend to receive more commonly HD than PD compared to those from wealthier families and have less access to preemptive kidney transplantation [46, 68]. This suggests delayed referral as well as concerns by healthcare providers and/or caregivers themselves about their ability to safely provide dialysis at home. Further investigation is needed to clarify whether social inequities exist in the provision of KRT in children. Although the published data is limited in pediatric nephrology, there has been growing recognition of the importance of patient and caregiver involvement in planning CKD care and shared decision-making regarding KRT modality [68–70]. The modality selection should therefore reflect informed child and family choice adapted to their health and social conditions and appropriate to the healthcare system and local resources.

Dialysis Preparation and Transplant Workup

Dialysis Preparation

Many children, for various reasons, require a variable period of dialysis before transplantation is possible. Dialysis is considered a bridge to kidney transplantation and should be as short as possible to improve outcomes [19, 31]. Adequate dialysis preparation enables better understanding of the dialysis process, reduces fear and anxiety regarding long-term dialysis, thus ensuring improved therapy adherence and better overall outcomes.

Before starting dialysis, patients and their caregivers should meet the following criteria: (i) they need to have a good understanding of the different treatment options available, (ii) they should have a functioning permanent access for the dialysis modality of their choice, and (iii) they should not require hospitalization for untreated complications of acute or chronic uremia [30, 35, 38, 71].

1. Education

   Patient education and decision support are essential in helping patients and families to better understand ESKD and available KRT options, improve literacy and possibly outcomes. In adults, early education has been associated with better survival after dialysis initiation [72]. Effective education programs should be offered to the child if appropriate, and the family or caregivers when the eGFR is <30 mL/min/1.73 m^2 (CKD stage 4). Comprehensive education material should

provide information related to all forms of dialysis. In addition, a thorough psychological, social and economic evaluation of the family or caregivers is required to determine their ability to cope with the burden of care associated with the provision of home KRT (PD or HHD) [63]. The multidisciplinary care team can therefore individualize the training plan to meet the needs of the parents or caregiver.

2. Dialysis access

PD access should be prepared whenever possible at least 2 weeks before starting KRT. For late-referred patients, this might imply switching to HD temporarily or definitively. However, urgent-start PD has been proven safe in adults, and early catheter use feasible in children. Gastrostomy tube feeding in children already receiving PD is not contraindicated but a gastrostomy tube should be ideally inserted prior to the placement of a PD catheter [73]. There are guidelines regarding optimal timing and placement technique for PD catheters, access type, PD training, and prevention of complications in the pediatric population [74–76]. Constipation has been associated with increased risk of post placement PD catheter migration and should be addressed preoperatively. The use of a double-cuff Tenckhoff catheter has been recommended [75], and omentectomy to prevent PD catheter occlusion should be considered at the time of catheter insertion.

Among children receiving HD, central venous catheter (CVC) is the predominant vascular access choice. The International Pediatric HD Network reported the use of an arteriovenous fistula (AVF) in 26% of children initiating HD; this rate was higher in an ESPN/ERA-EDTA study (45%), and much lower (< 15%) in a recent USRDS report [3, 77, 78]. A vascular access with an AVF has been associated with better dialysis efficacy, fewer medical complications, less vascular access procedures, lower costs, and higher deceased donor transplantation rate than an access with a CVC [77, 78]. It is therefore recommended that children start HD with a functioning AVF which should be created at least 3 months before anticipated use [79]. However, in some circumstances such as in infants and young children depending on size and surgical expertise, those requiring unplanned HD, those with an anticipated short period of dialysis before (living donor) kidney transplantation, or patient choice, a cuffed CVC is preferred [79]. The choice for access thus requires individualization for each patient and a dedicated vascular access clinic may optimize the timing and quality of vascular access in this setting [79–81].

Kidney Transplant Workup

1. General principles

The pre-transplant evaluation (Table 64.3) is an essential part of the kidney transplant process and aims to reduce complications and increase graft and patient survival [41, 82]. The transplant workup should be started 6 to 12 months before the patient is likely to require KRT in known CKD patients, but should start as soon as possible in patients with a late referral. It includes a medical and psychosocial evaluation, and patient/caregiver education. The initial phase is to rule out any contraindications (Table 64.2). The medical evaluation also includes the detection of anti-Human Leukocyte Antigen (HLA) antibodies to the donor, the detection and treatment of any infection prior to transplantation, the completion of immunizations, screening for coagulation and thrombosis abnormalities, and the correction of any significant urinary tract abnormality. In certain cases, the indication for native nephrectomy should be discussed. Patients and their families or caregivers should be provided with sufficient information regarding patient survival, transplant complications and immunosuppressive drugs used and their side effects.

2. Blood group

The majority of kidney transplantations are ABO compatible and most allocation schemes allocate donor kidneys on the basis of blood

Table 64.3 Proposed pre-transplant assessment check list

	Relevant workup		Date/frequency
Planned transplant type	Living or deceased donor		
Chronic kidney disease evaluation	Diagnosis (± genetic testing when relevant)		
	Dialysis history		
	Previous transplants (living donor – deceased donor)		
	Creatinine		
	eGFR		
	PTH, calcium and phosphorus levels		
Immunology	Blood group		
	Tissue typing		
	Cross match		
	HLA antibodies		/3 months
Infectious disease workup	Serologies/PCR	CMV	/3 months if negative
		EBV	/3 months if negative
		Hepatitis B	
		Hepatitis C	
		Hepatitis A	
		Varicella	
		Measles/mumps/rubella	
		Toxoplasmosis	
		HIV	
		Syphilis (VDRL/TPHA)	
	Immunization history		
	Interferon gamma release assay (Quantiferon)	All patients who have not had BCG	
	Chest X-ray for latent TB if required	All patients who have not had BCG	
	Urine culture		
Metabolic workup	Liver function		
	Fasting glucose level		
	CYP3A5/TPMT	If azathioprine used	
Coagulation	Family/patient history – bleeding and clotting		
	Protein C		
	Protein S		
	Anti-thrombin functional		
	Lupus anticoagulant (DRVVT)		
	Anti-cardiolipin IgG - B2GP1 (if required)		
	Factor VIII		
	Factor XII		
	Activated Protein C Resistance		
	Factor V Leiden mutation		
	Prothrombin		
	Factor II mutation		
	G6PD deficiency screen		
Imaging	Ultrasound of aorta, inferior vena cava and iliac vessels		
	Magnetic resonance angiography/venography	Under 20 kg / previous transplants	
	Angiography/Venography (if required)		
	Bladder ultrasound scan	Pre/Post micturition	
	Voiding cystourethrogram	Patients with urinary tract abnormalities	
	Urodynamics (If required)		
	Cardiac ultrasound and electrocardiogram		/6–12 months

(continued)

Table 64.3 (continued)

	Relevant workup		Date/frequency
Reviews/ Referrals	Detailed interview with nephrology consultant		
	Detailed interview with transplant surgeon	Including transplant plan (anatomical position, transplant ureter…)	
	Urology review if required	Bladder plan	/12 months
	Detailed interview with anaesthetist	Access plan for transplant	/6 months
	Ear Nose and Throat (ENT) review		
	Dental review		
	Dermatology review		
	Ophthalmology review if required		
	Other if required (neurology, liver)		
Psychosocial assessment	Psychologist		
	Pediatric psychiatrist		
	Social worker		

BCG Bacille Calmette-Guerin, *CMV* cytomegalovirus, *CYP3A5* cytochrome P450 3A5, *EBV* Epstein-Barr virus, *eGFR* estimated glomerular filtration rate, *G6PD* glucose-6-phosphate dehydrogenase; *HIV* human immunodeficiency virus, *HLA* human leukocyte antigen, *PCR* polymerase chain reaction, *PTH* Parathormone, *TB* tuberculosis, *TPMT* Thiopurine methyltransferase

group identity. However, given donor shortages, an increasing number of centers provide living donor blood group incompatible transplantation. Recipient anti-B or anti-A titers are measured and depending on the level, the decision is made regarding the kidney transplantation feasibility and the treatment required to prevent a reaction (rituximab, plasmapheresis, immunoadsorption) [83].

3. Histocompatibility and immunogenetics

HLA typing is performed for all patients undergoing kidney transplantation and refers to the characterization of the individual's HLA genes and the identification of the HLA molecules expressed on the surface of their cells. DNA technology has led to the identification of many more alleles and is the gold standard for HLA typing. Sensitization is the development of antibodies to HLA antigens and their presence is likely to result in antibody mediated rejection and early graft loss. Sensitization can happen in the following main situations: previous transplant, blood, platelet or fresh frozen plasma transfusions, and pregnancy. It can also happen spontaneously through cross-sensitization from infection and pro-inflammatory events [84]. Several different assays are available to determine the sensitivity of a potential transplant recipient to donor HLA antigens. Microbead array and ELISA techniques have been proven to be more sensitive than cytotoxicity. Pre-transplant cross-matching is mandatory and aims to confirm the absence of donor-directed HLA antibodies. A positive cross-match is a potential contraindication for kidney transplantation.

Assays for Cross-match Testing

- The complement-dependent **cytotoxic crossmatch** is the most common test where donor lymphocytes are incubated with recipient serum in the presence of complement. If donor specific antibodies are present, cell lysis is observed, and the crossmatch is deemed positive.
- The **flow cytometry cross-match** is a more sensitive cross-match. Recipient serum is added to the donor lymphocytes, followed by the addition of a secondary fluorochrome-conjugated antibody that detects human IgG. Samples with the patient's serum incubated with the donor cells are compared with a negative control containing pooled sera

from normal, healthy, unsensitized individuals. It is particularly useful in sensitized patients and can be performed with peripheral blood cells.

– **Virtual cross-matching** involves reviewing the donor HLA profile against the patient's HLA antibody profile to determine whether the patient has donor-directed antibodies that would cause a positive cross-match test result. This technique can be performed in a certain number of patients with a low immunological risk and reduces the cold ischemia time. A further cross-match test must be performed retrospectively to confirm the negative virtual cross-match.

4. Infectious Disease Issues

Infections are an important cause of morbidity and mortality post-transplant. As such, pre-transplant infectious screening is an essential step in preparing for kidney transplantation [85]. The urinary tract, skin, teeth, and sinuses should be carefully examined for signs of infection or site of chronic infection. Lack of prior exposure to certain pre-transplant viruses may affect transplantation outcomes. Post-transplant viral disease may be more severe (CMV, influenza), increase the risk of chronic allograft dysfunction (CMV, BK virus) and can increase the risk of post-transplant lymphoproliferative disease (EBV) [86].

Routine childhood immunizations should be completed prior to kidney transplantation according to age-appropriate guidelines and should be updated using expedited regimens where necessary [86, 87]. Live vaccines, such as varicella, tuberculosis and measles, should be administered at least two months prior to transplantation. Live vaccines are contraindicated after transplantation [87].

5. Coagulation and thrombosis screening

The patient's history of bleeding and/or clotting is an important aspect. All patients should have a pre-transplant coagulation and thrombophilia screen to avoid bleeding/clotting complications.

6. Urinary tract abnormalities

The lower urinary tract should be evaluated using a voiding cystourethrogram to detect any abnormality that should be corrected prior to transplantation in patients with history of congenital abnormalities of the urinary tract or infection. Bladder and urethra abnormalities may be responsible for ureterohydronephrosis of the transplant, thereby increasing the risk of graft loss. These and other abnormalities may increase the risk of urinary tract infection. Patients with lower urinary tract obstruction are at the highest risk of post-transplant bladder dysfunction. These patients should be assessed carefully with urodynamic studies. It may be necessary to create an appendicovesicostomy in patients that require clean intermittent catheterization and/or to enlarge the bladder with an intestinal segment in patients with a small pathologic bladder [85, 86].

7. Native nephrectomy

The need for native nephrectomy is controversial. The benefits of retaining native kidneys may include preservation of residual urine output, production of native erythropoietin and better vitamin D/calcium homeostasis. However, in certain cases, native nephrectomy may be beneficial, although data are limited and no guidelines exist. Native nephrectomy prior to transplant may be considered in patients with glomerular disease with significant proteinuria (to reduce risk of thrombosis due to low albumin), autosomal dominant polycystic kidney disease (to avoid symptoms secondary to extremely large polycystic kidneys such as pain, hematuria and pressure effects causing gastrointestinal or respiratory symptoms), high-grade vesicoureteral reflux (to reduce post-transplant infection/bladder dysfunction), severe uncontrollable hypertension, and malignancy or malignant predisposition (WT1 gene variants) [88–90]. These indications are center-dependent and no clear guidelines detailing for example proteinuria/polyuria cut-offs or the degree of hypertension exist.

8. Donor options

Access to kidney transplantation is highly variable globally and even regionally, with a limited number of low-middle and low-income countries offering pediatric kidney transplantation [3, 5–8, 11]. While well-established national policies and organizations for organ procurement and allocation account for higher deceased donor (DD) transplantation rates in high-income countries, most low-middle and low-income countries initiate pediatric kidney transplantation through a living related donor (LD) program [3, 7, 11]. The transplanted kidney has a limited survival and most children with ESKD will require two, three or even more kidney transplants in their lifetime. In this regard, a LD transplant provides a better long-term graft survival and is a better option for children [86]. Even in LD kidney transplant recipients, there is a benefit in graft survival for preemptive transplantation (vs. non preemptive) [19, 31]. When there is no LD identified or available, the choice of accepting or declining a DD kidney is a difficult decision-making process involving recipient's age and size, current morbidity, time spent on dialysis and sensitization, as well as donor history, age and size, kidney risk profile, HLA matching and expected cold ischemia time [91].

An important dilemma for pediatric nephrologists is, if there is only one possible living donor, should the first kidney transplant be from a LD or a DD. The pros for receiving a DD kidney first are that children have access to good-quality donors in a relatively short period of time. Therefore, the LD could be saved for a later time after first transplant failure when the waiting time may become longer. An argument against this would be that later, the potential donor will be older and may have health issues preventing re-transplantation with a LD [91]. This question has been addressed in the US by data from the Scientific Registry of Transplant Recipients (SRTR) showing in almost 15,000 pediatric kidney transplant recipients that the cumulative graft life was similar with both strategies, i.e. living first-deceased second and deceased first-living second [92]. The same authors examined this question of optimal timing regarding the order of DD and LD kidney transplantation using a Markov decision process model to compare the relative survival benefit of each strategy over a time horizon of 20 years [93]. It was shown that for the most highly sensitized patients (PRA > 80%), a DD-first strategy was associated with a survival advantage, but for all other patients (with PRA < 80%), a LD-first strategy conferred a better patient survival [93].

Although the choice of donor should be made on an individual basis, we, and others, feel that the best treatment should be proposed for children with ESKD and recommend that a preemptive living donor kidney transplantation should always be considered as the first option.

Summary: Lifetime Perspectives

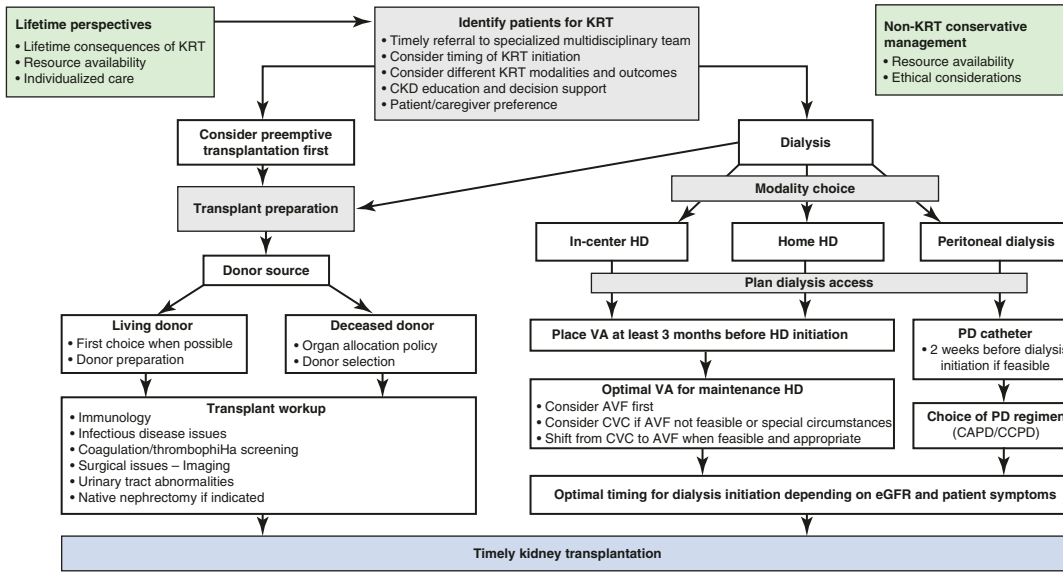
The initiation of KRT and subsequent changes of modality are intrinsic processes in the life of a child with ESKD and should be planned and reflect patients' life goals and preferences when possible. There are pros and cons of each KRT modality (CAPD/CCPD, in-center HD, home HD, preemptive/post-dialysis transplantation, living/deceased donor) to be considered in order to provide personalized care for a child with ESKD (Table 64.4). Both the short- and long-term benefits and risks of each KRT modality across the entire patient's lifespan should be carefully evaluated, considering the widely variable practice patterns and access to KRT around the world. From a lifetime perspective, it is essential for pediatric nephrologists not only to provide the best initial KRT modality available but also to keep in mind the subsequent best treatment modality and access for an individual patient.

We suggest that an integrated life plan approach for pediatric KRT should take into account the long-term consequences of the decisions and choices to be made such as dialysis

Table 64.4 Advantages and disadvantages of different kidney replacement therapy modalities in children

Modality		Advantages	Disadvantages
Kidney transplantation (KT)	KT (compared to dialysis)	Avoids dialysis morbidity and mortality	Variable access to transplantation
		Better patient survival	Not suitable for smaller children (< 10 kg)
		Better psychosocial outcome	Surgical complications
		Better quality of life	Infections
		Fewer restrictions (dietary, time, medications)	Malignancy
		Better growth	Burden of graft failure/repeated KRT modalities
		More cost-effective	
	Preemptive (compared to non-preemptive) KT	Better graft survival	Psychosocial difficulties
	Non-preemptive KT		Increased risk of graft failure
	Living donor (compared to deceased donor) KT	Better long-term graft survival	Kidney health risk for the donor
		Shorter waiting time	Psychological and socioeconomic issues for the donor
		Better graft quality	Possibility of poorer HLA matching
		Reduced delayed graft function	
		Reduced hospital costs	
	Deceased donor (compared to living donor) KT	Priority in allocation schemes	Increased risk of graft failure
Peritoneal dialysis (PD)		Increased patient freedom	Dependent on parental/caregiver participation
		Longer preservation of residual kidney function	Caregiver burden
		Avoidance of vascular access	Medicalization of the home
		Improved school attendance	Infections
		Fewer dietary restrictions	Risk of peritoneal membrane failure if on PD for years
		Technically more feasible in smaller children	
		Possible for patients who live far away from HD centre	
		Lower cost compared to HD	
Hemodialysis (HD)	In-centre HD (ICHD)	Long-term technique survival	Limited by availability of vascular access
		Suitable if patients/parents/caregivers unable to perform dialysis at home	Availability dependent on local resources
		Decreased treatment duration	Increased cost when compared to other modalities
	Home hemodialysis (HHD)	Increased flexibility with dialysis times	Dependent on parental/caregiver participation
		Improved school attendance compared to ICHD	Caregiver burden
		Reduced intradialytic symptoms and hypotensive episodes	More rapid loss of residual kidney function
		Improved cardiovascular outcomes compared to ICHD	Medicalization of the home
		More cost-effective compared to ICHD	Increased AVF complications
		Improved nutrition and growth compared to ICHD	Increased home utility bills (water, electricity)

AVF arteriovenous fistula, *HD* hemodialysis, *HHD* home hemodialysis, *HLA* human leukocyte antigen, *ICHD* in-center hemodialysis, *KRT* kidney replacement therapy, *KT* kidney transplantation, *PD* peritoneal dialysis



Abbreviations: AVF, Arteriovenous Fistula; CAPD, Continuous Ambulatory Peritoneal Dialysis; CCPD, Continuous Cyclic Peritoneal Dialysis; CKD, Chronic Kidney Disease; eGFR, Estimated Glomerular Filtration Rate; CVC, Central Venous Catheter; HD, Hemodialysis; KRT, Kidney Replacement Therapy; PD, Peritoneal dialysis; VA, Vascular access

Fig. 64.4 Integrated ESKD life plan approach for kidney replacement therapy modalities in children

AVF arteriovenous fistula, *CAPD* continuous ambulatory peritoneal dialysis, *CCPD* continuous cyclic peritoneal dialysis, *CKD* chronic kidney disease, *eGFR* estimated glomerular filtration rate, *CVC* central venous catheter, *HD* hemodialysis, *KRT* kidney replacement therapy, *PD* peritoneal dialysis, *VA* vascular access

access sustainability, cardiovascular morbidity and mortality associated with prolonged dialysis, cumulative functioning graft survival according to the order of donor type, long-term morbidity of cumulative immunosuppressive load post-transplant, family/caregiver burden, interference of modalities with social life and relationships, growth and cognitive outcomes, QoL, and patient-centered outcomes (Fig. 64.4).

The (very) long-term outcome studies clearly favor early (living donor) kidney transplantation in children with ESKD, which appears to have a beneficial effect on overall mortality, morbidity, and psychosocial maturation. The cumulative duration of dialysis in relation to years with a functioning kidney graft has been strongly associated with many adverse outcomes, especially with cardiovascular mortality, but also short stature, impaired cognitive performance, locomotor disorders, social dependence and depression [94–99]. The impact of dialysis on physical condition is also reflected by the sharp difference in physical health perception of dialysis and transplanted patients. On the other hand, some registry data (ANZDATA, ERA-EDTA) suggested that a short period of dialysis (up to 1 year) did not affect overall mortality [17, 100]. A longer period of dialysis has been associated with a higher risk of death regardless of donor source [19]. Although PD patients may have short-term survival advantage compared to HD, no studies showed significant differences in long-term outcome of PD and HD. However, PD may certainly be favorable over HD to achieve the longest and most feasible dialysis access life plan for the individual patient.

Although dialysis is the most unfavorable mode of KRT, kidney transplantation is associated with considerable late morbidity. Disabling co-morbidity was reported by 40% of all kidney transplant recipients in the Dutch late outcomes study (LERIC) [98]. Apart from clinical bone disease, the most frequently reported disabling problems were daily headaches, tremors and severe itching, most of them appearing in transplanted patients. Malignancies, infection, cumulative cardiovascular burden (hypertension-related LVH and arterial wall stiffening) are the most life-threatening problems after kidney transplan-

tation [99, 101]. Although current data shows a much lower mortality in pediatric kidney transplant recipients than in those remaining on dialysis, the need for many decades of kidney graft function will expose more patients to risk of life-threatening infections and malignancies at relatively young adult age.

The approaches to optimize long-term outcomes and QoL in pediatric patients with ESKD include timely referral for multidisciplinary CKD care, education and support for patients and caregivers, promotion of pre-emptive and living-related transplantation, reduction of KRT time on dialysis to the minimum, favor home dialysis therapies, consider intensive dialysis, individualization of the subsequent KRT modality and dialysis access plan in a lifespan perspective. There is general consensus that providing personalized care which incorporates patient goals and preferences has become a priority.

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Management of Peritoneal Dialysis in Children

65

Alicia M. Neu, Bradley A. Warady, and Franz Schaefer

Introduction

Peritoneal dialysis (PD) is the most frequently prescribed maintenance dialysis therapy for children with kidney failure worldwide, particularly in infants and very young children [1–3]. Technical advances and increasing efforts to minimize risk for infection and cardiovascular disease, the leading causes of morbidity and mortality, have contributed to improvements in technique and patient survival among children on maintenance PD [4–7]. However, mortality for children on dialysis remains unacceptably high and notably higher than for children who receive a kidney transplant [2, 3, 7]. Ongoing efforts to further improve outcomes in children on maintenance PD must include prescribing, monitoring and adjusting the dialysis treatment to meet the unique needs of the child [8, 9]. This chapter focuses on the principles involved in developing and monitoring the PD prescription, establishing a functioning access to perform the dialysis procedure and the infectious and non-infectious complications seen in children on maintenance PD. Kidney failure is an incredibly complex condition and therefore comprehensive care of the child on maintenance peritoneal dialysis must not only include tailoring the PD prescription to provide optimal solute and fluid removal, but also maximizing growth and neurocognitive development, managing anemia, minimizing bone and mineral metabolism disorder and cardiovascular disease, and addressing the psychosocial well-being of the child and their family [8, 9]. Each of these important topics is therefore covered in a separate chapter of this book.

The Peritoneal Dialysis Prescription

The directly modifiable components of the PD prescription include the composition and volume of the dialysis fluid and the schedule by which that fluid is instilled and removed from the peritoneal cavity. Although empiric recommendations for prescribing maintenance PD in children are often used when initiating dialysis, optimal care requires that the PD prescription be modified to meet the unique needs of the individual child or adolescent with kidney failure [8–10]. This requires a basic knowledge of the physiology of dialysis which, in turn, relies on an under-

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standing of the peritoneal membrane as the primary barrier to solute and fluid transport. This chapter therefore begins with a brief overview of the structure of the peritoneal membrane, followed by a discussion of the physiology of dialysis, that is, the driving forces for the exchange of solute and fluid across the peritoneal membrane. The application of these principles to guide selection of the modifiable components of the PD prescription is then presented.

The Peritoneal Membrane

The peritoneal membrane is a thin structure lining the inner surface of the abdominal wall and the majority of visceral organs. It is lined by the mesothelium, a continuous layer of flattened epithelial cells covered with numerous microvilli, and includes a dense network of capillaries distributed within a thin interstitium [11–13]. The pathway for the solute and water exchange between the plasma in the peritoneal capillaries and the dialysate in the peritoneal cavity of the child on PD includes the continuous capillary endothelium, the peritoneal interstitial space, and the mesothelium [14]. Of these, the capillary endothelium appears to be the primary determinant of resistance to transport, and microvascular density is therefore a major determinant of transport characteristics [11, 15–18]. The permeability of the endothelium lining the peritoneal capillaries has been functionally described by the three-pore model proposed by Rippe and colleagues [19]. In this model, the major route for small-solute and water movement is represented by the spaces between the endothelial cells, the so-called small pores, which have a radius of 40–50 Å, slightly larger than albumin (36 Å) [12, 19]. Ultrasmall pores, with a radius of approximately 2.5 Å, are the most abundant type of pores and are involved in sodium-free water transport [12, 19]. Several lines of evidence have demonstrated that the water channel aquaporin-1 corresponds to the ultrasmall pore [20, 21]. The third group of pores is the transendothelial 'large pore' pathways, which have a radius of approximately 250 Å, and which account for only 0.01% of the total population of capillary pores and through which macromolecules are transported [19].

The Physiology of Dialysis

The driving forces for exchange of solute across the peritoneal membrane include diffusion and convective mass transfer through the small pores in the capillary endothelium. The rate of solute movement by diffusion is determined by the concentration gradient of the solute between the dialysate in the peritoneal cavity and the plasma in peritoneal capillaries, the effective surface area of the peritoneal membrane in contact with the dialysate, the so called "wetted membrane," and the permeability of the peritoneal membrane to that solute, which, in turn, is influenced by the molecular weight of the solute [13, 22]. Convective mass transfer occurs as water moves through small pores from capillaries to dialysate, "dragging along" dissolved solutes. The amount of solute removed by convective mass transfer is, therefore, determined by the amount of water removed and by the membrane permeability, or sieving coefficient for that solute. While small molecular weight solutes, like urea, move by both diffusion and convective mass transfer, the movement of larger molecular weight compounds, including the uremic "middle molecules," is driven primarily by convective mass transfer [23].

The bulk movement of water, or ultrafiltration, is driven by Starling forces, i.e. osmotic and hydrostatic pressure [12, 23]. Figure 65.1 depicts the Starling forces (P, hydrostatic pressure; Π, oncotic or osmotic pressure) that operate across each of the pore types in the three-pore model [12]. Movement of water through the ultrasmall pores is driven by the osmotic gradient between the plasma in peritoneal membrane capillaries and the interstitium and, ultimately, the dialysis fluid in the peritoneal cavity. The osmotic pressure in the plasma is generated primarily by albumin, whereas osmotic pressure in the dialysate is typically generated by crystalloid, i.e. glucose, or the glucose polymer icodextrin. This "water only" movement through the ultrasmall pores explains the transient decrease in dialysate sodium concentration during the early phase of a dialysis dwell, which is referred to as sodium

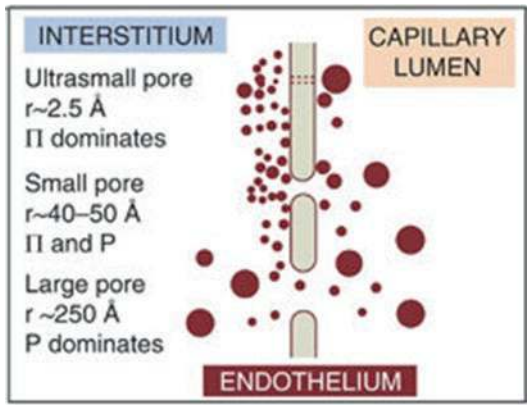

Fig. 65.1 The Starling forces (P, hydrostatic pressure; Π, oncotic pressure) operating across each type of pore in the three-pore model of peritoneal membrane capillary permeability. *Å* angström, *r* functional radius. (From [12], with permission)

sieving. Movement of water through small pores is influenced by both hydrostatic and osmotic forces (Fig. 65.1) [12]. In simplest terms, hydrostatic forces in plasma and osmotic forces in the dialysate promote ultrafiltration, while osmotic forces in plasma and hydrostatic pressures in the peritoneal cavity oppose it [24]. Several factors contribute to the generation of these forces; however, the critical component for ultrafiltration during PD is the difference in osmotic pressure between the dialysate and the plasma, which, in turn, is largely dependent on the osmotic agent present in the dialysate [24]. The amount of water removed from the person on PD, or net ultrafiltration, is also influenced by water movement from the peritoneal cavity back to the capillaries in the late stages of a dwell, when the osmotic gradient generated by dialysate glucose may have dissipated, and by uptake of water from the peritoneal cavity into tissue and lymphatics [25, 26]. The contribution of water movement through the relatively rare large pores to net ultrafiltration is felt to be minimal [12].

These principles of solute and fluid movement during PD should be used to guide selection of the various components of the dialysis prescription, including dialysate composition, fill volume and the schedule by which dialysis is instilled and removed from the peritoneal cavity (PD modality/dwell time), to optimize solute and fluid removal.

Determination of Fill Volume

As discussed above, the movement of solutes and water during PD is intrinsically dependent on the amount of peritoneal membrane surface area available for exchange, or the "wetted membrane" [13]. Although the peritoneal membrane has an estimated surface area of 1 m^2 in adults, computed tomography studies in people on maintenance PD have demonstrated that only 30–60% of this anatomic area is in contact with dialysate [27]. The peritoneal membrane contact area can be influenced by position, increasing in the supine position, and by increasing the volume of the infused dialysate, or fill volume [28]. In children, where body size varies considerably, the concept of scaling the fill volume to body size is intuitive. Fill volume should be based on body surface area (BSA), rather than weight, as the relationship between peritoneal membrane surface area and BSA is constant and age-independent [29]. Body surface area can be calculated from anthropometric data, i.e. height and weight. The most commonly used equation is that of Gehan and George [30]:

$$\text{BSA } (m^2) = 0.0235 \times (\text{height, cm})^{0.42246} \times (\text{weight, kg})^{0.51456}$$

As stated above, increasing the fill volume will promote solute and fluid removal by maximizing peritoneal membrane contact area [31]. In addition, increasing fill volume will facilitate movement by diffusion. The impact of fill volume on diffusion rests on the principle of geometry of diffusion, that is, the larger the dialysate volume, the longer the transperitoneal concentration gradient will persist to drive diffusion [32]. However, increasing fill volume also increases intraperitoneal pressure (IPP) which may lead to patient discomfort and other complications including hernia formation, hydrothorax and gastroesophageal reflux (See Non-Infectious Complications) [10, 26, 28, 33]. In addition, elevated IPP may increase lymphatic uptake of fluid, thereby reducing net ultrafiltration [10, 33]. Studies in children on PD revealed that the peritoneal membrane vascular surface area available for exchange increased by a mean of 21% as fill

volumes were increased from 800 to 1400 ml/m^2, with no further improvement as fill volumes increased to 2000 ml/m^2 [28, 34]. These data support current recommendations that, if required for solute clearance and fluid removal, the fill volume should be gradually increased to an upper limit of 1200–1400 ml/m^2 in children over age 2 years [10]. Infants may not tolerate such large fill volumes, and an upper limit volume of 800 ml/m^2 is currently recommended in this age group [10]. The maximal volume for individual children on PD should also be influenced by the child's comfort level and when indicated, an objective measure of IPP [35]. Measurement of IPP can be done at the bedside, using a manometer attached to the PD catheter. The mean IPP is calculated from the pressure measured during inspiration and expiration. Normal ranges of mean IPP for children over age 2 years have been reported to be 7–14 cmH_2O, with an upper tolerated limit of 18 cmH_2O [35, 36].

Choice of PD Fluid

Conventional PD Solutions

PD solutions typically contain an osmotic agent, a buffer and sodium, chloride, calcium and magnesium in varying concentrations, in an effort to provide not only removal of fluid and waste products, but also electrolyte homeostasis, and acid-base and calcium balance. The composition of the most widely used commercially available dialysis solutions attempt to mimic normal plasma, while allowing mass production and storage stability [37]. These constraints led to the selection of glucose in supraphysiologic concentrations as the osmotic agent and lactate alone as the buffer, with a resultant low pH of the dialysis fluid. This allows heat sterilization without caramelization of the glucose, and minimizes precipitation of calcium and magnesium from the solution, which may occur when bicarbonate is used as the buffer [37]. From the description of the Starling forces involved in water movement during PD, it follows that increasing the concentration of glucose in the dialysis fluid increases the osmotic gradient driving ultrafiltration. From a functional standpoint, because glucose is a diffusible solute, it is absorbed from the dialysate to plasma via the small pores, resulting in a time-dependent loss of the crystalloid osmotic gradient. Thus, glucose is unable to provide sustained ultrafiltration during extended exchange dwell times. In addition, absorption of glucose can contribute to anorexia and lead to elevated serum glucose and hyperinsulinemia, even in non-diabetic patients [38]. This increased carbohydrate load can predispose to abnormalities of lipid metabolism and insulin resistance (See Non-Infectious Complications) [37, 39]. In addition to the negative effects associated with glucose absorption, the heat sterilization process used with conventional PD solutions produces high levels of glucose-degradation products (GDP), which are directly toxic to the peritoneal mesothelium and are systemically absorbed [40]. GDPs also enhance production of advanced glycation end products, which along with high concentration of glucose have been implicated in the development of structural changes in the peritoneal membrane including vascular proliferation and progressive fibrosis, both of which contribute to peritoneal membrane failure [31, 37, 41, 42].

Alternate Osmotic Agents

In light of these findings, minimizing the exposure of the peritoneal membrane to hypertonic glucose is a therapeutic aim [43]. Currently, there are two commercially available PD solutions that contain osmotic agents other than glucose; one contains icodextrin and the other amino acids. Icodextrin is a glucose polymer with a molecular weight of approximately 16,000 Daltons, which exerts its osmotic effect through the small pores in the capillary endothelium. Thus, there is little to no salt-free water movement through the ultrasmall pores (sodium sieving) and sodium removal is typically higher than with glucose-based solutions [44]. Because icodextrin does not diffuse through the peritoneal membrane, the osmotic gradient, and therefore ultrafiltration, is typically sustained, and icodextrin solutions are therefore used during dialysis exchanges with a prolonged dwell time [45, 46]. The net ultrafiltration seen in individual people on PD can be variable, probably owing to variability in the peritoneal residual volume, i.e. the amount of

non-icodextrin containing fluid remaining in the peritoneal cavity from the previous exchange, which modifies the concentration of icodextrin and, therefore, the osmotic pressure difference between the peritoneal cavity and plasma [47, 48]. Another factor influencing net ultrafiltration is lymphatic absorption of icodextrin, which has been reported to be as much as 45% within 12–14 h in children on PD. [49] Reabsorption may be particularly high in infants on PD, limiting the ultrafiltration achieved with icodextrin in this age group [50]. Finally, a minimum daytime fill volume of 550 ml/m^2 has been suggested to optimize ultrafiltration with icodextrin in children [51]. Icodextrin is metabolized to maltose and a number of oligosaccharides which reach systemic steady state levels within 2 weeks of initiating treatment, and concerns about higher levels of these non-degradable compounds limits the use of icodextrin containing solutions to a single daily exchange [43, 45]. Hypersensitivity reactions have also been reported with icodextrin-containing solutions [45].

Amino acids, in a 1.1% solution, are also used as an osmotic agent in a commercially available, non-glucose PD solution. This solution is as efficient an osmotic agent as a 1.36% glucose-based solution. Amino acid-based solutions initially appeared particularly appealing for children on PD because of the potential nutritional benefit; however, studies revealed conflicting impact on nutrition, as well as increases in blood urea nitrogen and metabolic acidosis [52]. Given these findings, it is not recommended that amino acid solutions be used as a nutritional source in children on PD. [43] The benefits and potential drawbacks of each of the three solutions described here are summarized in Table 65.1 [37].

Table 65.1 Characteristics of currently available single-chamber peritoneal dialysis solutions, based on osmotic agent. Modified from [37], with permission

Buffer	Potential drawbacks	Potential benefits
Glucose	Low pH High GDP Poor peritoneal membrane biocompatibility Infusion pain Local and systemic glucose exposure	Ease of manufacture Low cost
Icodextrin	Hypersensitivity Low pH Systemic accumulation of oligosaccharides Lactate containing	Sustained ultrafiltration Preservation of RKF Hypertonic glucose replacement Reduced hyperglycemia Desirable effects on metabolic profile and body composition
Amino acid	Low pH Exacerbation of uremic symptoms and acidosis	No GDP Avoid systemic and peritoneal glucose exposure Peritoneal membrane protection Enhanced nutrition in adults

GDP glucose degradation product, *RKF* residual kidney function

Biocompatible Solutions

The supraphysiologic concentrations of glucose and the presence of GDPs are not the only contributors to the bio-incompatibility of standard dialysis solutions. Low pH is associated with infusion pain and directly induces neoangiogenesis and mesothelial cell damage [53, 54]. Even at a neutral pH, lactate-based peritoneal dialysis solutions have been associated with impaired mesothelial cell viability and function [55, 56]. The effort to provide truly biocompatible solutions therefore includes not only the use of alternative osmotic agents, but also a solution composition that results in a more neutral pH and reduced exposure to lactate. The development of multi-chamber dialysis solutions has allowed these issues to be addressed at the commercial level. These bags isolate the buffer during storage, thus allowing glucose to be stored at low pH, ensuring stability, and avoiding the creation of GDP during heat sterilization. This also avoids bicarbonate-induced precipitation of calcium and magnesium in the solution [37]. A summary of the benefits and potential drawbacks of the currently available multi-chamber PD solutions is shown in Table 65.2 [37]. All of these solutions provide lower GDP levels than standard glucose-containing solutions. Although numerous in vitro studies have supported the biocompatibility of

Table 65.2 Characteristics of currently available multi-chamber peritoneal dialysis solutions, based on buffer. Modified from [37], with permission

Buffer	Potential drawbacks	Potential benefits
Lactate alone	More physiologic, but not neutral, pH Local and systemic glucose exposure	Lower GDP levels More physiological pH Improved peritoneal membrane biocompatibility Preserved membrane defense
Lactate/ bicarbonate	Local and systemic glucose exposure Does not eliminate peritoneal lactate exposure	Lower GDP levels More physiologic pH Improved peritoneal membrane biocompatibility Preserved membrane defense Reduced infusion pain
Bicarbonate alone	Local and systemic glucose exposure	Lower GDP levels More physiologic pH Improved peritoneal membrane biocompatibility Preserved membrane defense Improved correction of acidosis

GDP glucose degradation product

these solutions, a study of peritoneal biopsies in children at the time of PD catheter insertion and then after receiving maintenance PD with neutral pH, low GDP fluids revealed a doubling of peritoneal microvascularization and exchange area within a few months of initiating PD, calling into question the ability of these fluids to preserve membrane function and structure [41]. A subsequent analysis found that the duration of dialysis and dialytic glucose exposure were the primary determinants of the alterations to the peritoneal membrane [57]. Although biocompatible fluids may, in turn, not eliminate the structural changes to the peritoneal membrane, there may be some benefit of using bicarbonate, rather than lactate, as the dialysis solution buffer. A multicenter randomized controlled trial in 37 children on PD compared two multi-chamber, neutral pH, low GDP PD solutions that differed only with regard to the buffer, lactate versus bicarbonate. This study found equivalent correction of metabolic acidosis with the two solutions, but bicarbonate-based solutions were associated with better long-term preservation of peritoneal membrane function as measured by ultrafiltration capacity [58]. In addition, data from the International Pediatric Peritoneal Dialysis Network (IPPN) revealed that young infants exposed to neutral-pH, low-GDP PD solutions exhibited significant catch-up growth, whereas patients using conventional PD fluids showed no improvement in height standard deviation scores over the same time period. These findings led investigators to speculate that reduction of the inflammatory processes associated with conventional solutions might improve growth in children undergoing maintenance PD [59]. Finally, a Cochrane Review revealed that use of a neutral pH, low GDP PD solution is associated with improved preservation of residual kidney function and urine volume in adults on PD [60]. Given these findings, use of the more biocompatible solutions is encouraged, while recognizing that cost and availability of these solutions may limit widespread use [43]. In fact, data from IPPN reveals significant regional variability in the prescription of neutral pH PD solutions among children on PD enrolled in that registry [61]. When excluding children from the United States, where neutral pH PD solutions are not approved, 8% of children from low-income countries are prescribed these solutions, compared to 68% of children in high-income countries [8, 61].

Determination of PD Modality/ Dwell Time

CAPD vs. APD

There are two major PD modalities utilized for maintenance PD, continuous ambulatory peritoneal dialysis (CAPD), in which 3 or 4 exchanges

are performed manually during the day with an exchange with a long dwell time conducted overnight, and automated peritoneal dialysis (APD), in which multiple exchanges are provided, typically overnight, by a cycler. The most commonly prescribed APD schedules are continuous cycling PD (CCPD) and nightly or nocturnal intermittent PD (NIPD). Both provide multiple exchanges overnight, but in CCPD, 50–100% of the nightly fill volume is instilled at the end of the APD session for a daytime exchange. For NIPD, no daytime exchange is used, and the person on PD is said to have a dry day with no dialysate being present in the peritoneal cavity. Other modifications can include the addition of a mid-day manual exchange, sometimes referred to as semi-automated PD, and tidal PD, where only a portion of the initially instilled fill volume is drained and replaced with each exchange overnight, with the full volume drained only at the completion of the APD session. Tidal therapy has been found to be particularly beneficial in patients who experience "drain pain."

The selection of PD modality should be individualized for each child based on a number of factors, including age, residual kidney function, nutritional status, tolerance/comfort and the preference of the child and their caregivers [8, 9]. The physiology of PD should be considered so that the modality selected meets the child's solute and fluid removal requirements. Because APD allows more exchanges to be conducted during a 24-h period than CAPD, the peritoneal membrane is exposed to a larger total volume of dialysate in this time period which may enhance clearance of small solutes. In addition, during APD the majority of exchanges occur at night, when the child is in the supine position, which optimizes peritoneal membrane contact area and minimizes increases in IPP [28]. Conversely, CAPD allows increased clearance of middle molecules, which is dependent on the duration of contact between dialysate and the peritoneal membrane [62]. The requirement for fluid removal will also impact modality selection. In CAPD, daytime dwell times are typically 4–6 h long, as more frequent exchanges may be too cumbersome for the child/caregivers to perform. These long dwell times may result in reduced ultrafiltration, due to the loss of glucose-generated osmotic gradient, and necessitate higher glucose-containing solutions to maintain that gradient. Recall that in the early part of an exchange, sodium-free water movement occurs via the ultrasmall pores. Thus, frequent exchanges with short dwell times characteristic of APD may result in a relatively higher contribution of free water transport to total fluid removal, that is, more water than sodium is removed. Conversely, an exchange with a longer dwell time, as occurs with CAPD, allows more time for convective losses of sodium, but also allows back-diffusion and back-filtration, and may result in net fluid and sodium retention [26].

From a practical standpoint, because a cycler is not required for CAPD, the training and equipment required are less than for APD. However, because APD, is performed at night, this therapy minimizes the restriction on daytime activities, such as school attendance for children and work for adult caregivers, which is a significant benefit associated with the use of this modality [63].

Empiric Dialysis Prescriptions

A typical empiric APD prescription includes 5–10 exchanges over 9–12 h overnight, with an identical fill volume and duration for each exchange. A daytime exchange is usually prescribed, particularly in children who are anuric. More recently the concept of adapted PD, with initial cycles using a relatively small fill volume and short dwell times to maximize ultrafiltration, followed by a larger fill volume with longer dwell times to promote solute clearance, has been suggested as a means of improving dialysis efficiency, and in particular sodium and fluid removal [26, 64]. Not all commercially available PD cyclers are able to provide adapted PD and further prospective crossover studies in children on PD are required for validation. As stated previously, the typical CAPD prescription includes 3–4 exchanges during the day and a long overnight exchange.

Measures of Peritoneal Membrane Function

Because peritoneal membrane transport characteristics may vary considerably between people on PD, and even in a single person over time, it is important to evaluate these characteristics to optimize the PD prescription. Pediatric guidelines recommend evaluating peritoneal membrane function within the first month of initiating PD and then

after any event that may impact peritoneal membrane transport capacity, such as peritonitis [65]. The most commonly used test to characterize peritoneal membrane transport capacity is the peritoneal equilibration test or PET, developed by Twardowski [66]. The PET measures the rate at which solutes, specifically urea, creatinine and glucose, equilibrate between the blood and the dialysate. In the PET, dialysate is infused into the peritoneal cavity using a standardized fill volume and glucose concentration. Because the fluid used in the exchange immediately preceding the PET may influence results, the solution used for the PET should also be used for the dialysis session the night prior [67, 68]. Once the dialysis solution is instilled, the concentrations of creatinine and urea in the dialysate and in plasma are measured after 2 and 4 h of dwell time to derive dialysate to plasma ratios (D/P). The concentration of glucose in the dialysate at 2 and 4 h after instillation is compared to the concentration of glucose in the dialysate at the time of instillation (D/D_0). The D/P and D/D_0 ratios are then compared to standard curves to characterize the child as having high, high average, low average or low peritoneal membrane solute transport capacity [66]. People on PD with low or low average transport capacity may benefit from exchanges with longer dwell times, which will allow maximal diffusion of solutes. Conversely, rapid diffusion of glucose in patients with high peritoneal membrane transport capacity necessitates the use of exchanges with short dwell times to achieve ultrafiltration. The crossing point of the urea and glucose equilibration curves obtained from the standardized PET, referred to as the Accelerated Peritoneal Examination (APEX) time, has been proposed as a means to identify the dwell time to be used to optimize ultrafiltration [69]. The characteristics seen with the various peritoneal membrane transport types, and the percent of children enrolled in the IPPN with each are shown in Fig. 65.2.

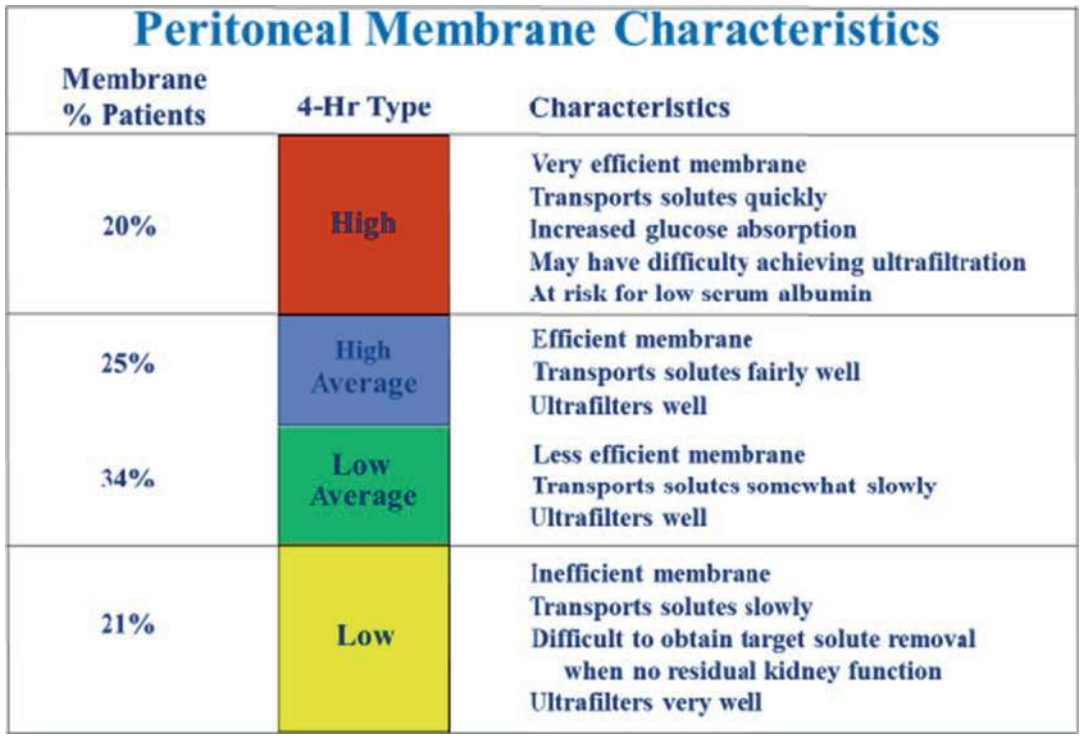

Fig. 65.2 Characteristics of the various peritoneal membrane transport types (high, high average, low average and low) and the percentage of children with each of the types enrolled in the registry of the International Pediatric Peritoneal Dialysis Network (personal communication, B Warady)

The PET has been validated in children on PD, using 2.5% dextrose, or 2.3% glucose PD solution and a fill volume of 1100 ml/m^2 [70, 71]. In infants, the fill volume used for the PET is usually the clinically prescribed fill volume [23]. Figures 65.3 and 65.4 show the standardized D/P creatinine and D/D$_0$ glucose curves, respectively, from which a child's peritoneal membrane transport capacity can be characterized [70]. In a study of 20 children on maintenance PD, nearly identical characterization of peritoneal membrane function was found with the D/P creatinine or D/D$_0$ glucose at 2 and 4 h, and it has therefore been suggested that a 2 h or short-PET may be reasonable in children on PD [72]. The sequential PET, in which the standard PET is followed by a "mini-PET," has been proposed as a method for providing more complete characterization of both solute and fluid transport [73]. The mini-PET is a modification of the

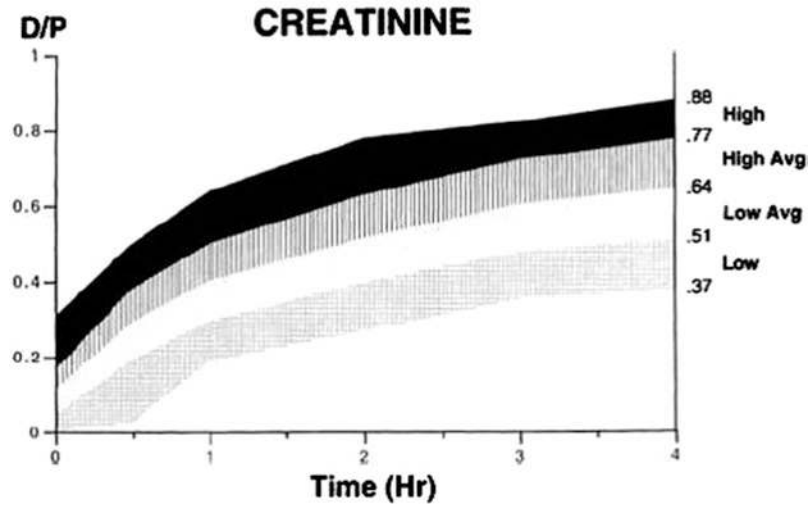

Fig. 65.3 Peritoneal equilibration test results for creatinine. Shaded areas represent high, high average, and low transport rates. The white band represents the low average transport rate. The four categories are bordered by the maximal, mean + 1 SD, mean, mean − 1 SD, and minimal values for the population. D/P, dialysate to plasma ratio. (From [70], with permission)

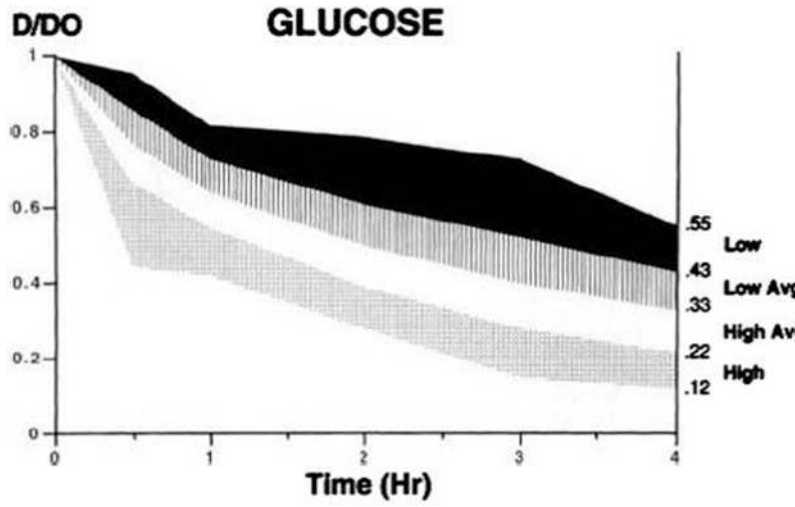

Fig. 65.4 Peritoneal equilibration test results for glucose. Shaded areas represent high, low average, and low transport rates. The white band represents the high average transport rate. The four categories are bordered by the maximal, mean + 1 SD, mean, mean − 1 SD, and minimal values for the population. D/D0, dialysate glucose to initial dialysate glucose concentration ratio. (From [70], with permission)

standard PET which uses a 3.86% glucose solution instilled for 1 h. Dialysate sodium concentration is measured just prior to infusion and after 60 min, providing more accurate information about the ultrafiltration capacity and assessment of sodium sieving [74].

Data obtained from the PET can also be used to calculate the mass area transfer coefficient (MTAC) [70, 75, 76]. The MTAC has been variably defined as the area available for solute transport divided by the sum of resistances to peritoneal diffusion. The MTAC represents the maximal clearance of a solute theoretically achievable at a constantly maximal gradient for diffusion, i.e. when the dialysate concentration of the solute remains at zero. Unlike the D/P ratio, MTAC is essentially independent of dialysate glucose or fill volume. Calculation of MTAC requires rigorously performed PD exchanges and complex mathematical equations. However, with the assistance of computer programs, data from a carefully performed PET can be used to derive MTAC. These programs, which have been validated in children on PD, can also be used to predict solute and fluid removal for individualized dialysis prescriptions [77, 78]. It must be recognized that the results predicted by these programs assume optimized conditions and therefore the actual amount of dialysis delivered by any prescription needs to be measured (See Goal-directed Approach to Prescribing PD).

Goal-Directed Approach to Prescribing PD

Solute Clearance

Historically, modification of the empiric PD prescription has been driven by the concept of achieving "dialysis adequacy," i.e. the dose of dialysis delivered is measured and adjustments are made to exceed a minimum dose below which patient outcomes are unacceptable. For decades, adequacy targets focused on the delivered dialysis dose in terms of small solute clearance. Peritoneal dialysis adequacy guidelines recommended the use of urea removal, scaled for the urea volume of distribution, Kt/V_{urea}, to monitor solute clearance and guidelines published in 2006 in the United States and internationally suggested a minimum target of a total weekly (residual kidney and dialysate) Kt/V_{urea} of 1.8 or 1.7 for adults on PD, respectively [65, 79]. These targets were largely based on studies in adults on PD which suggested improved survival with increasing small solute clearance [80, 81]. However, a reanalysis of data from a large prospective study in Canada and the United States (CANUSA) found the association between small solute clearance and mortality to be completely explained by the clearance contributed by residual kidney function, with no association between increasing dialysate small solute clearance and survival [82]. Similarly, two large prospective randomized trials did not demonstrate an association between increasing small solute clearance and mortality in adults on PD [83, 84]. A retrospective analysis of administrative data in the United States did reveal an increased risk for mortality with a $Kt/V_{urea} < 1.7$ in anuric adults on PD [85].

Although a prospective study of 171 children on PD demonstrated a positive correlation between dialytic creatinine clearance and change in height standard deviation score, and cross-sectional and retrospective studies have suggested improved growth and cardiac function with increasing small solute clearance, there are no large-scale, prospective, randomized studies of the influence of small solute clearance on outcomes in children on PD to more definitely define adequacy targets [86–88]. In light of this, the 2006 guidelines recommended that the total weekly Kt/V_{urea} in children should meet or exceed the adult standard [65].

Measurement of total weekly Kt/V_{urea} should incorporate both dialysate and residual kidney clearance [65]. This is accomplished by collecting the volume of urine from a 24-h period, as well as the peritoneal dialysis effluent from the PD exchanges during those 24 h. The volume is recorded and urea measured on each sample. Blood urea nitrogen concentration is also measured.

The total dialysate Kt/V_{urea} is then calculated by:

(24 h Dialysate/Plasma urea×24 h drained volume × 7) / Volume of distribution of urea

The residual kidney urea clearance is calculated by:

(Volume of 24 h urine in mL×urine urea nitrogen concentration)/(1440 min/day × blood urea nitrogen concentration)

From this, the residual kidney Kt/V_{urea} can be calculated as:

(Kidney urea clearance (ml/min) × 1440 min/day × 7 days)/1000 mL × Volume of distribution of urea.

The total weekly Kt/V_{urea} is the sum of the weekly dialysis and residual kidney Kt/V_{urea} [65].

The volume of distribution of urea, V, is assumed to be equal to total body water. Therefore, accurate estimates of total body water are important to accurately determine Kt/V_{urea}. The gold standard method for determining total body water, the heavy water dilution technique, is rarely applied in the clinical setting. Equations using anthropometric information (height and weight) are more commonly used to estimate total body water, and sex-specific nomograms developed in children on PD are available [89].

Guidelines for children on PD recommend that total weekly Kt/V_{urea} be measured within the first month after initiating dialysis and then at least twice yearly, and following any change in the child's clinical status that could influence solute clearance or ultrafiltration capacity [10, 90]. Given these recommendations, measurement of small solute clearance is standardly performed, and achievement of the minimal target for Kt/V_{urea} in adults and children on PD is used as a measure of the quality of care by dialysis organizations around the globe and regulatory and payment agencies in the United States. However, the data linking small solute clearance to outcomes in people on PD remains relatively weak, with no prospective intervention trials since publication of the 2006 KDOQI guidelines, and prospective cohort and retrospective studies in adults on PD only confirming that patient outcomes are more closely linked with residual kidney function than clearance of solute by dialysis [91–98]. In addition, it has been increasingly acknowledged that optimal care requires that all aspects of management, including the PD prescription, be driven by the unique needs of the person with kidney failure, and not solely by small solute clearance [8, 91]. In 2018, a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference focused on dialysis proposed a change in terminology from "adequate" to "goal-directed" dialysis, where shared decision-making between the person on PD and the care team is utilized to establish realistic care goals that allow the person on PD to meet their life goals and allow the care team to provide individualized, high quality dialysis care [99]. In this framework, solute removal targets are interpreted and implemented in the context of the overall goals and clinical status of the person on PD [99]. In alignment with this statement, the International Society for Peritoneal Dialysis (ISPD) published new practice points for prescribing high-quality, goal-directed PD in 2020, including specific practice points for children on PD [8, 9, 91]. These documents suggest that modifications to the PD prescription should be based on regular assessment of clinical well-being, volume status (see below) and other laboratory parameters, in addition to Kt/V_{urea}, with a minimum target total weekly Kt/V_{urea} of 1.7 [9]. The guidance document specifically states that children on PD with $Kt/V_{urea} < 1.7$ should not have their PD prescription modified for the sole purpose of achieving the target, if close and repeated assessment of clinical and laboratory parameters suggest that the child is otherwise doing well [9].

The 2020 ISPD guidance document for children on PD also suggests that the PD prescription be adjusted with the goal of achieving a normal serum phosphate level [9]. Because phosphate clearance is related to contact time between dialysate and the peritoneal membrane, optimizing the long daytime exchange is suggested to enhance phosphate removal [100]. It is recognized, however, that phosphate control cannot be achieved with dialytic clearance alone and dietary restriction and phosphate binders are required in most children on peritoneal dialysis [9].

Fluid Removal

Cardiovascular disease, as manifested by hypertension and left ventricular hypertrophy, is unfortunately quite common in children on dialysis, and fluid overload is a major contributor to both [4, 5, 61, 87, 101–104]. PD guidelines have therefore consistently emphasized the importance of adjusting the dialysis prescription to provide adequate salt and water removal [9, 79, 105].

Routine assessments of fluid status should be included in the care of children on PD. Casual blood pressure should be monitored, both in the clinic and at home, and ambulatory blood pressure monitoring may be performed to more accurately assess blood pressure and detect masked hypertension [106]. Central to the evaluation of fluid status is assessing the "dry" body weight of the child on PD, which should be performed routinely. However, determination of fluid overload may be inaccurate when based on clinical assessment alone and is further complicated by the expected weight gain in the growing child. Bioimpedance, if available, may be used as a component of the assessment of fluid status, and a recent study demonstrated that multifrequency whole-body bioimpedance spectroscopy successfully quantified total body water and acute changes of extracellular and intracellular water in children with chronic kidney failure, including those on dialysis [9, 107–109]. Data from the IPPN found that anemia tended to be associated with characteristics of the patient with fluid overload, including low urine output, high ultrafiltration requirements, high transport status on PET, hypertension, and left ventricular hypertrophy [101]. In addition, serum albumin and hemoglobin levels were closely associated, suggesting that fluid overload could result in dilution of both markers [101]. These findings led the authors to speculate that ESA-resistant anemia and hypoalbuminemia may be indicators of "occult" fluid overload in children on PD.

Adjustment to the dialysis prescription should, in turn, be made to achieve "dry weight" and blood pressure control. Efforts to optimize ultrafiltration while avoiding exposure to high glucose containing solutions include the use of icodextrin-containing dialysate for an extended daytime exchange, modifying dwell time using the APEX time, and potentially the use of adapted PD, as discussed previously [9, 26, 69, 110].

The amount of sodium removal required will depend on salt intake. Infants have very low sodium intake from formula or breast milk, and may have significant urinary losses of sodium associated with underlying congenital anomalies of the kidney and urinary tract. As a result, additional sodium losses from dialysis may result in hyponatremia, hypovolemia and hypotension. Therefore, infants on PD often require sodium supplementation [111]. On the other hand, older children and adolescents on PD are typically salt overloaded. In these children, the sodium gap, defined as the difference between the calculated theoretical sodium removal (plasma sodium concentration multiplied by ultrafiltration volume) and the amount actually removed (dialysate sodium concentration multiplied by ultrafiltration volume), is positive, reflecting inadequate sodium removal [112]. Most commercially available PD solutions have a sodium concentration of 132–134 mmol/L, just slightly lower than the concentration in normal serum. Studies of PD solutions containing 115–126 mmol/L sodium in adults on PD have shown increased sodium removal and a lower sodium gap, with associated improvements in blood pressure and fluid status [112–114]. However, very low sodium solutions require slightly higher glucose concentrations to maintain osmolarity and therefore may increase overall glucose exposure [112]. There are currently no studies of the impact of lower sodium-containing dialysis solutions on sodium and fluid balance in children or adolescents on PD.

Peritoneal Dialysis Access

Catheter Configuration

Successful PD requires a catheter that provides reliable, rapid dialysate flow rates without leaks or infections. The first description of placement of an indwelling catheter for maintenance PDs was in 1968 by Tenckhoff, and the Tenckhoff catheter continues to be the most commonly used

PD access in children [3, 115, 116]. Despite significant improvements in catheter design, however, the catheter has continued to be a significant barrier to successful PD because of catheter-related complications. A recent analysis of 824 incident PD catheters in the IPPN revealed that more than 20% required revision and 83% of those revisions occurred in the first year after placement [116]. Need for access revision increased the risk of peritoneal dialysis technique failure or death [116]. This section will review the currently available catheter configurations and placement techniques. Associations between the various configurations and risk for catheter-associated infectious and non-infectious complications, including catheter malfunction, are discussed later in this chapter.

The most commonly used catheters for maintenance PD are constructed of soft material, such as silicone rubber or polyurethane. There are a wide variety of catheter configurations available, which differ in their intraperitoneal configurations (curled or straight), the number of Dacron cuffs (one or two) and the subcutaneous tunnel configuration (straight or "swan-neck"). Figure 65.5 shows the most common combinations of these configurations [117]. Table 65.3 reveals the percentage of catheters with the various configurations in children on PD from large national and international collaborative projects: IPPN, the North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS), and the Standardizing Care to Improve Outcomes in Pediatric End stage kidney disease (SCOPE) collaborative [3, 115, 116]. These data demonstrate that a curled intraperitoneal configuration is most commonly used in children on PD [3, 115, 116].

The next catheter characteristic to consider is the number of Dacron cuffs on the catheter. If a single cuff catheter is used, it is generally recommended that the cuff be positioned between the rectus sheaths in the rectus muscle, and not be located in a superficial position. The addition of a second cuff was prompted by the potential to better secure the catheter and reduce migration of bacteria into the peritoneal cavity. Early data in children on PD demonstrated a lower incidence of infection with catheters having two cuffs, rather than one [3]. Based on these data, current guidelines recommend use of a 2-cuff PD catheter in children, except possibly in the very small infant in whom it may not be technically feasible [118]. Accordingly, the percentage of children on PD with 2-cuff catheters has increased, from roughly half of children in the NAPRTCS report from 2011, to more than 70% and 80% in recent reports from SCOPE and IPPN, respectively (Table 65.3) [3, 115, 116]. If two cuffs are used, the second cuff should be located at least 2.0 cm from the exit site to reduce the risk for cuff extrusion [117, 119]. If cuff extrusion occurs, prompt

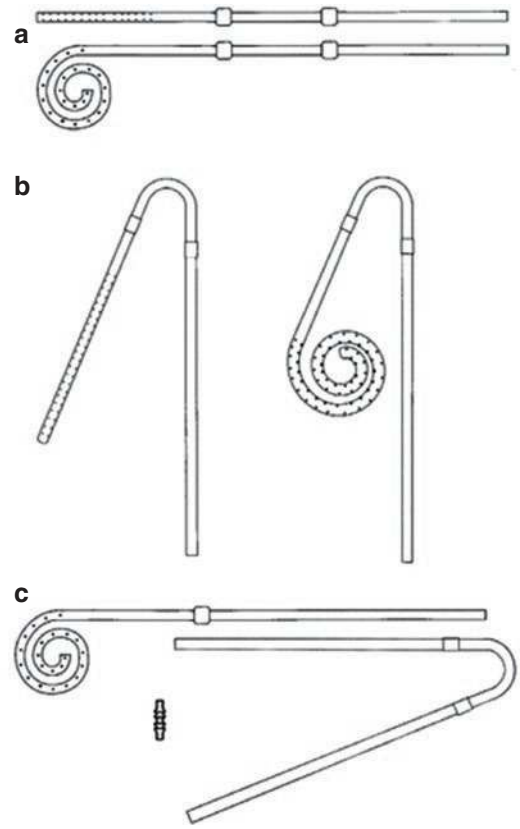

Fig. 65.5 Commonly used peritoneal catheters. (**a**) Catheter with straight tunnel segment, 2 cuffs, and straight or coiled intraperitoneal segment. (**b**) Catheter with preformed arc tunnel segment ("swan neck"), 2 cuffs, and straight or coiled intraperitoneal segment. (**c**) Extended catheter with 1-cuff, coiled-tip abdominal catheter, 2-cuff extension catheter with swan neck. Catheters with a straight tunnel segment are also available with a single cuff. (From [117], with permission)

Table 65.3 Catheter configurations from national and international collaborative registries and projects in children on maintenance peritoneal dialysis

Catheter configuration	NAPRTCS [3]	SCOPE [115]	IPPN [116]
	N (%)[a]	N (%)[a]	N (%)[a]
Number of catheters	4687 (100%)	1201 (100%)	2453 (100%)
Intraperitoneal segment			
Tenckhoff Curled	2909 (62.1%)	1070 (89.1%)	1681 (68.5%)
Tenckhoff Straight	1213 (25.9%)	66 (5.5%)	673 (27.4%)
Cuffs			
One	2375 (50.7%)	264 (22.0%)	346 (13.7%)
Two	2124 (45.3%)	873 (72.7%)	2117 (86.3%)
Tunnel			
Swan neck	1590 (33.9%)	793 (66.0%)	1542 (62.9%)
Straight	2895 (61.8%)	313 (26.1%)	911 (37.1%)
Exit-site orientation			
Up	564 (12.0%)	52 (4.3%)	346 (14.1%)
Down	1537 (32.8%)	613 (51.0%)	1299 (53.0%)
Lateral	1816 (16.4%)	459 (38.2%)	808 (32.9%)

NAPRTCS North American Pediatric Renal Trials and Collaborative Studies, *SCOPE* Standardizing Care to Improve Outcomes in Pediatric End Stage Kidney Disease, *IPPN* International Pediatric Peritoneal Dialysis Network
[a]Percentages may not add to 100% due to missing/other

shaving of the cuff off the catheter has been advocated to reduce infection risk [120, 121].

The shape of the extraperitoneal portion, or tunnel, of the catheter can be straight or have a preformed angle ("swan neck"), in which there is an inverted U-shape arc (170–180°) between the deep and the superficial cuffs (Fig. 65.5). The purpose of the catheter arc is to allow the catheter to exit the skin in a downward pointing direction, which may be associated with a decreased likelihood for the accumulation of dirt and debris within the catheter tunnel which, in turn, may reduce the development of a tunnel infection/peritonitis (see Infectious Complications) [3, 122]. In addition, the swan neck configuration allows the distal end of the catheter to enter the peritoneal cavity in an unstressed condition (i.e. without too much torque because of the synthetic material's memory), thereby decreasing the chance for its migration out of the pelvis, and the associated risk for impaired drainage [123, 124]. Since its introduction, the use of swan neck catheters has been increasing in children on PD and is now placed in the majority (Table 65.3) [3, 115, 116].

A modification of the swan neck catheter is the swan neck presternal catheter, which has a very long subcutaneous portion (Fig. 65.5). This catheter has been utilized when it is necessary to make the exit-site remote from the abdomen, such as in infants and children with incontinence, intestinal stomas, and suprapubic catheters, and the catheter exit-site is typically located in the anterior chest wall [125–127]. However, infants with complex congenital anomalies often have minimal subcutaneous tissue over the chest, which makes cuff erosion more likely in that location. One suggested approach to this problem is to place the two cuffs below the costal margin and then have the catheter exit high on the chest wall [126]. Conversely, a single cuff catheter may be used.

Preoperative Evaluation and Preparation

Careful preoperative evaluation is required for all children and adolescents prior to PD catheter placement. The preoperative evaluation should include screening and treatment of constipation, which is common in children with kidney failure and has consistently been associated with an increased risk for post placement PD catheter migration and malfunction [128]. The preoperative physical examination should include evaluation for the presence of hernias. The incidence of hernias is inversely proportional to age, with an

overall frequency of 8.0–57.0% [129–132]. The highest frequency of inguinal hernias occurs in the first year of life; they are often bilateral and all require surgical correction. Umbilical hernias can worsen in a child on PD as a result of the increase in intra-abdominal pressure generated by instillation of dialysis solution (see Non-infectious complications). As a result, some have advocated peritoneography or laparoscopic inspection for hernias at the time of catheter placement [130]. If detected, the hernias can then be fixed at the same time the PD catheter is inserted [133, 134].

A critical portion of the pre-catheter assessment is deciding upon the most appropriate location of the exit-site. In infants, the exit-site of the catheter needs to be outside of the diaper area to help prevent contamination. In older children, it should be either above or below the beltline. The presence of a vesicostomy, ureterostomy, colostomy or gastrostomy will also influence the preferred exit-site location. The exit-site must be planned so that it is either on the opposite side of the abdomen from any stoma site or, as stated previously, the exit-site may be placed on the chest to increase the distance from any stoma.

Catheter Placement Technique

Since Moncrief and Popovich first reported on the use of CAPD, there have been a number of modifications of the technique for the implantation of the PD catheter [135]. The two most common PD catheter insertion techniques are open and laparoscopic. Although there are no randomized trials in children comparing outcomes in PD catheters placed using these two approaches, several case series report excellent outcomes with the laparoscopic approach, including excellent revision free survival and a lower incidence of catheter flow problems [117, 136–138]. SCOPE data reveals that more than 65% of catheters in the collaborative were placed using a laparoscopic procedure, with no statistically significant difference in placement technique (open versus laparoscopic) between children with and without peritonitis in the first 60 day after catheter placement [115].

Infectious Complications

PD-associated infections include PD catheter-related infections, i.e. infection at the catheter exit-site and/or the subcutaneous tunnel, and peritonitis. Infectious complications remain the most significant cause of morbidity and PD technique failure in children on maintenance PD [2, 3, 139–141]. In addition, infection is a leading cause of death in children on PD [2, 3, 141]. Analyses of data from large pediatric dialysis registries have revealed associations between many factors and the risk for PD-related infections in children on PD. Recognition of these risk factors is important, as they may prompt modification of care practices, which, in turn, may lower infection rates as well as the rates of patient morbidity and mortality.

Risk Factors and Prevention

Patient Age

Data from collaborative registries have consistently identified young age at dialysis initiation, and specifically age less than 2 years, as a risk factor for peritonitis [3, 122, 142–144]. It seems intuitive that the relatively close proximity of the PD catheter to the diaper region or urinary or gastrointestinal ostomy sites in a small infant would increase the risk for bacterial contamination and subsequent infection. As stated previously, efforts to maximize the distance between the catheter exit-site and the diaper area and stomas are important to decrease the risk for infection [125, 145].

PD Catheter Design, Insertion and Post-operative Exit-Site Care

As discussed previously, early studies of data from children on PD suggested a higher incidence of infection and a higher risk for relapsing peritonitis with a one cuff rather than a two cuff catheter, and current guidelines recommend a catheter with two cuffs in children on maintenance PD [3, 118, 146]. However, more recently the SCOPE collaborative has failed to show any relationship between the number of catheter cuffs

and the development of either exit-site/tunnel infections or peritonitis [122, 147]. Data in adults on PD suggest that benefit of a second cuff for infection prevention may have been reduced by widespread adoption of application of antibiotics at the catheter exit-site [117, 148].

While some studies in adults have found the use of the swan neck catheter to be associated with less frequent exit-site/tunnel infections, other studies have been unable to confirm these results [149–151]. As stated previously, one advantage of the swan neck catheter is that it allows a downward, rather than upward, pointing exit-site. Data from NAPRTCS has consistently identified an upward facing exit-site as a risk factor for peritonitis, a finding confirmed by a recent analysis of SCOPE data [3, 122]. Accordingly, current guidelines for children on PD recommend that the exit-site orientation be in the downward or lateral position [118].

Efforts to minimize the risk for peritonitis at the time of catheter placement include the provision of antibiotics prior to surgical incision [118, 152, 153]. Although vancomycin may be slightly more effective than a first-generation cephalosporin in the prevention of post-operative peritonitis, use of the latter is recommended because of concern for the generation of vancomycin resistance [118, 153–155]. The ultimate choice of antibiotic for perioperative prophylaxis should be influenced by the PD unit's antibiotic susceptibility patterns [118, 154]. Current guidelines also recommend that while securing the newly inserted catheter and minimizing movement at the exit site is important, sutures should not be placed at the catheter exit-site at the time of surgical placement, as they may increase risk of bacterial colonization and subsequent infection [118].

In the immediate post-operative period, PD catheter and exit-site care are aimed at optimizing healing and minimizing bacterial colonization [156]. Current guidelines suggest that the sterile dressing placed in the operating room following PD catheter placement remain in place for at least 7 days, and subsequent dressing changes should be performed by trained staff, using aseptic technique, no more frequently than weekly until the exit-site is healed [118, 157]. More frequent dressing changes should be performed only if the dressing becomes loose, damp, or soiled [118]. The catheter should be immobilized to optimize healing and minimize trauma [118, 158]. It is generally recommended that initiation of dialysis be delayed for at least 2 weeks following catheter placement to minimize the risk of leak at the peritoneal insertion site, although exit-site healing may take as long as 6 weeks [156–158]. In support of this, an analysis of SCOPE data demonstrated an association between use of the PD catheter for dialysis within 14 days of placement and an increased risk for early peritonitis, defined as peritonitis occurring within the first 60 days following catheter insertion [115].

Training

Because PD is a home dialysis therapy, appropriate training of the child with kidney failure and caregivers is essential to minimize the risk for infection. Unfortunately, there are no randomized controlled trials to evaluate the relationship between various training elements or the training process itself and outcomes [159–161]. Several observational studies have shown associations between shorter training time (<15 h), training in the 10 days after catheter insertion and small center size with an increased risk for peritonitis [159–164]. Current guidelines for children on PD suggest that training should include the use of a formalized teaching program that has clear objectives and criteria, with incorporation of adult learning principles [118, 159]. The training should be performed by an experienced PD nurse with pediatric training and should include core topics, including those related to infection prevention such as hand hygiene, aseptic technique, exit-site care and appropriate treatment for contamination [118, 159]. It is suggested that PD training should include no more than one child/family simultaneously [118, 159]. A syllabus for teaching PD to patients and caregivers has been published by the ISPD, and includes a checklist for PD assessment and another for PD training [165]. It remains to be determined if widespread use of this syllabus and the associated tools leads to a decrease in infection rates.

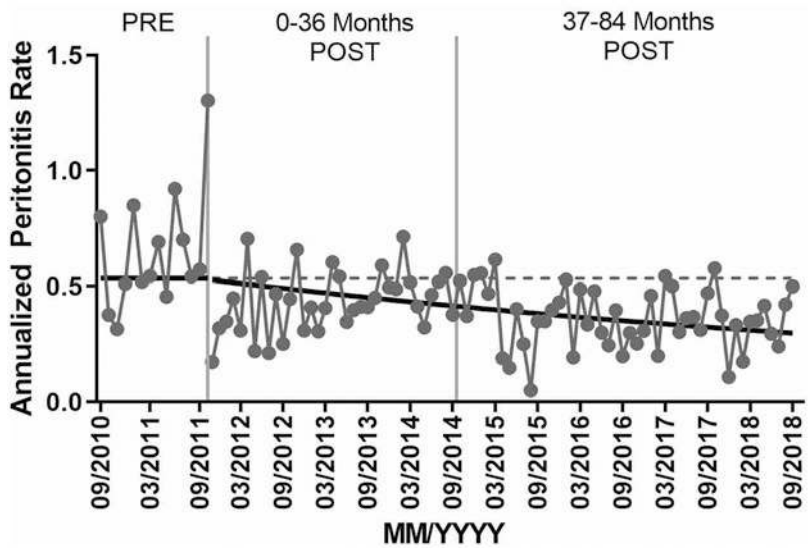

Fig. 65.6 Average monthly peritonitis rates, expressed as annualized rates, among 19 pediatric dialysis centers in the United States participating in the Standardizing Care to Improve Outcomes in Pediatric End Stage Kidney Disease (SCOPE) Collaborative from collaborative launch on October 1, 2011 through September 30, 2018. Differences between peritonitis rates in the 13 months prior to launch (pre-launch period) and the post-launch period were modeled using Generalized Linear Mixed Models techniques and revealed that the decrease in infection rate observed in the first 36 months persisted and there was a significant reduction in the average monthly peritonitis rates from 0.53 (95% CI 0.37, 0.70,) pre-launch to 0.30 infections per patient year (95% CI 0.23, 0.43) at 84 months post launch, $p < 0.001$). From [170], with permission)

Current guidelines suggest periodic retraining of the persons performing PD in the home, particularly after a peritonitis episode [118, 159]. The Trial on Education and Clinical outcomes for Home PD patients (TEACH), compared PD-related infections in adults on PD randomized to receive home visits for retraining every 1–3 months over a 24-month period compared to no re-training [166]. The study failed to demonstrate a significant difference in peritonitis rates between the two groups, although a sub-analysis demonstrated a significantly lower risk for first peritonitis episodes in patients >60 years of age who received frequent home visits [166]. The SCOPE collaborative includes a "follow up" care bundle, which requires a review of key aspects of hand hygiene, exit-site care, and aseptic technique at each monthly follow up visit in the clinic, redemonstration of competency with these procedures every 6 months, regular scoring of the PD catheter exit-site and treatment of touch contaminations according to ISPD guidelines [118, 167, 168]. SCOPE centers demonstrated a significant increase in compliance with this care bundle over the first 3 years of the collaborative, accompanied by a significant reduction in peritonitis rates [169]. A more recent analysis demonstrated that centers participating in the collaborative for 7 years were able to achieve and then maintain high level compliance with the follow up bundle and had continued reduction in center peritonitis rates over the collaborative's entire post-launch period (Fig. 65.6) [170]. An analysis of SCOPE data at the patient level also demonstrated that compliance with the follow up care bundle was significantly associated with a lower rate of peritonitis [122]. These data suggest that in addition to comprehensive training at the initiation of dialysis, ongoing review with regular testing of competency of PD catheter care and the dialysis procedure may minimize the risk for peritonitis.

Chronic Exit-Site Care

Once the catheter exit-site has healed, regular exit-site care is vital to minimize the risk for PD catheter-related infection. Current guidelines rec-

ommend regular cleansing of the exit-site with a sterile antiseptic solution and sterile gauze [118, 171]. Several cleansing agents are available and none has been shown to be superior in the prevention of catheter-related infection [118, 171]. In addition, there is no clear guidance for the optimal frequency of exit-site care, e.g. daily, every other day, or weekly [118, 171]. Not surprisingly, data from the International Pediatric Peritonitis Registry (IPPR) revealed significant variability in exit-site practices around the globe, including the frequency of exit-site care as well as the type of antiseptic agent used [172]. IPPR data also revealed that peritonitis due to *Pseudomonas* species was significantly more common at centers where exit-site care was performed more than twice weekly and where non-sterile cleansing agents (e.g. saline, soap) were used [172]. Among SCOPE participants, compliance with the specific recommendation to review exit-site care at each visit was associated with lower exit-site infection rates [147].

In addition to regular exit-site cleaning, current guidelines suggest application of a topical antibiotic during routine care, in an effort to minimize colonization of the exit-site with *Pseudomonas aeruginosa* (*P. aeruginosa)* and *Staphylococcus aureus (S. aureus),* both of which are widely accepted as risk factors for exit-site infection and subsequent peritonitis [118, 171, 173–176]. A number of observational studies, randomized controlled trials and meta-analyses have demonstrated that mupirocin applied to the skin around the exit-site reduces the risk for exit-site infections [118, 153, 171, 177–181]. However, there is concern that routine use of mupirocin may be associated with an increased risk for gram-negative infections and the emergence of mupirocin resistant *Staphylococcus* species [172, 182, 183]. Topical gentamicin is an alternative therapy, and a randomized trial in adults showed that daily application of gentamicin cream to the exit-site was not only effective in reducing exit-site infections caused by *Pseudomonas* species, but it was as effective as topical mupirocin in reducing *S. aureus* infections [180]. There are concerns, however, about the possible development of gentamicin-resistant organisms and an increased risk of fungal infections with this therapy.

Touch Contamination

Accidental contamination of the sterile portions of the PD catheter transfer set or dialysis tubing, or touch contamination, is a leading cause of peritonitis [122, 184, 185]. Current guidelines recommend that contamination prior to the infusion of dialysis fluid into the peritoneal cavity be treated with a sterile transfer set change alone, without antibiotics [118]. If the contaminating event occurs after dialysate has been infused into the peritoneal cavity, both a sterile transfer set change and antibiotic prophylaxis is recommended [118, 163]. Intraperitoneal administration of a first-generation cephalosporin for 1–3 days is typically recommended, unless the patient has a history of methicillin-resistant *S. aureus* (MRSA), in which case a glycopeptide (vancomycin or teicoplanin) should be used [118, 163]. Gram-negative coverage may be appropriate if the contamination may have included enteric organisms, e.g. from stool in a diapered infant [118]. An effluent sample should be obtained for cell count, differential and culture prior to delivery of antibiotics, if possible, and culture results and susceptibility testing used to guide any subsequent antibiotic usage [118].

Ostomies

As stated previously, ostomy sites, including gastrostomy, ureterostomy, nephrostomy and colostomy, may increase the risk of bacterial contamination of an adjacent PD catheter. In fact, data from the IPPN demonstrated an increased risk for peritonitis in the presence of any ostomy [186]. Data in children on PD have not revealed a consistent association between presence of a gastrostomy tube (GT) and risk for infection, including fungal infection; however, among infants on PD enrolled in SCOPE, placement of a GT after PD catheter placement was associated with increased risk for bacterial peritonitis [122, 144, 185, 187–190]. Although data on the subject is limited, current guidelines suggest that an open procedure should be used to place a GT in patients who are already receiving PD, while either open or laparoscopic placement may be used if the gastrostomy is placed prior to initiating PD. [118, 191]. Prophylactic

antibiotics, typically a first-generation cephalosporin, and antifungal therapy should be provided during gastrostomy tube placement in a patient with a PD catheter [118].

While an analysis of SCOPE data did not find an association between the presence of a colostomy and the risk for peritonitis in multivariable analysis, a recent study from IPPN revealed a significantly higher rate of peritonitis among patients with a colostomy [122, 192]. The number of children on PD with colostomies in these analyses was relatively small at 14 and 20, respectively [122, 192].

Antibiotic and Antifungal Prophylaxis

Although fungal peritonitis is relatively uncommon in children on PD, it is associated with an increased risk for significant morbidity and mortality [190, 193–195]. Observational data suggests that risk factors for fungal peritonitis include prior treatment with antibiotics, recurrent peritonitis, and immunosuppression [189, 190, 195–199]. Antifungal prophylaxis with either oral nystatin or fluconazole is currently recommended whenever antibiotics are administered to children on PD, although data from SCOPE reveal that this practice is not uniformly implemented, particularly when antibiotics are prescribed for infections other than bacterial peritonitis [118, 190, 200–204].

Prophylactic antibiotic and antifungal therapy should also be provided when children on PD undergo certain procedures, including gastrostomy tube placement, as previously discussed, as well as invasive dental, gastrointestinal or genitourinary procedures [118, 205, 206].

Other Factors

The risk factors listed in this section were largely derived from data collected by observational registries and quality improvement collaboratives that identified associations between various factors and risk for infection among a cohort of children on PD. There are clearly many other factors that may impact the risk for infection in individual children. The dialysis unit should perform a formal review, or apparent cause analysis, of each infection in search of causation [118, 162, 163]. This review should include nurses and physicians at a minimum. Inclusion of the child on PD and their caregivers/family, social worker, infection preventionist and infectious disease specialist is encouraged. Identification of causation will allow appropriate intervention for the individual, and potentially other children on PD in the unit.

Catheter-Related Infections

Infections of the PD catheter include exit-site and tunnel infections. PD catheter-related infections are associated with an increased risk for peritonitis. However, even without subsequent peritonitis, exit-site and tunnel infections require exposure to antibiotics with the subsequent risk for fungal infection and drug resistant organisms, both of which may require catheter removal [147, 207–209]. Catheter-related infections also carry a high risk of recurrence. In a Japanese multicenter study, 15% of all infections and 40% of MRSA infections relapsed [210].

Routine use of an objective scoring system is recommended to monitor the status of the catheter exit site and to optimize the diagnostic accuracy of exit-site infections. The pediatric Exit Site Score (ESS) considers pericatheter swelling, crust, redness, tenderness and secretion with a score range from 0 to 10 (Table 65.4) [118, 168]. An exit site infection is diagnosed by an ESS >1 in the presence of a pathogenic organism, or >3 irrespective of culture results. A tunnel infection is defined by an ESS >5 [118]. Sonographic examination may help to evaluate the extent of infection along the catheter [211, 212]. Data from SCOPE, which requires scoring of the exit-site at every monthly visit, revealed that an ESS of anything more than

Table 65.4 Catheter exit-site scoring system. From [118], with permission [168]

	0 Points	1 Point	2 Points
Swelling	No	Exit only (<0.5 cm)	Including part of or entire tunnel
Crust	No	<0.5 cm	>0.5 cm
Redness	No	<0.5 cm	>0.5 cm
Pain on pressure	No	Slight	Severe
Secretion	No	Serous	Purulent

zero is associated with an increased risk for an exit-site infection in the following month [147]. However, significant variability in exit-site scoring using this tool has been noted at SCOPE centers, and the collaborative is currently modifying the tool in an effort to promote more consistent scoring and, therefore, greater uniformity in the diagnosis of exit-site infections.

Uncomplicated catheter exit-site infections can be treated with oral antibiotics according to culture results and susceptibilities [118]. Empiric therapy for tunnel infections may be via the oral route; however, intraperitoneal or intravenous antibiotics are often indicated, particularly if signs of severe infection and/or a history of *S. aureus* or *P. aeruginosa* are present. Infections with gram-positive bacteria should be treated with a first-generation cephalosporin or a penicillinase-resistant penicillin. Intraperitoneal or intravenous glycopeptide therapy should be reserved for cases with proven MRSA infection [118]. The use of oral ciprofloxacin for infections due to *P. aeruginosa* had previously been recommended, with the addition of a second antibiotic such as cefepime, piperacillin, or a carbapenem, if resolution of the infection is slow, or there is recurrence [118]. However, recent reports from observational studies have suggested an increased risk for aortic aneurysm or dissection associated with fluoroquinolone use, particularly in the setting of other risk factors such as hypertension, which led the United States' Food and Drug Administration to issue a safety announcement (https://www.fda.gov/Drugs/DrugSafety/ucm628753.htm) [213–216].

Adjunctive therapy for exit-site/tunnel infections should include daily or twice daily dressing changes, and cautious removal of exuberant granulomatous tissue ("proud flesh") with silver nitrate.

Antibiotic treatment should be administered for a minimum of 2 weeks and for at least 7 days beyond complete resolution of the infection. Treatment for at least 3 weeks is recommended for infections caused by *S. aureus* or *P. aeruginosa*. Extension of antibiotic therapy beyond 4 weeks should be avoided. In case of persistence of symptoms or recurrence after discontinuation of antibiotic treatment, the catheter should be removed and replaced [118]. Surgical shaving of the external cuff may be an alternative to catheter removal for treatment of a persistent exit-site infection if the inner cuff is not involved [121, 217].

Peritonitis

The diagnosis of peritonitis should be considered in any child on PD with abdominal pain and/or cloudy PD effluent, with an effluent white blood cell count of greater than 100/mm^3 and at least 50% polymorphonuclear neutrophils (PMN) confirming the diagnosis [118]. For children on automated PD, the effluent white blood cell count should be obtained from an exchange with the dialysis solution instilled for at least 1–2 h [118]. In this setting, the presence of 50% or more PMN is highly suggestive of peritonitis when the clinical features of peritonitis are present, even if the total white blood cell count is below 100/mm^3. Bacterial growth in the effluent confirms the diagnosis, whereas a negative culture does not rule out a bacterial etiology. The efficiency of microbiological diagnostics can be maximized by incubating the effluent in 3–4 blood culture bottles, and by centrifuging large effluent volumes. A culture-negative rate of less than 10% should be aimed for according to consensus guidelines [218]. However, international surveys have shown that this target is far from being achieved by pediatric PD centers around the globe [172]. Data from the SCOPE collaborative revealed an overall culture negative rate of 26.6% and significant variability in the culture negative rate and culture techniques among centers, although no associations between practices and culture negative rate could be elucidated [219]. In response to these data, the SCOPE collaborative has implemented a standardized PD effluent culture bundle and has already demonstrated a decrease in the both the culture negative rate and the percentage of cultures that are negative per month (unpublished finding). Among culture-positive cases, IPPN discovered wide regional variability in causative organisms, but in general gram-positive organisms predominate, with coagulase-negative *Staphylococci* and *S. aureus* most frequently cultured [122, 172].

Empiric intraperitoneal antibiotic treatment should be initiated as soon as the diagnosis of peritonitis is considered, and include coverage for both gram-positive and gram-negative organisms

[118]. Monotherapy with cefepime may be considered for empiric therapy, while a first-generation cephalosporin or a glycopeptide combined with ceftazidime or an aminoglycoside may be used if cefepime is not available [118]. However, global peritonitis data from children on PD reveals not only significant variability in the causative organisms, but also associated antibiotic susceptibilities, prompting the additional recommendation that empiric coverage be guided by the center-specific antibiotic susceptibility pattern [172]. Specifically, the empiric use of glycopeptides should be restricted to centers where the rate of MRSA exceeds 10%. Antibiotic therapy should be modified based on culture and antibiotic susceptibility results. Dosing recommendations are given in Table 65.5 [118]. If cultures remain sterile and signs and symptoms of peritonitis are

Table 65.5 Dosing recommendations for anti-infective agents in children with peritoneal dialysis catheter-related peritonitis. Administration should be via intraperitoneal route unless specified otherwise. Intermittent doses should be applied once daily unless specified otherwise. From [118] with permission

	Continuous therapy[a]		Intermittent therapy
	Loading dose	Maintenance dose	
Aminoglycosides[b]			
Gentamicin	8 mg/L	4 mg/L	
Netilmicin	8 mg/L	4 mg/L	Anuric: 0.6 mg/kg Non-anuric: 0.75 mg/kg
Tobramycin	8 mg/L	4 mg/L	
Amikacin	25 mg/L	12 mg/L	
Cephalosporins			
Cefazolin	500 mg/L	125 mg/L	20 mg/kg
Cefepime	500 mg/L	125 mg/L	15 mg/kg
Cefotaxime	500 mg/L	250 mg/L	30 mg/kg
Ceftazidime	500 mg/L	125 mg/L	20 mg/kg
Glycopeptides[c]			
Vancomycin	1000 mg/L	25 mg/L	30 mg/kg; Repeat dosing 15 mg/kg q 3–5 days
Teicoplanin	400 mg/L	20 mg/L	15 mg/kg q 5–7 days
Penicillins[b]			
Ampicillin	—	125 mg/L	—
Quinolones			
Ciprofloxacin	50 mg/L	25 mg/L	—
Others			
Aztreonam	1000 mg/L	250 mg/L	—
Clindamycin	300 mg/L	150 mg/L	—
Imipenem/Cilastin	250 mg/L	50 mg/L	—
Oral			
Linezolid	<5 years.: 30 mg/kg/day divided TID; 5–11 years: 20 mg/kg/day divided BID; ≥12 years 600 mg/dose BID		
Metronidazole	30 mg/kg/day divided TID (max daily dose 1.2 g)		
Rifampin	10–20 mg/kg/day divided BID (max daily dose 600 mg)		
Antifungals			
Fluconazole	6–12 mg/kg IP, IV or PO q 24–48 h (max daily dose 400 mg)		
Caspofungin	IV only: initial dose 70 mg/m^2 on day 1 (max daily dose 70 mg); Subsequent dosing 50 mg/m^2 daily (max daily dose 50 mg)		

[a] For continuous therapy, the exchange with the loading dose of antibiotics should dwell for 3–6 h, followed by the use of the maintenance dose for all subsequent exchanges

[b] Aminoglycosides and penicillins should not be mixed in dialysis fluid because of the potential for inactivation

[c] Accelerated glycopeptide elimination may occur in patients with residual renal function. If intermittent therapy is used in this setting, the second dose of antibiotic should be time-based on a blood level obtained 2–4 days after the initial dose. Redosing should occur when the blood level is <15 mg/L for vancomycin, or 8 mg/L for teicoplanin. Intermittent therapy is not recommended for patients with residual renal function unless serum drug levels can be monitored in a timely manner

improved, empiric antibiotic therapy should be continued for 2 weeks with the exception of aminoglycosides, which should be discontinued after 72 h in culture-negative peritonitis [118].

General adjunctive measures include the reduction of the peritoneal fill volume during the initial 24–48 h of therapy in children with significant abdominal discomfort, and intraperitoneal administration of 500–1000 IU/L heparin until complete resolution of dialysate cloudiness [118].

Most children with PD-associated peritonitis achieve clinical improvement within two to three days following the initiation of antibiotic treatment [168, 185]. The initial treatment response is predictive of the functional recovery of PD and the risk of peritonitis relapse [142, 146]. Prolonged attempts to treat refractory peritonitis and to "save the catheter" should be avoided to minimize permanent injury to the peritoneal membrane [220]. In children who fail to respond clinically within 72 h of initiation of appropriate antibiotic therapy, repeat effluent cell count, differential and culture should be performed and potential sources of persistent infection should be sought. Treatment failure in peritonitis with *S. aureus* or *P. aeruginosa* points to a concomitant tunnel infection and requires catheter removal [118]. Treatment-resistant peritonitis with anaerobic bacteria or multiple gram-negative organisms is suspicious of intraperitoneal pathology (*e.g.,* ruptured appendix). Catheter removal is also recommended for any bacterial or culture-negative peritonitis that fails to resolve within 5 days of appropriate antibiotic treatment [118, 221, 222].

Ten to twenty percent of peritonitis episodes recur within 4 weeks of completion of antibiotic treatment with the same bacterial strain as indicated by identical antibiotic susceptibilities ('relapsing peritonitis') [146, 168, 185]. Repeated bouts of peritonitis are a risk factor for incomplete functional recovery and PD technique failure [146]. In relapsing peritonitis, empiric therapy should be reinitiated using an antibiotic combination covering the susceptibilities of the previous causative organism. Slime-forming bacteria can survive antibiotic therapy in a biofilm matrix or fibrinous adhesions on catheter surfaces. Accordingly, intraluminal fibrinolytic therapy may expose sequestered bacteria and render them susceptible to antibiotic activity. Local instillation of fibrinolytic agents (urokinase or recombinant tissue plasminogen activator), followed by instillation of high-dose antibiotics, has been shown to be efficacious in preventing further peritonitis relapses [223–225]. Hence, intraluminal fibrinolytic therapy should be considered in patients with a first peritonitis relapse which is not explained by extraluminal pathology such as a tunnel infection or an intraabdominal abscess. If a second relapse occurs, the catheter should be removed as soon as peritonitis is controlled by antibiotic therapy [118].

Fungal peritonitis is an infrequent but potentially serious complication of PD fraught with a high risk of PD technique failure and sometimes life-threatening, systemic infection [189, 190, 195]. Fungal infections represent 1–4% of all peritonitis episodes, although roughly 8% of peritonitis episodes reported to the SCOPE collaborative have been due to fungi [122, 185, 189, 190]. Treatment of fungal peritonitis consists of prompt catheter removal and appropriate antimycotic therapy [193, 226]. Fungi avidly grow on PD catheter surfaces, and resolution of infection is usually not possible as long as the catheter is in place. Fluconazole is the treatment of choice for most *Candida* species due to its excellent bioavailability and peritoneal penetration [227]. Alternative agents are echinocandins (e.g. caspofungin) for non-responding, non-albicans candida, and posaconazole or voriconazole for filamentous fungi such as Aspergillus [227]. Following catheter removal, effective antimycotic therapy should be administered for at least 2 weeks beyond complete resolution of clinical symptoms [118]. Reinitiation of PD following the treatment of fungal peritonitis in children has been successful [189].

Non-Infectious Complications

Non-infectious complications can result in significant morbidity, including the need to terminate PD [116, 228]. Non-infectious PD complications can be divided into catheter-related complications, and complications related to the dialysis procedure itself (Table 65.6) [229–231].

Table 65.6 Non-infectious complications of peritoneal dialysis [229–231]

Catheter related complications
Obstruction/reduced inflow or outflow
Migration of catheter out of pelvis
Catheter kinking
Catheter blockage
Fibrin
Blood clot
Omentum
Catheter compression
Stool/Constipation
Tumor or other intraabdominal mass
Peri-Catheter leak
Catheter cuff extrusion
Complications associated with the dialysis procedure
Related to increased intraperitoneal pressure
Subcutaneous leak
Gastroesophageal reflux and delayed gastric emptying
Abdominal and back pain
Hernia
Hydrothorax
Related to transfer of solutes during dialysis (electrolyte and metabolic derangements)
Hypokalemia
Hypo/hypermagnesemia
Hyperglycemia
Hyperinsulinemia
Hypertriglyceridemia
Related to exposure of peritoneal membrane to dialysis fluid
Membrane failure
Pancreatitis
Encapsulating peritoneal sclerosis

Catheter-Related Complications

Catheter-related complications include catheter malfunction, i.e. poor inflow and/or outflow, and leaks at the catheter exit-site. A recent analysis of data from the IPPN revealed a catheter revision rate of 1 per 83.2 patient-months, and the leading indication for revision was catheter malfunction, particularly in the first year after placement (Fig. 65.7) [116]. In that study, the need for access revision increased the risk of PD technique failure or death, and access dysfunction due to mechanical causes doubled the risk of technique failure compared with infectious causes [116]. The risk of access revision was associated with younger age, diagnosis of congenital anomalies of the kidney and urinary tract, coexisting ostomies, presence of a swan neck tunnel with curled intraperitoneal portion, and a high gross national income [116].

Catheter malfunction may be caused by obstruction from the omentum, kinking or migration of the catheter out of the pelvis, or blockage by fibrin or clots. Omentectomy at the time of catheter placement may reduce the risk of obstruction, and is practiced in most centers [133, 232, 233]. In practical terms, the omentectomy does not have to be complete. The remnant amount needs to be such that it cannot reach the distal catheter once it is positioned in the pelvis. However, one group of investigators, despite reporting a 20% decrease in the incidence of catheter blockage with omentectomy, calculated that eleven omentectomies would be required to prevent two omental PD catheter blockages. Therefore, the authors felt that nine patients would undergo an unnecessary omentectomy. In their hands, a secondary omentectomy was not difficult, resulting in their conclusion that omentectomies should only be carried out after a blockage occurs [232].

Migration of the catheter out of the pelvis can lead to poor dialysate inflow or outflow, as well as increased pain with dialysis. As mentioned previously, constipation is a risk factor for migration and should be monitored for and treated aggressively. Once migration has occurred, interventional radiology (IR) techniques may be used to reposition the catheter, with laparoscopic repositioning if IR repositioning fails [234]. For catheters that are occluded by fibrin or blood clot, installation of fibrinolytic agents can be very effective in restoring catheter flow [235–237].

Leaking of fluid from the peritoneal cavity through the PD catheter tunnel is a significant risk for the development of peritonitis. As previously discussed, delaying use of the PD catheter for routine dialysis for at least 14 days is advised to minimize the risk for leaks, and subsequent infection [115, 118]. The use of fibrin glue in the PD catheter tunnel has been reported to be both effective in treating established leaks and, when used at the time of catheter implantation, may help prevent the development of peritoneal leaks around catheters that are used soon after being placed [238, 239].

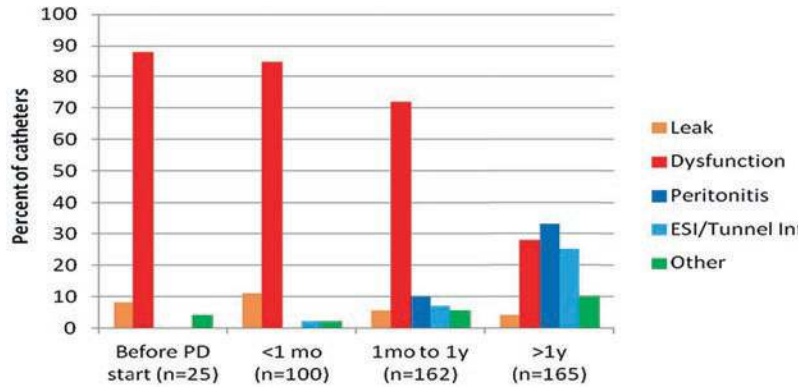

Fig. 65.7 Characterization of 452 access revisions by indication and time on peritoneal dialysis (PD) among children enrolled in the International Pediatric Peritoneal Dialysis Network (IPPN) Registry. ESI, exit site infection; tunnel inf, tunnel infection. (From [116], with permission)

Hemoperitoneum, or blood in the dialysate effluent, is common immediately after PD catheter placement and typically clears with dialysis exchanges. Heparin may be added to the dialysate to reduce the risk of clotting within the PD catheter. Strictly speaking, most cases of hemoperitoneum beyond the post-implantation period are not a complication of PD *per se*, but rather diagnosed because of the ability to visualize peritoneal fluid during the dialysis procedure. A common benign cause of hemoperitoneum in female adolescents and young adult women is menstruation. Blood may appear a few days prior to menstruation and arise from shedding of intraperitoneal endometrial tissue if endometriosis is present, or from the uterus in a retrograde fashion through the fallopian tubes [230, 231]. Hemoperitoneum can also be seen at the time of ovulation. Other causes of hemoperitoneum include trauma, bleeding disorders, anticoagulation therapy, and rupture of a hepatic, ovarian or renal cyst. Finally, bleeding into the peritoneal cavity may be associated with intraperitoneal calcifications, which may occur as a consequence of chronic kidney disease bone and mineral metabolism disorder, or in the setting of encapsulating sclerosing peritonitis (see below) [231].

Complications Related to the Dialysis Procedure

Complications related to the dialysis procedure can be divided into those due to the increased intraperitoneal pressure that arises with instillation of dialysis fluid into the peritoneal cavity, those occurring as a consequence of the transfer of solute between plasma and dialysate during the dialysis exchange (i.e. metabolic or electrolyte derangements), and those that are either directly related to or exacerbated by exposure of the peritoneum and other intra-abdominal organs to dialysis fluid (Table 65.6) [230, 231].

Complications Related to Increased Intraperitoneal Pressure

Since the efficacy of the dialysis procedure is dependent on the area of the peritoneal membrane in contact with the dialysis fluid, increasing this contact area by increasing the fill volume is a therapeutic aim. However, an increase in the fill volume is associated with an increase in the intraperitoneal pressure (IPP) [35]. Elevated IPP can lead not only to patient discomfort and intolerance of the dialysis procedure, but may also increase the risk of dialysis leaks, gastroesophageal reflux, hernia formation and hydrothorax [10, 33, 34]. While leaks at the catheter exit-site occur most frequently around the time of catheter placement, more subtle leaks may occur well after the catheter exit-site has healed [240]. These leaks typically present with accumulation of fluid in the subcutaneous tissue, weight gain, and peripheral and/or genital edema, and often resolve with reduction in fill volume, avoiding a daytime fill, or temporary cessation of PD. Complaints of back or abdominal pain and gastroesophageal reflux may be eased by efforts to lower the IPP, including a reduction of the fill volume, particularly during the day.

As stated previously, hernias are common in children and their frequency is inversely related to age. Ideally, hernias are identified and repaired at the time of PD catheter placement [133, 134, 241]. Hernias may develop after PD is initiated at the sites of surgical incisions or areas of anatomic weakness such as the umbilicus or the linea alba. Small hernias may be followed with careful monitoring for incarceration, with efforts to reduce IPP as described above. However, many hernias in children ultimately require surgical repair.

Hydrothorax in the patient on PD occurs when an elevated IPP causes fluid to enter the pleural space by way of a pleuroperitoneal leak, presumably at the site of a diaphragmatic defect [242]. This defect is almost always on the right side; the presence of the heart and pericardium may limit leak of fluid across the left hemidiaphragm. Hydrothorax usually presents with shortness of breath and chest discomfort and must be differentiated from congestive heart failure. In addition, other causes of transudative pleural effusion, including volume overload, should be ruled out. Diagnosis is typically made by measuring glucose in the fluid obtained by way of thoracocentesis, with an elevated pleural fluid glucose relative to serum glucose verifying the peritoneal dialysate origin of the fluid [231, 242]. Confirmatory tests can include CT peritoneography or a technetium scan, followed by serial imaging [243]. First line treatment of hydrothorax is transient cessation of PD, which may allow closure of the diaphragmatic defect [244]. If conservative therapy fails, chemical pleurodesis with tetracycline, talc or autologous blood may be successful [244, 245]. Other therapeutic options include thoracoscopic pleurodesis, and thoracoscopic or open diaphragmatic repair [231, 244].

Complications Related to Transfer of Solutes During the Dialysis Procedure

The bidirectional transfer of solutes between the plasma in peritoneal capillaries and the dialysate in the peritoneal cavity is the therapeutic goal of the PD procedure. However, the transfer of solutes cannot be precisely controlled and so certain electrolyte and metabolic derangements should be anticipated. The most common of these is hypokalemia, the result of potassium losses into the potassium-free dialysis solution. Liberalization of dietary intake will typically restore normal potassium, but enteral supplementation may be required, particularly in infants or young children on low potassium formulas. The possible association between hypokalemia and the risk for peritonitis should prompt attention to and correction of this issue.

Hypermagnesemia is relatively common in children with kidney failure secondary to reduced kidney clearance of magnesium. Magnesium concentrations in commercially available dialysis solutions range from 0.25–0.75 mmol/L. Elevated serum magnesium levels are typically seen with use of solutions containing 0.75 mmol/L magnesium and high magnesium levels may contribute to adynamic bone disease [246–248]. On the other hand, use of solutions containing both 0.5 mmol/L and 0.25 mmol/L magnesium has been associated with hypomagnesemia in adults on PD [246]. Given the lack of data available on magnesium homeostasis in children on PD, current recommendations suggest choosing a solution that allows maintenance of a high normal serum magnesium, i.e. 0.9–1.0 mmol/L, in this population [43].

Hyperglycemia, hyperinsulinemia and dyslipidemia are present even in the early stages of kidney failure in children [249, 250]. These conditions persist or worsen on dialysis [251–253]. The pathophysiologic mechanisms contributing to disturbances in glucose and lipid metabolism seen in children with kidney failure are quite complex, and beyond the scope of this chapter. It is important to recognize, however, that in people on PD, exposure to glucose-containing dialysis solutions provides a substantial glucose load, which induces insulin resistance and an atherogenic lipid profile [38, 229, 254]. Thus, PD may contribute to the development of disturbances of glucose and lipid metabolism, or exacerbate them if already present. These findings reinforce recommendations to minimize exposure to glucose by using the lowest dialysate glucose concentration possible, with the addition of icodextrin if required to maintain euvolemia [9, 43]. The pri-

mary therapeutic approach for dyslipidemia is lifestyle modifications, including nutrition and dietary counseling to address obesity if present. Although several pharmacologic therapies for dyslipidemia are available, given the lack of data on safety and efficacy of these agents in children, KDIGO guidelines suggest that statins or statin/ezetimibe combinations not be initiated in children less than 18 years of age with kidney failure, including those on maintenance dialysis [255].

Complications Related to Exposure of Peritoneal Membrane to Dialysis Fluid

Peritoneal membrane failure, or the inability of the membrane to provide adequate removal of fluid and/or solutes, is an important complication of PD as it typically necessitates conversion to hemodialysis. International and national registry data suggest that 4.2–8% of the children on PD in these large cohorts required transfer to HD due to membrane failure, and the percentage is as high as 27% in smaller series [3, 116, 256]. Severe, persistent or recurrent peritonitis is a significant contributor to membrane failure, but as previously discussed, an increasing body of experimental evidence suggests that exposure of the peritoneal membrane to PD solutions, and high concentrations of glucose in particular, is a predominant contributor to progressive fibrosis [41, 57, 257].

Pancreatitis in people on PD may be caused by the same precipitating factors as in people who are not on PD, such as infection, medications, hypercalcemia and hyperlipidemia. However, irritation from the peritoneal dialysis fluid and/or PD catheter has also been reported as a cause of pancreatitis in people on PD [258]. The presenting symptoms of abdominal pain, emesis and cloudy dialysis effluent may mimic peritonitis, and thus the diagnosis can be missed. The diagnosis should be considered in people on PD with sterile peritonitis, particularly if their symptoms do not improve. Most episodes may be treated conservatively, although recurrence with reintroduction of dialysate into the abdomen may prompt at least the temporary cessation of PD [231].

Encapsulating peritoneal sclerosis (EPS) is a rare, but extremely serious complication of PD defined by the ISPD as ‘a clinical syndrome continuously, intermittently or repeatedly presenting with symptoms of intestinal obstruction due to adhesions of a diffusely thickened peritoneum’ [259]. EPS has been reported in 0.7–3.3% of adult cohorts, with a mortality rate of 35–69% [259–262]. A 10-year survey of 1472 children on PD revealed a similar prevalence of EPS at 1.5% or 8.7 cases per 1000 patient-years on PD, but a lower mortality rate with 3 deaths among 22 cases after a median follow-up of 4.8 years [263]. Non-PD related risk factors for the development of EPS include previous intra-abdominal surgery, beta-blockers, and cirrhosis with ascites [259]. Among people on PD, the cause of EPS is likely multifactorial, but recurrent infection and long term exposure to dialysate are thought to be the major contributors [259, 261, 264]. As in adults, data from children on PD reveal that increasing time on PD is associated with an increased risk for EPS [263, 265, 266]. Efforts to prevent EPS, therefore, have included pre-emptive transfer to hemodialysis in people who remain on PD for more than 8 years. Ongoing treatment with PD beyond this time period can be considered if the person on PD has a stable dialysate/plasma creatinine (D/P Cr) ratio based on PET, no evidence of high peritoneal transport capacity, no requirement for frequent use of hypertonic dialysis solution, normal or only intermittently elevated serum C-reactive protein level, absence of recurrent peritonitis and clinical stability defined as “good appetite and no signs of fluid overload.” [265, 267].

People on PD with EPS typical present with symptoms of bowel obstruction, including abdominal pain, emesis, anorexia, abdominal mass, weight loss, ascites and hemoperitoneum, and EPS is almost universally associated with progressive loss of ultrafiltration [259]. The diagnosis is usually confirmed radiographically, with either ultrasound or CT demonstrating loculated/septated ascites, adherent bowel loops, peritoneal thickening, and peritoneal calcification. Treatment typically consists of transfer to HD and bowel rest with provision of parenteral nutri-

tion [261, 264]. Treatment with several immunosuppressive agents, including prednisolone, sirolimus, mycophenolate mofetil, and tamoxifen, has been reported with variable success [259, 260, 268–271]. EPS can develop in patients on immunosuppression following kidney transplantation, calling into question the role of immunosuppression in this condition [264]. In adults with EPS, surgical intervention at specialized centers has shown improvement in symptoms and survival [272, 273]. Ongoing prospective efforts to monitor peritoneal membrane function and ultrastructural changes in people on PD should provide valuable information about the risk factors for developing EPS and ultimately strategies to minimize the risk for its development [71, 274].

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Management of Hemodialysis in Children

66

Daljit K. Hothi, Rukshana C. Shroff, and Benjamin Laskin

Abbreviations

AAMI	Association for the Advancement of Medical Instrumentation
ACE	Angiotensin converting enzyme
ARB	Angiotensin II receptor antagonists
BMI	Body mass index
BP	Blood pressure
BUN	Blood urea nitrogen
BVM	The Blood Volume Monitor ™
cIMT	Carotid intima-media thickness
CKD	Chronic kidney disease
CRP	C-reactive protein
DDS	Dialysis disequilibrium syndrome
DOPPS	Dialysis Outcomes and Practice Patterns Study
ECV	Extracellular volume
eKt/V	Equilibrated Kt/V
ESA	Erythropoiesis stimulating agent
ESKD	End-stage kidney disease
G	Urea generation rate
HD	hemodialysis
HDF	Hemodiafiltration
IVC	Inferior vena cava
IVC	Intracellular volume
K	Urea clearance
KDOQI	National Kidney Foundation Dialysis Outcomes Quality Initiative
KoA	Mass transfer coefficient of urea
K_{uf}	Ultrafiltration coefficient
LAVI	Left atrial volume indexed to height
L-carnitine	Levocarnitine
LMWH	Low molecular weight heparin
LV	Left ventricular
LVH	Left ventricular hypertrophy
NCDS	National Cooperative Dialysis Study
NIVM	Non-invasive blood volume monitoring
nPCR	Normalized protein catabolic rate
PTH	Parathyroid hormone
RBV	Relative blood volume
spKt/v	Single pool method
TAC-urea	Timed-average-concentration of urea
TBV	Total blood volume
TBW	Total body water
UF	Ultrafilter/ultrafiltration
UFH	Unfractionated heparin
URR	Urea reduction rate
USRDS	United States Renal Data System
V	Volume of distribution unless otherwise specified

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Introduction

Hemodialysis (HD) was introduced as a treatment for uremia at the end of World War II [1]. A decade later, Mateer et al. reported the first experience using HD to treat five uremic adolescents using 15 meter cellophane tubing and a 32 liter dialysis bath. Each dialysis procedure was 13 h, and although the metabolic and fluid status of their patients improved, there were challenges related to anticoagulation of the circuit and achieving normal plasma calcium and potassium concentrations [2]. Maintenance HD was not practical because vascular access required cannulae placed in the radial artery and saphenous vein prior to each session. This problem was overcome by the development of silastic arteriovenous cannula by Scribner et al [3] which were inserted in the forearm vessels and could be used for repeated blood access. What followed was the report by Fine et al [4] describing the use of HD for maintenance treatment of end-stage kidney disease (ESKD) in five adolescents who were dialyzed three times weekly for 7–8 h per session using a concentrated dialysis solution mixed with tap water. A urea clearance of 45 ml/min resulted in a urea reduction rate (URR) of 48% during each 7–8 h treatment. While maintenance HD was now a realistic option for children with ESKD, technical difficulties persisted in small children and the need for 20 h of treatment per week required long periods of time in the hospital.

In 1971, Kjellstrand et al. reported their experience treating 10 children <15 kg [5]. Applying data from adults receiving dialysis, the authors recommended a urea clearance in children based on body weight, with a goal urea clearance of 2–3 ml/kg/min during each dialysis session. This clearance, multiplied by the number of hours of dialysis, allowed an accurate prediction of the expected fall in urea during a single HD session. This reduced the risk of disequilibrium syndrome from excessive reductions in urea and established a standard formula for dialysis urea clearance in children that is still used today.

Despite the initial success of the Scribner shunt, clotting and infection of the vascular access remained common. Arteriovenous fistulae reduced this problem and remain the gold standard for dialysis access. However, creation of fistulae in small children is technically challenging and requires surgical expertise and a critical mass of patients to maintain skills, which are not available in many pediatric centers [6]. These technical challenges, combined with the desire to avoid repeated needle punctures in small children, led Mahan et al. to use a Hickman central venous catheter for prolonged HD vascular access in children [7]. Central venous catheters have since become the most widely used HD access [8]. While allowing children to obtain puncture-free HD, catheters have a high rate of clotting, infection, and decreased blood flow and therefore should only be used for long-term access when creation of a suitable fistula or access to expert fistula teams is not possible.

Technological improvements over the last 50–60 years have made HD widely available for children with ESKD. While overall and cause-specific mortality have decreased for children initiating maintenance dialysis over the last several decades, children with ESKD continue to experience unacceptably high rates of morbidity and mortality compared to the healthy pediatric population [9]. Improvements in dialysis equipment, medications, and consensus treatment guidelines are likely responsible for better patient outcomes (Fig. 66.1) [9]. Today, children often receive less than half the weekly HD treatment time compared to when the therapy first became widely available, as described above [4]. To dramatically improve outcomes further may require a fundamental change to the "standard" thrice weekly HD prescription. Initial pediatric experiences using short daily or nocturnal HD dialysis and hemodiafiltration (HDF) have been very positive, although widespread application remains futuristic due to logistic and funding barriers in some countries.

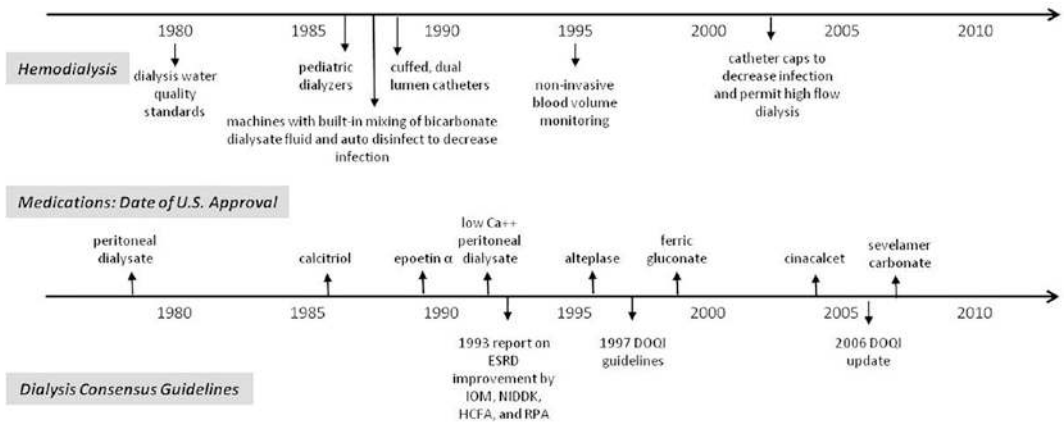

Fig. 66.1 Improvements in hemodialysis from 1980–2010

Prescribing Hemodialysis

Most of the HD literature reports on adults, with less data available in pediatric patients. In theory, the principles learned from adults are universal and applicable to children, but adjustments are required to accommodate the spectrum of age, weight, growth, and physiological development that are specific to children. Ideally, children should receive ESKD treatment at specialized pediatric centers with the necessary technical expertise, staffing, and multidisciplinary resources (physicians, nurses, psychologists, dieticians, social workers, teachers, and child-life specialists) to provide optimal care [10]. The two primary objectives of HD are to clear metabolic waste products and to UF excess fluid. To achieve these goals, the prescriber must calculate adequate clearance and estimate the patient's dry weight. To complete the HD prescription, one must then choose the blood flow rate, dialyzer, extracorporeal tubing, dialysate electrolyte composition and temperature, and anticoagulation.

Adequacy

The publication of the National Cooperative Dialysis Study (NCDS) in 1981 [11, 12] addressed how dialysis might best reduce patient morbidity and mortality by comparing four groups of patients with high or low timed-average-concentration of urea (TAC-urea) and with either long (4.5–5 h) or short (2.5–3.5 h) dialysis sessions. The results showed that the TAC-urea was the most important determinant of patient morbidity and hospitalization. In a subsequent analysis of the NCDS using a single-pool urea kinetic model, Gotch and Sargent argued for the use of Kt/Vurea to measure the adequacy of dialysis [13]. This unitless measure is an estimate of the clearance of urea from the blood during a dialysis session, standardized by total body water (which reflects the urea distribution volume). Kt/Vurea has become the standard measure of the delivered dialysis dose and the adequacy of dialysis.

Various methods for calculating Kt/V have been proposed. The single pool (spKt/V) method assumes urea is removed from a single pool and so a delayed postdialysis sample is not required. However, this method overestimates urea clearance because it ignores urea rebound postdialysis from access recirculation, cardiopulmonary recirculation, and tissue redistribution**.** Access recirculation becomes insignificant within 15–20 s of the blood flow being reduced to <50–80 ml/min. Cardiopulmonary recirculation only occurs with arteriovenous access and not with central lines. Cardiopulmonary recirculation is a

result of blood returning to the dialyzer after circuiting the heart and lungs without passing through the other tissues and ceases 1–2 min after slowing blood flow. Conversely, urea tissue rebound continues over a longer time because there is diminished blood flow to muscle, which has high urea content, during dialysis [14]. Urea rebound is minimized by using either of the following two methods proposed by National Kidney Foundation Dialysis Outcomes Quality Initiative (KDOQI) guidelines [15]. With the slow blood flow method, the dialysate is turned off and the UF is minimized at the end of dialysis. The blood flow is then decreased to <100 ml/min for 15 s and then the urea sample is obtained. Using the stop dialysate method, the same protocol is used, except the blood flow is maintained at a normal rate for 3 min prior to drawing the postdialysis urea sample. Standardization is important, especially because different results for Kt/V have been shown in children depending on which day of the week laboratory studies are performed [16].

The double pool Kt/V recognizes that postdialysis rebound of plasma urea may be substantial. Therefore, a urea sample drawn 60 min postdialysis is required to avoid the overestimation of urea removal. This method is probably the most accurate estimate of Kt/V, but the need for a delayed postdialysis blood sample and lack of validation studies have limited its use.

Calculating Kt/V with urea kinetic modeling requires sophisticated computer algorithms which may not be available in many pediatric dialysis units. However, websites including Hypertension Dialysis and Clinical Nephrology (www.hdcn.com) and www.Kt-v.net provide programs for calculation of single and double pool Kt/V measurements, some of which have been used in pediatric studies [17]. The major advantage of kinetically modeled methods to estimate Kt/V is that they also provide an estimate of the urea generation rate from which the normalized protein catabolic rate (nPCR), an estimate of dietary protein intake, can be calculated. Nevertheless, several potential inaccuracies are intrinsic to the measurement of kinetically derived Kt/V. Urea clearance (K) for individual dialyzers is derived from the manufacturer's specifications, which do not account for recirculation or reductions in dialyzer efficacy due to clotting of dialysis fibers or interruptions in treatment from kinked lines. Also, determining the urea distribution volume (V) may be imprecise, particularly in children [18].

To overcome these limitations, more simplified equations for calculating Kt/Vurea have been proposed [19]. One such formula [Kt/V = – Log n (R – 0.008 t) + (4–3.5 R) UF/BW] estimates the spKt/V where R is the ratio of the predialysis to postdialysis urea, t is the time of dialysis in hours, UF is the UF volume in liters, and BW is body weight in kg. This formula varies by only 6% from formal urea kinetic modeling in children [17]. To correct for postdialysis urea rebound, additional equations have been developed to calculate the equilibrated Kt/V (eKt/V) in patients with arteriovenous [eKt/V = spKt/V – (0.6 × spKt/V/T) + 0.03] or venovenous [eKt/V = spKt/V – (0.47 × spKt/V/T) + 0.02] access. Standardized (stdKt/V) formulas are available to estimate the Kt/V over a week, which are useful in patients regularly receiving more frequent or intensified dialysis regimens, or for those requiring occasional extra sessions for UF [18, 20].

Finally, the URR measures the percentage decrease in blood urea during a dialysis session. The URR as a marker of dialysis adequacy was evaluated retrospectively in 13,473 patients, and the mortality rate increased by 28% when URR values of <60% were obtained [21]. Despite its validation as a measure of morbidity, URR is not recommended as the primary measure of dialysis adequacy because significant variations of Kt/Vurea may be obtained with each URR value, particularly when URR is greater than 65%. Also, with increasing UF, URR underestimates urea removal. Nonetheless, targeting a URR of <50% for the first several treatments in a patient initiating chronic dialysis is a useful means of preventing dialysis disequilibrium. As no upper limit of Kt/Vurea has been established, care must be taken with aggressive treatment. Even

in patients who have been on dialysis for a long time as excessive urea removal can lead to symptoms of dialysis disequilibrium.

KDOQI guidelines published in 2000 recommended that the delivered dose of HD in both adults and children should be measured using formal urea kinetic modeling with a spKt/V urea of at least 1.2. In 2002, the HEMO Study randomized 1846 patients on conventional thrice weekly HD to either a standard or high-dose of dialysis as well as to a low-flux or high-flux dialyzer. In high-dose patients, the URR was 75% and spKt/V 1.71, compared with standard-dose patients whose Kt/V was 1.32 with a URR of 66%. Neither dialysis dose nor dialyser flux affected the relative risk of death. The authors concluded that there was no major benefit from a higher dialysis dose than recommended by KDOQI or from the use of high-flux dialysis membranes [22].

However, dialysis dose may be associated with mortality in association with body mass index (BMI). In patients with lower BMI, a URR of >75% was associated with a lower risk of death compared to patients with a URR of 70–75% [23]. Daugirdas et al. found that by normalizing Kt/V to body surface area (BSA), most children less than 10 years of age would receive less dialysis compared to older patients, despite acceptable eKt/V and stdKt/V values [18]. Theoretically, it is tempting to postulate that there may be a survival advantage in increasing the HD dose in women and patients with a low BMI, such as children.

The Dialysis Outcomes and Practice Patterns Study (DOPPS) review of 22,000 adult HD patients from seven countries found that a higher dialysis dose, as reflected by a higher Kt/V, was important and an independent predictor of lower mortality. Survival was greatest when combining a higher Kt/V with a longer treatment time. For every 30 min longer on HD, the relative risk of mortality was reduced by 7% [24]. Reports from the Australian and New Zealand Dialysis and Transplant Registry and the United States support that longer treatment times, notably those >4–4.5 h, are associated with a lower risk of death, independent of adequate clearance [25, 26]. Such research establishes the basis for intensified dialysis programs (see below), namely a move away from conventional 3–4 h, three-times-per-week dialysis to more frequent and/or more prolonged dialysis sessions.

The KDOQI guidelines for HD adequacy were revised in 2005 to recommend a minimum spKt/V urea of 1.2 per session, with a target spKt/V of 1.4 and URR of 70%. These recommendations were consistent with the minimal Kt/V reported in the HEMO Study and also the European Guidelines for Hemodialysis, which endorsed a spKt/V of 1.4–1.5 [27]. However, no large scale studies have assessed HD adequacy in children. Buur and colleagues compared two urea kinetic models with direct quantification of urea removal and found that although each method produced different results, correlation between the methods was very high [28]. The authors commented that for practical purposes, and to limit blood sampling, one of the direct single-pool methods of urea kinetic modeling should be used. A study of 8 children <18 years of age compared an online urea monitor (UM 1000™, Baxter Healthcare) with single and double-pool formulas and separately with single-needle dialysis [29]. The study reported considerable differences in Kt/V urea between single and double pool formulae and concluded that online urea monitoring was inaccurate during single-needle dialysis.

Despite the limited data in children, expert working groups have developed guidelines for HD in children both in Europe and North America [10] together with European adult guidelines [27]. These are summarized in Table 66.1. We recommend maintaining a spKt/Vurea between 1.4–1.8 in children dialysed for 3–4 h per session. It is imperative that the prescribed dialysis dose for an individual child should be based on more than just an estimate of urea removal. Achieving optimal dialysis must also include a careful clinical assessment including growth, nutrition, cardiovascular health (especially blood pressure (BP)), anemia treatment, and the bone and mineral health of a developing child [30].

Table 66.1 Published guidelines for hemodialysis adequacy

Source	Urea Clearance	Other
KDOQI, adults	Minimum spKt/V ≈ 1.2target spKt/V ≈ 1.4	URR ≈ 65%URR ≈ 70%
European, adults	eKt/V > 1.2spKt/V ≈ 1.4	Double-pool urea kinetics preferred
KDOQI, children	spKt/V > 1.4	Assess nutrition (nPCR)optimize ultrafiltration
European, children	eKt/V ≥ 1.2–1.4	Assess nutrition (nPCR)monitor growth and cardiac function

Estimation of Dry Weight

UF is targeted to an estimated 'dry weight.' Dry weight is most commonly defined as the post-HD weight at which the patient is close to euvolemia without experiencing symptoms. Overestimation of the dry weight places patients at risk of developing volume-dependent hypertension, left ventricular hypertrophy (LVH), and congestive heart failure. An underestimation of the dry weight increases the risk of symptoms from intradialytic volume depletion. In children, growth and changes in lean body mass and body habitus necessitate regular and frequent re-evaluation of the dry weight to detect subtle differences in the ratio of total body water to body mass. This is especially important in infants and young children receiving HD for ESKD as they are growing rapidly, Therefore, tests for evaluating dry weight have to be easily accessible, reproducible, and ideally non-invasive.

The clinical examination is the most widely used test, but at best it only provides a crude assessment of volume status. In children with ESKD, even the most sensitive signs are rendered imprecise. For example, as dialysis patients have fluid removed there are a number of factors that can result in hypotension (cardiac dysfunction, low vascular tone, hypoalbuminemia, medications) and thus changes in orthostatic vital signs are not diagnostic of true hypovolemia. Jugular venous distension, though infrequently assessed in children, is a useful sign in the assessment of central venous pressure, provided heart failure is absent [31].

Biochemical markers including plasma atrial natriuretic peptide and brain natriuretic peptide correlate with increased plasma volume in ESKD [32], but levels can also remain elevated in volume contracted individuals and hence they lack the ability to detect volume depletion. Brain natriuretic peptide appears superior to atrial natriuretic peptide in predicting LVH and dysfunction. However, in the context of defining dry weight, results have been variable [33].

Ultrasound guided supine inferior vena cava (IVC) diameter measurement and its decrease on deep inspiration, better known as the collapse index (CI = end expiratory IVC diameter minus end inspiratory IVC diameter)/end expiratory IVC diameter), have been shown to correlate with right atrial pressure and circulating blood volume [34]. Results are influenced by wide interpatient variability, lack of validated normal values for children, the timing of the measurement in relation to dialysis, and the presence of heart failure or tricuspid regurgitation. As a result of these limitations, although it is a non-invasive test which could conceivably become available at most centers, it cannot reliably predict dry weight in children.

Bioelectrical impedance technology can directly assess extracellular volume (ECV), intracellular volume (ICV) and total body water (TBW) by detecting differences in the degree of resistance (impedance) as electric currents pass through each fluid compartment. At low frequencies, current cannot cross cell membranes and only flows through ECV; at higher frequencies it flows through both the ICV and ECV. Three methods for assessing dry weight using bioimpedance are available: (1) The normovolemia/hypervolemia slope method uses whole body multi-frequency bioimpedance spectroscopy to measure the ECV (Fig. 66.2) [35]; (2) The resistance-reactance graph method uses whole body single-frequency bioimpedance analysis to estimate TBW, but is unable to separate ECV from ICV, and therefore only useful when trying to

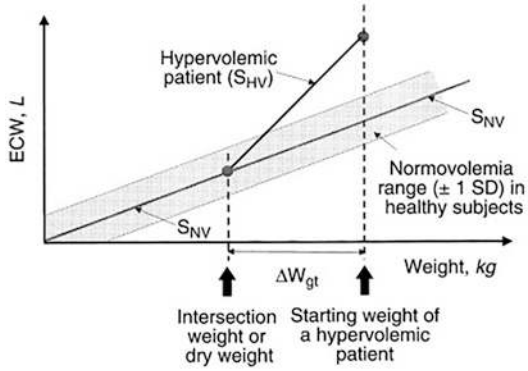

Fig. 66.2 Bioelectrical impedance to estimate dry weight using the normovolemia/hypervolemia slope method with whole body multi-frequency bioimpedance spectroscopy

differentiate between excessive body water and true weight gain [36]; (3) The continuous intradialytic calf bioimpedance method records changes in extracellular resistance in real-time, generating a curve whose slope flattens as excess ECV is removed, dry weight defined as flattening of the curve over a period of 20 minutes [37]. Premature flattening of the curve may occur in the presence of venous thrombosis or lymphatic edema. The value of bioimpedance techniques to estimate dry weight in pediatrics is unknown and limited by incomplete data in children and from patients with ESKD, and the inherent underestimation of TBW with multi-frequency bioimpedance methods. Importantly, changes in electrolyte, red cell, and protein concentrations and patient temperature are all known to influence bioimpedance. Bioimpedance may be used more frequently as increasing evidence from clinical studies validate its assessment of fluid status [38, 39].

Finally, on-line non-invasive blood volume monitoring (NIVM) is commonly used in clinical practice. NIVM provides information on intradialytic blood volume changes and vascular refilling rates. The magnitude of blood volume changes differs between patients and dialysis sessions, but if combined with post-dialytic vascular compartment refilling rates, dry weight can be assessed. Vascular refilling typically occurs in the first 10 min after stopping UF and is characterized by an increase in the relative blood volume (RBV), which can continue for up to 60 min. Steuer et al. achieved a twofold reduction in intradialytic symptoms using NIVM in 6 hypotension prone adults, without reducing the UF volume or increasing treatment times [40]. Others have shown an increase in the UF potential, lowering of the dry weight, improved patient well-being and reduced hospitalization due to fluid overload.

NIVM is based on the principle of mass conservation: the concentration of measured blood constituents (hemoglobin/haematocrit/plasma protein) confined to the vascular space is proportional to changes in the vascular volume. Individual NIVM devices differ by their intrinsic sensing technique. Optical devices measure the absorbance of monochromatic light via an optoprobe in the arterial line to estimate the hematocrit because the optical density of whole blood is a measure of red blood cell concentration. The Crit-line™ (Fresenius) is a stand alone device, while the Hemoscan™ (Hospal-Dasco, Medolla, Italy) is a component of the dialysis machine. Blood density monitors are dependent on the total protein concentration (plasma protein concentration + mean cellular hemoglobin concentration). The Blood Volume Monitor™ (BVM, Fresenius AG, Bad Homburg, Germany) measures the velocity of sound through blood, as a reflection of blood density, by means of a cell inserted in the pre-pump segment of the arterial line. Schneditz et al. demonstrated a 2% difference in RBV changes between the Crit-line and BVM which developed 1 h into dialysis and persisted thereafter [41].

NIVM is used to divide patients into 4 groups. Group 1: Absence of postdialysis refilling with no symptoms suggestive of intradialytic hypovolemia or post-dialytic fatigue: the patient is likely to be at their dry weight. Group 2: Postdialysis refill, lack of a substantial change in blood volume during HD, and no intradialytic or post dialytic symptoms: indicative of extracellular fluid expansion and the need to lower the patient's dry weight. Group 3: Absence of postdialysis refill, intradialytic and/or post dialytic symptoms: indicative of hypovolemia and the need to increase the dry weight. Group 4: Postdialysis refill but intradialytic symptoms of hypovolaemia: indicative of slow vascular refilling rates, but ECV expansion at the end of dialysis. This suggests that the dry

weight needs to be reduced incrementally and slowly following changes to the dialysis prescription to increase the UF potential. Extended duration of dialysis sessions may be necessary.

Information on blood volume status can be particularly helpful in the pediatric HD setting as the prevalence of intra- and interdialytic morbidity may be underestimated because children often do not verbalize early warning symptoms. Jain et al. show reduced dialysis associated morbidity with NIVM, with the greatest impact on children weighing less than 35 kg [42]. Michael observed improved targeting of the dry weight in children, which reduced the requirement for antihypertensive medication [43]. Using a constant dialysate sodium concentration of 140 mmol/L, Jain also defined a safe UF rate as an RBV change of <8% per hour in the first 90 mins and then <4% thereafter, with no more than a 12% net RBV change per dialysis session [42]. Hothi et al. reported in 11 pediatric HD patients that the gradient of the RBV curve in the first hour, as well as changes in heart rate, were the strongest predictors of treatment-related complications [44].

In summary, evaluating a patient's dry weight can be a challenge. The limitations and benefits of the available tests to estimate dry weight are summarized in Table 66.2. As of yet no gold standard has been defined and for the majority, applicability in pediatrics has not been validated. We recommend the use of NIVM combined with clinical assessment. NIVM also offers the advantage of accurate assessment of the patient's hemoglobin, allowing for tracking of responses to changes in erythropoietin stimulating agent and iron administration. This is especially important in young children in whom frequent blood draws for lab testing can exacerbate anemia secondary to ESKD and blood loss from HD.

Blood Flow Rate

The blood flow rate is a major determinant of solute clearance on dialysis. With increased blood flow, more solute is delivered to the dialyzer, resulting in higher dialyser "flow limited clearance." However, clearance is also determined by the membrane's permeability to the solute, which is known as "membrane limited clearance." With poorly permeable solutes, increasing the blood flow will only produce a mild increase in clearance (Fig. 66.3). The dialyser flow clearance is limited and starts leveling off at blood flows of 250–300 ml/min, and therefore some adult dialy-

Table 66.2 Summary of Methods for Assessing Dry Weight [246]

Modality	Pros	Cons
Biochemical markers	-ease of use -noninvasive	-wide variability -poor correlation with volume depletion -not available at most laboratories -inaccurate in patients with congestive heart failure
Inferior vena cava diameter	-correlated with right heart pressure and intravascular volume -noninvasive	-no normative values for children -technician dependent -cost -limited availability -unclear which time after HD to measure
Bioimpedance	-measures ECFV and ICFV, estimating fluid shifts from various compartments -strong correlation with ultrafiltration volume	-limited normative values for children -unclear which time after HD to measure -cost -underestimates volume shifts from trunk
Non-invasive blood volume monitoring	-ease of use -ease of interpretation -decreases risk of intradialytic hypotension -validated use in children	-no standardization across devices -requires active intervention by providers -only measures fluid shifts from intravascular space and refilling rates Cost

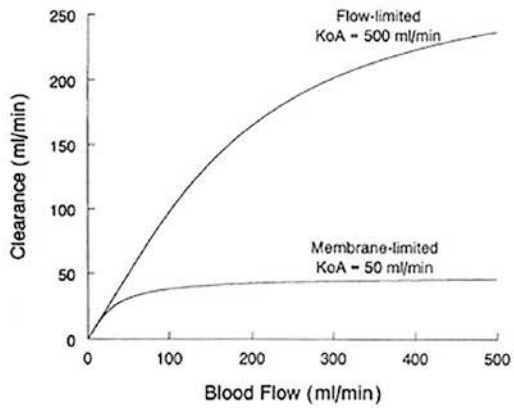

Fig. 66.3 Blood flow-limited and membrane-limited clearance

sis units have set this as their maximum blood flow rate.

The effective blood flow rate is largely determined by the vascular access, especially in pediatrics. For chronic HD we recommend a blood flow rate that is equivalent to 4–6 ml/kg/min urea clearance obtained from dialyser urea clearance estimates provided by the manufacturer. In infants, a minimum blood flow of 20–30 ml/min avoids the risk of clotting the circuit. Effective blood flows are often lower than those prescribed due to partially occlusive pumps, malposition of the vascular access needle, access failure, tubing diameter changes, and shear effects. The efficacy of dialysis is also reduced by recirculation effects, which are more pronounced with higher dialyzer blood flows, vascular access inflows lower than dialyzer blood flow, stenosis at the access outflow, single lumen access particularly with small stroke volumes, increased length of blood lines, and small needle and tubing diameter [45]. This places infants with blood flows determined by small, high resistance double-lumen central venous catheters or single lumen catheters with high recirculation rates at the highest risk of inadequate dialysis with conventional dialysis regimens. This can be improved by increasing the dialysis time, which in our experience is best tolerated by increasing the frequency and not the duration of treatment.

Choice of Dialyser

When selecting a dialyser for maintenance HD, several membrane characteristics need to be taken into consideration [10]. To improve efficacy, dialysers are designed to maximize the surface area available for diffusion. Two designs have predominated, namely hollow fibre and parallel plate dialysers. In the latter, parallel layers of membranes are separated by flat supporting structures. Their greatest disadvantage is their high compliance and thus large filling and priming volumes. Therefore, in children, they have largely been replaced by hollow fibre dialysers, which consist of a bundle of capillaries potted at both ends into a plastic tubular housing unit with sealing material. Hollow fibre dialysers have virtually no compliance and lower priming volumes, but the sealing materials are at risk of releasing solvents or ethylene oxide after gas sterilization, and thus producing anaphylactic reactions. As a general rule, the dialyser membrane surface area (which is readily available on the label of most dialysers) should be approximately equal to the patient's BSA. It is important to regularly assess the choice/size of the dialyser in growing children and those who are receiving inadequate dialysis.

Because the dialysis membrane is in direct contact with the patient's blood, it can initiate leucocyte and complement activation. The extent of the inflammatory response reflects the biocompatibility of the material that makes the dialyser. Three types of membranes are presently available, those made from unmodified cellulose, modified/regenerated cellulose, and synthetic membranes. Unmodified cellulose membranes, such as cuprophan, are relatively inexpensive but also the most bioincompatible. The modified cellulose membranes such as the cellulose acetate or hemophan® have some or all of the hydroxyl groups esterified to make them more biocompatible. However, such modifications may result in increased activation of the coagulation cascade and thus increase the anticoagulation requirement of the HD circuit. Synthetic membranes are made from polysulfone, polycarbonate, polyamide or polyacryl-polyam-

ide acrylate. These membranes are relatively biocompatible, except for the negatively charged AN69 polyacrylonitrile membranes, which are known to cause hypotension, inflammatory hyperemia, oedema and pain secondary to a bradykinin mediated reactions. Dialysis patients most at risk are infants requiring blood to prime their HD circuit [46] and children that are concurrently taking angiotensin converting enzyme (ACE) inhibitors[47] or angiotensin II receptor antagonists (ARB) [48]. Synthetic membranes are generally more hydrophobic than cellulose membranes and therefore have higher adsorption properties [49]. Their increased ability to bind proteins may be partly responsible for their improved biocompatibility, and also makes them the membrane of choice for therapies such as albumin dialysis or in the treatment of acute toxicities where the undesired toxin is highly protein bound.

Membrane solute permeability refers to the clearance of middle molecular weight molecules, and is assessed by measuring the rate of β2-microglobulin clearance. Solute permeability is determined by the number of pores, the size of the pores, and the membrane wall thickness. A highly permeable membrane is one that is thin, with a high pore density and large diameter pores. Efficiency, represented as the KoA or mass transfer coefficient of urea, is a measure of urea clearance, a surrogate marker of small molecule clearance. Traditionally, membranes have been characterized as low-flux or high-flux according to their solute permeability. High-flux membranes are highly permeable membranes that can permit convective solute clearance of molecules weighing between 5000–25,000 Daltons, but urea clearance rates vary. Highly efficient membranes have high urea clearance rates but differ in their hydraulic permeability, and thus may be limited in their ability to clear middle molecules (Table 66.3).

A useful measure of the hydraulic permeability of a membrane is the K_{UF}, the UF coefficient, defined as the volume of UF produced per hour per mmHg transmembranous pressure, which is determined at a blood flow of 200 ml/min. K_{UF} is most directly influenced by the membrane's mean pore size. In turn, the mean pore size influences the solute sieving coefficient and molecular weight cut-off for a membrane. High-flux dialysers with larger mean pore sizes have a higher molecular weight cut-off and are most efficient in clearing larger uremic compounds. The UF rate and the dialyzer membrane's sieving coefficient are the most important determinants of convective solute removal [50]. Therefore, in consideration of predominantly convective therapies such as hemofiltration or HDF, high-flux dialysers are required.

Analyzing the United States Renal Data System (USRDS) database, Bloembergen et al. demonstrated a 20% decrease in the relative risk of death for modified cellulose and synthetic membranes compared with cellulose membranes [51]. In a retrospective analysis of 715 patients, Woods et al [52] compared mortality in a group treated exclusively with low-flux polysulfone dialysers with another treated for at least 3 months with high-flux polysulfone dialysers. The high-flux group had a significant 65% reduction in the risk of death compared with the low-flux group. A Kaplan-Meier analysis suggested a higher 5-year survival in the high-flux group, but a statistically significant difference was only seen after 4 years of dialysis.

In conclusion, epidemiological studies suggest improved morbidity and mortality in dialysis patients treated with modified cellulose or synthetic membranes but few have been able to demonstrate whether the effects were due to differences in flux, biocompatibility or middle

Table 66.3 Dialyser classification [247]

Class	Surface area	K_{UF} (ml/h/mmHg)	Urea clearance	β2 microglobulin clearance
Conventional	< 1.5 m^2	< 12	Moderate	Negligible
High efficiency	> 1.5 m^2	> 12	High (KoA > 600 ml/min)	Negligible
Mid-flux	Variable	12–30	Variable	Moderate
High-flux	Variable	> 30	Variable	High (>20 ml/min)

molecule clearance. Few, if any, paediatric centers practice dialyzer reuse. Reuse is associated with a reduction in the incidence of "first use" reactions, but may be associated with allergic reactions to residual sterilizing agents, such as formaldehyde. Inadequate sterilization of dialyzers may cause pyrogen reactions or frank infection, manifest by fever, chills and rigors [53].

Extracoporeal Circuit

Multiple dialysis machines are on the market, each with different sizes, weights, capabilities for home therapy, and interfaces with providers (reviewed in [54]). Regarding the extracorporeal circuit, during pediatric dialysis, if the total blood volume of the circuit is greater than 10% of the estimated total blood volume (TBV) a circuit prime with 5% albumin or blood is recommended. Even though traditionally blood has been preferred, these recommendations come from an era when severe anaemia was the rule for children with ESKD. However, minimizing exposure to blood products may decrease the risk of human leukocyte antigen sensitization in young children awaiting transplantation. The TBV is approximately equal to 100 ml/kg body weight in neonates and 80 ml/kg for infants and children. As a general rule, we use blood primes if the patient is anaemic. To avoid the risk of clotting the circuit, priming can be achieved with packed red blood cells diluted with normal saline or 5% albumin to achieve a final haemtocrit of 30–35%. Alternatively, the circuit can be primed with undiluted packed red blood cells from the blood bank when manipulation of the product is not permitted. The potassium load to the patient can be minimized by using fresh blood, and once priming is completed, recirculating the blood through the dialyser for 10 min, without connecting to the patient. At the end of dialysis, we do not recommend retransfusing the blood back into the infant, and if a blood transfusion is required, to give this during the dialysis session infused through a peripheral line or via a Y-connection at the venous return site to reduce the possibility of clotting the circuit.

Dialysate Water

Dialysate contaminants can be both chemical and biological, and can cause significant morbidity. It is therefore imperative that each dialysis unit ensures that disinfection practices are in place to achieve these standards, combined with regular surveillance to ensure that they are sustained. Dialysate quality is known to be an important component of the biocompatibility of the HD procedure and therefore also contributes to the chronic inflammation of dialysis [55]. In vitro studies have shown that bacterial products can cross both high-flux and low-flux dialysis membranes and stimulate synthesis of inflammatory mediators such as cytokines within the blood compartment [56]. The degree of cytokine stimulation is related to the concentration of endotoxin and other 'cytokine-inducing substances' in the dialysate compartment [57, 58] and the permeability of the dialysis membranes to these substances. In general, polysulfone and polyamide based membranes are effective barriers to endotoxins because of their high adsorptive properties [59] whereas high-flux membranes and low-flux cellulose based membranes are less protective [60, 61]. With an increasing use of high-flux membranes, especially with HDF therapy, a very high degree of water purity is essential (see section on 'backfiltration' below).

Water Purification Systems

A standard water treatment device consists of a water softener, an activated carbon filter, a sediment filter, and a reverse osmosis system [62]. Water softeners contain a resin that exchanges sodium cations for calcium, magnesium, and other polyvalent cations. Water softening not only prevents hard water, but also protects the reverse osmosis membrane used in the final step of water treatment from the build-up of scale and subsequent failure. The resin is regenerated periodically with concentrated sodium chloride solution, which also reduces bacterial growth in the resin bed. Activated carbon filters remove chloramines and organic solvents, but

tend to release carbon particles and therefore require a sediment filter placed downstream. The final purification step is performed by reverse osmosis where the water is forced through a semipermeable polyamide or polysulfone membrane at 14–28 bar. This step removes 90–99% of inorganic and organic substances, pyrogens, bacteria, and particulate matter. The purified water is pumped from the reverse osmosis module to the individual treatment stations in a recirculating ring loop which delivers the water produced in excess back to the reverse osmosis module, avoiding wastage of high-quality water. The ring loops themselves require regular disinfection, and this is performed either by heat or chemical disinfection.

Testing Water Quality

The International Organization for Standardization (ISO) has published a series of standards addressing fluids for extracorporeal therapies. Specifically, ISO 11663:2009, Quality of dialysis fluid for hemodialysis and related therapies, requires that replacement fluid used for HDF be sterile and pyrogen-free; the currently accepted norms for ultrapure dialysate are defined as containing <0.1 colony-forming unit/ml and <0.03 endotoxin unit/ml. In addition, the chemical composition of water must be tested at least once per year [63].

Dialysate Composition

The composition of the dialysate fluid will influence the exchange of electrolytes during the dialysis treatment and thus ultimately creates an opportunity to modify adverse events during dialysis and the net transfer of electrolytes to or from the patient.

Sodium

Following a sodium load, even in the presence of renal failure, the mechanisms responsible for preserving plasma tonicity will maintain plasma sodium within narrow limits by changing the plasma volume. During HD, dialysate sodium generates a crystalloid osmotic pressure and thus influences fluid shift between the different body compartments, but it also permeates the dialysis membrane and thus has the potential for becoming a sodium load.

Diffusive sodium transport is proportional to the difference in sodium activity between blood and dialysate compartments. Dialysate sodium activity is approximately 97% of the measured sodium concentration, but varies with changes in dialysate temperature, pH, and the presence of additional ions. The proportion of plasma water free sodium ions that are unbound to protein and other anions can be measured by direct ionemetry. Plasma sodium activity is influenced by the Donnan effect: negatively charged proteins (mainly albumin) produce a small electrical potential difference across the membrane (negative on the plasma side) that prevents movement of the positively charged sodium ions. In the absence of UF, the concentration of dialysate sodium needed to achieve isotonic dialysis can be approximated by correcting the blood sodium measured by direct ionometry for a Donnan factor of 0.967.

Hyponatremic dialysis causes osmotic fluid shift from the extracellular to intracellular compartment, contributing to dialysis disequilibrium and intradialytic hypotension. Hypernatremic dialysis transfers sodium to the patient, causing interstitial edema, interdialytic thirst, increased interdialytic weight gain and worsening hypertension. A therapeutic advantage can be gained by manipulating the dialysate sodium concentration throughout dialysis, known as sodium profiling, and typically utilizes a sodium concentration that falls in a step, linear, or exponential fashion (Fig. 66.4). The higher dialysate sodium at the start allows a diffusive sodium influx to counterbalance the rapid decline in plasma osmolarity due to clearance of urea and other small molecular weight solutes. Low dialysate sodium at the end aids diffusive clearance of the sodium load and minimizes hypertonicity. Compared with a constant dialysate sodium bath, profiling has been shown to increase stability of intradialytic blood volume and reduce both intradialytic cramps and interdialytic fatigue in children and adults [64,

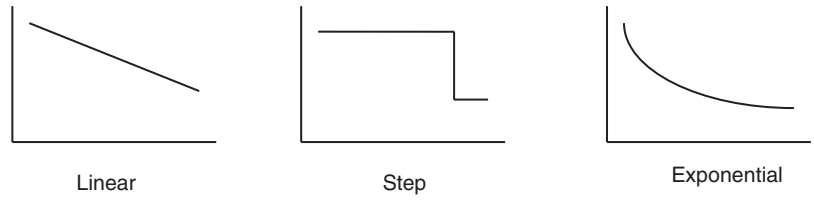

Fig. 66.4 Sodium profiling options

65]. Compared with exponential profiles, step profiles are most effective at attenuating postdialytic hypotension and early intradialytic hypotension, while linear profiles best reduce cramps and late intradialytic hypotension. Sodium profiling is also indicated in the prevention of dialysis dysequilibrium.

The difficulty with sodium profiling is finding the concentration gradient that offers the benefits of cardiovascular stability without exposing the patient to a small but repeated sodium load. A net sodium gain of 1 mmol/L will result in a 1.3% expansion of the extracellular space. Based on concerns of inducing hypervolemia, neutral sodium balance profiles may be preferred. Protocols of isonatremic dialysate are similar, with time averaged dialysate sodium 2–3 mmol/L lower (Donnan effect) than the predialysis sodium [66]. Results indicate benefits similar to those described with sodium profiling, but with a significant decrease in the interdialytic weight gain and thirst score [67, 68]. The difference is likely to be due to an improvement in sodium balance, but as neutral balance is unlikely even with the 'isonatraemic' protocols we recommend monitoring for changes in interdialytic weight gain and BP.

Potassium

One of the key objectives of HD is to maintain the plasma potassium levels within a narrow normal range, both during the intradialytic and interdialytic periods. The risk of arrhythmia, QT dispersion [69] and ventricular ectopic beats is increased with hypokalemia and also if the rate of decline is rapid early in dialysis, even if the actual plasma potassium levels are normal. This is one of the postulated mechanisms for the phenomenon of sudden cardiac death in HD patients. Conversely, failure to normalize serum potassium levels is also arrhythmogenic [70].

The challenge is that HD can only remove potassium from the extracellular compartment and that comprises 2% of total body potassium. In addition net potassium removed is influenced by a number of dialysis related factors. During HD, approximately 85% of potassium is removed by diffusion and this is influenced by the serum:dialysate potassium gradient. The rate of potassium removal is highest in the first hour of dialysis and then declines as the serum:dialysate potassium gradient falls. Therefore the greatest fall in plasma potassium levels are in the first hour, with a more gradual reduction in the subsequent 2 h, and almost no change in the plasma potassium level in the fourth and fifth hours as the serum:dialysate concentrations reach equilibrium. Postdialysis the plasma potassium rebounds and again the serum:dialysate gradient appears to influence the magnitude of this rebound, with a rapid postdialysis rebound of potassium levels with higher gradients compared with smaller gradients [71]. Convective clearance of potassium by UF accounts for approximately 6% of the total potassium mass removed. The glucose content of dialysate solutions is important with high glucose–containing dialysate solutions resulting in higher potassium removal as a result of osmotic shifts of intracellular potassium to the extracellular space [72]. The other major factor is the dialysate bicarbonate. Higher dialysate bicarbonate concentrations result in a rise in serum bicarbonate levels during HD, enhancing Na^+/K^+-ATPase activity with a larger shift of potassium into the intracellular space and a lowering of serum potassium levels, but total body potassium removal is not improved [73].

Optimizing the management of potassium removal in patients on HD involves reducing large intradialytic potassium shifts as well as providing adequate potassium removal to minimize hyperkalemia. However, there is no consensus on how best to achieve this. As a general rule, we would recommend dialyzing children against a potassium bath of 2 mmol/L. If patients are hyperkalemic and asymptomatic, use a 1 mmol/L potassium bath for the first 1–2 h of dialysis, and then switch to 2 mmol/L for the second half of dialysis. We discourage the use of zero potassium dialysate solutions unless the patient is severely hyperkalemic and symptomatic. If predialysis serum potassium levels are low or low normal, a potassium bath of 3–4 mmol/L may be required.

Bicarbonate

Acetate was originally used as the buffer in dialysate as it was inexpensive, offered equimolar conversion to bicarbonate, and was bacteriostatic. However, 10% of patients, especially women, are poor metabolizers of acetate. The high plasma acetate levels led to impaired lipid and ketone bodies metabolism, vasodilatation, depressed left ventricular function, intradialytic hypotension, and hypoxaemia, particularly in the first hour [74]. Consequently, most centers switched to sodium bicarbonate.

The preparation of bicarbonate based dialysate requires a second proportioning pump that mixes solution or dry powder bicarbonate to water, and an 'acid' compartment containing a small amount of acetate or lactate, sodium, potassium, calcium, magnesium, chloride and glucose. During the mixing procedure, the acid in the acid concentrate reacts with an equimolar amount of bicarbonate to generate carbonic acid and carbon dioxide. The generation of carbon dioxide causes the final solution pH to fall to approximately 7–7.4. It is this lower pH, combined with the lower concentrations of calcium and magnesium that prevents precipitation from occurring in the final solution. Cartridge systems containing pure, dry sodium bicarbonate powder are often preferred as they are less conducive to bacterial growth, and liquid bicarbonate has to be used within 8 h of opening the container to avoid significant bicarbonate loss.

Dialysis aims to correct the metabolic acidosis of ESKD by the removal of organic anions and restoration of the bicarbonate deficit. Plasma bicarbonate levels rise by 4–5 mmol/L and then fall to predialysis levels by 48 h. The adjusted survival of HD patients decreases with predialysis serum bicarbonate levels <18 mmol/L and >24 mmol/L [11], suggesting a "U" shaped correlation with mortality. The severity of metabolic acidosis also correlates with bone disease [75], muscle wasting [76], and ß2-microglobulin levels [77]. With standard dialysate bicarbonate concentrations of 35 mmol/L, the HEMO study showed that 25% of patients had predialysis levels below 19 mmol/L. [78] Increasing the dialysate bicarbonate concentration to 39–40 mmol/L will improve the predialysis bicarbonate levels but in some will result in a transient alkalosis. This has a hypothetical risk of facilitating calcific uremic arteriolopathy, reducing phosphate removal because of shift of phosphate into cells, and intradialytic vascular instability by causing a sudden drop in the plasma potassium and calcium levels.

Alkalosis has been shown to rapidly reduce dangerously high serum potassium levels, albeit with a potentially increased postdialysis rebound effect [79]. Finally on a more experimental level, the use of citric acid in place of acetic acid in the dialysate acid concentrate was shown to improve both acidosis and delivered dose of dialysis [80]. The role for citrate is expanding in the dialysis community; however, caution is advised as it increases aluminum absorption and therefore plasma aluminum levels must be monitored.

Calcium

Owing to fear of inducing extra-skeletal calcium deposition, KDOQI guidelines suggest maintaining plasma calcium levels in the low normal

range. Using a dialysate calcium concentration of 1.25 mmol/L permits higher doses of vitamin D and calcium based phosphate binders in the management of hyperparathyroidism. In a proportion of patients this can lead to hypocalcaemia and worsening hyperparathyroidism [81]. Hypocalcaemia also depresses myocardial contractility and reduced vascular reactivity [82] and thus increases the risk of intradialytic hypotension. This forms the basis for the short-term use of a higher calcium bath. In our experience, the only situation requiring routine use of 1.5 mmol/L calcium baths are in patients receiving nocturnal HD, who have a reduced need for calcium containing phosphate binders and increased calcium clearance [83].

Phosphate

Phosphate is the major anion in the intracellular compartment and the steep gradient between the intracellular and extracellular compartments is maintained by active transport systems. The factors that limit the removal of excessive phosphate are dialysis clearance and the kinetics of phosphate distribution within the body. During dialysis, plasma phosphate levels initially fall, but thereafter plateau or increase, with a postdialysis rebound effect persisting for up to 4 h [84]. The implication is slow mobilization of phosphate from the intracellular stores and bone and phosphate generation from reserves triggered by falling extracellular [85] or intracellular levels [86]. The point at which phosphate generation is initiated appears to correlate with predialysis phosphate levels. There is also evidence for a "switching on" effect to protect against critically low intracellular phosphate levels.

Phosphate supplementation to dialysate may occasionally be required in severely hypophosphatemic patients with tubulopathies, severely malnourished children who develop hypophosphataemia secondary to refeeding syndrome, and those receiving more frequent, daily, or nocturnal HD.

Magnesium

Typically the concentration of magnesium in dialysate is 0.5–1 mmol/L. If magnesium containing phosphate binders are used, a lower concentration may be required to avoid hypermagnesemia. Conversely low magnesium levels can result in cramping and arrhythmias and therefore higher magnesium baths may help to improve cardiovascular stability and reduce intradialytic symptoms.

Glucose

Glucose concentration of dialysate usually approximates 100–200 mg/dL (6–11 mmol/L). This level of glucose should ensure patients remain normoglycemic unless hyperglycemic or hypoglycemic at the start. If hyperglycemic, a dialysate glucose in the recommended range will remove glucose, and if hypoglycemic the dialysate will provide supplemental glucose. There is a theoretical risk of inducing hypertriglyceridemia by addition of glucose to dialysate but this should not be significant with dialysate values of 100–200 mg/dL. If the patient is hyperkalemic, less potassium might be removed when dialysate glucose is elevated, causing hyperinsulinemia, which pushes potassium into cells. However, this should not be a problem with the dialysate glucose levels recommended above.

Dialysis Flow Rate

Typically, dialysate flow rates of 300–500 ml/min are employed. During infant dialysis, the practice within our unit is to start with a dialysate flow rate of 300 ml/min. If clearance is inadequate, increasing the dialysate flow rate can produce improvements, but eventually plateaus. The HEMO Study provided in vivo confirmation of increased hemodialyzer mass transfer-area coefficients for urea at high dialysate flow rates [87]. A subsequent study showed that the relative gains in spKt/V for increasing the dialysate flow rate

from 300 to 500 ml/min and 500 to 800 ml/min were 11.7% ± 8.7% and 9.9% ± 5.1%, respectively [88].

Dialysate Temperature

By modifying skin blood flow, we can control heat exchange between the body and the environment. This is mediated by two sympathetic nervous system effects, an adrenergic vasoconstrictor and a lesser understood sympathetic vasodilator. During times of increased body core temperature, tonic sympathetic vasoconstriction is relaxed and active vasodilatation is initiated [89] and the skin blood flow rate can increase from a baseline of 5–10% of the total body cardiac output to approximately 60% [90, 91].

Traditionally, dialysate temperatures have been set at ≥37 °C, based to match physiological normal values and to compensate for losses of heat in the extracorporeal circuit. Both of these assumptions have in fact been found to be untrue. In a study of adult HD patients, 62.5% of 128 patients had predialysis body temp below 36.5, with marked inter- and intra-individual differences [92]. There is growing evidence in both adults and children of a net gain rather than loss of heat during dialysis. This is the result of higher resting energy expenditure in HD patients compared to the normal population, especially in those with residual renal function [93]. Secondly, UF activates sympathetic vasoconstriction, reducing skin blood flow and therefore heat exchange, with a direct correlation between UF volume and net heat gain [94]. If the accumulation of heat causes an increase in the body core temperature, UF induced vasoconstriction is overridden by active vasodilatation. Blood is redistributed to the skin [95], and the peripheral vascular resistance falls, resulting in decreased cardiac refilling and hypotension [51]. Fine and Penner [92] showed that dialysis patients with subnormal body temperature (below 36 °C) dialyzed against a 37 °C dialysate had a 15.9% incidence of symptomatic hypotensive episodes, which fell to 3.4% with 35 °C dialysate.

The hemodynamic advantage of "cool" HD has been documented, but may be uncomfortable for patients and reduce urea clearance as a result of compartmental dysequilibrium. Application of thermoneutral (no gain or removal of thermal energy from the extracorporeal circuit) and isothermic (patient temperature is kept constant) dialysis is technically possible, but the dialysis circuit has to be adapted to accommodate a feedback control circuit. A more practical option is to individualize the dialysate temperature based on the patient's predialysis temperature. Even then, efforts may be hampered by the current standards of the Association for the Advancement of Medical Instrumentation (AAMI) that requires the dialysate temperature at the dialyser to be maintained within ±1.5 °C of its set point.

Infants have an increased susceptibility to hypothermia. As a result, infants have traditionally been dialysed against higher dialysate temperatures of 37.5 °C to 38 °C. Alternatively, one may consider more physiological dialysate temperatures with the use of external warming methods to maintain normothermia. The impact of either strategy on thermal balance and cardiovascular stability has not been studied.

Anticoagulation

Anticoagulation of the extracorporeal circuit is usual but not mandatory and should be determined by estimating the risk of bleeding against that of clotting the circuit, which results in blood loss and reduced dialysis efficacy. In children, unfractionated heparin (UFH) remains the agent of choice for systemic anticoagulation, but low molecular weight heparin (LMWH) and citrate have been used.

UFH is a mixture of polyanionic branched glycosaminoglycans that bind with high affinity to antithrombin causing a structural change, converting it from a slow to a very rapidly (1000 times) acting inhibitor of thrombin. It interacts with other components of the coagulation cascade, producing a combined effect of inhibiting fibrin formation and thrombin-induced platelet activation and increasing vessel wall

permeability. The polyanionic nature of heparin allows non-selective binding to other proteins and cell membranes. This mediates the adverse effects associated with UFH use such as activation of lipoprotein lipase causing increased generation of free fatty acids, which can induce platelet aggregation, and loss of bone mass resulting in osteoporosis [96, 97].

UFH has to be administered intravenously as intestinal absorption from oral therapy is poor. Following a bolus injection, the non-specific interactions reduce bioavailability to approximately 30%. Consequently, an initial bolus is usually recommended to saturate these non-specific binding sites as the dose-response relationship becomes almost linear thereafter. UFH is metabolized by the liver, and the kidney clears desulfated fragments. Owing to a marked inter-individual sensitivity to heparin and the possibility of heparin inactivation in the extracorporeal circuit, it is essential to individualize heparin requirements during dialysis and review dosing needs with time (Fig. 66.5).

The consensus on the desired degree of anticoagulation varies amongst different dialysis units, ranging between 25 to 300% above baseline. In our experience, for the majority of patients, adequate anticoagulation is achieved with activated clotting time 50% above the baseline. Standard regimens consist of a bolus dose of 15–20 units/kg of heparin at the start of dialysis followed by a continuous infusion of 15–20 units/kg/h, stopping the heparin infusion over the last 30 min of dialysis. In children weighing less than 10 kg, the likelihood of clotting is increased. Nonetheless, safe, effective anticoagulation with lower activated clotting time target ranges is possible with tight heparin regimens [98].

In high-risk groups, there is a 10–30% risk of bleeding with unfractionated heparin. Alternative options include regional anticoagulation with citrate, use of prostacyclin infusion, high flow rate HD, calcium free dialysate with calcium infusion back to the patient in a closely monitored setting, or modification of the standard heparin regimen. Low dose heparin or

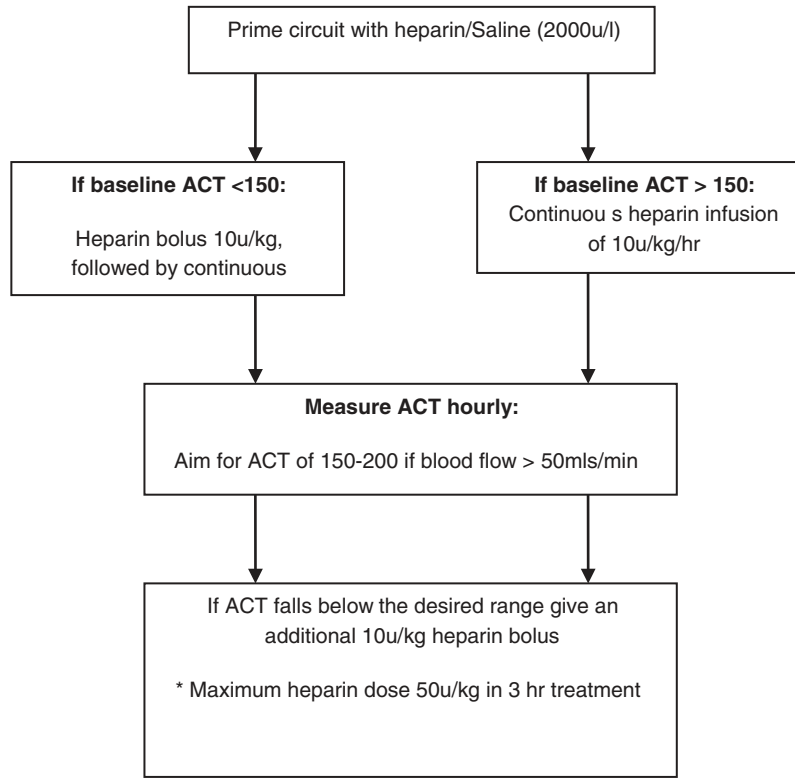

Fig. 66.5 Tight heparin regimen

heparin free dialysis combined with regular intermittent saline flushes is possible without compromising dialysis dose or causing unwanted bleeding complications in children at increased of bleeding [98].

LMWH is composed of smaller molecules prepared from UFH through enzymatic or chemical depolymerization. They act predominantly by inhibiting factor Xa, but also cause a variable degree of thrombin inactivation. Following a single subcutaneous injection, bioavailability reaches 100%, but with differences in inter-individual sensitivity, fixed dosing is inappropriate. LMWHs are principally cleared by the kidney, and therefore in ESKD the drug's pharmacokinetics are unpredictable.

Due to the prolonged half-life in kidney failure and lack of a commercially available antidote, there has been a reluctance to use LMWH. However, several adult trials show that sustained intradialytic anticoagulation can be achieved following a single bolus dose at the start of dialysis, making it a very convenient option. The negative charge of the LMWH complexes makes them impermeable across dialysis membranes and therefore, in spite of their low molecular weight, there is no relevant elimination either through HD or hemofiltration [99, 100]. One meta-analysis comparing the safety and efficacy of LMWH compared with UFH showed no difference in preventing extracorporeal thrombosis and demonstrated comparable bleeding risks [101].

The use of LMHH was first described in children on HD by Bianchetti et al. who successfully hemodialyzed 7 children for an average time of 4 h, using enoxaparin 24–36 mg/M^2 [102]. More recently, Davenport has reviewed the issue of anticoagulation for children on HD and has proposed doses for LMWH in children receiving HD [103].

It has become our routine practice to use LMWH in our home HD population in London. All patients are commenced on 50 units/kg of dalteparin as a single intravenous dose at the start of dialysis. The dose is then adjusted in 20% increments according to percentage of visible clot formation in the dialyser at the end of dialysis, predialysis anti-Xa levels and, in those with fistulae, the presence of prolonged bleeding times after removing fistulae needles. All patients with fistulae are also placed on low dose aspirin. No patient has lost a circuit from excessive clotting. The final dose of dalteparin ranges from 21 to 58 units/kg, with a trend for infants and young children to be on higher doses of dalteparin (52–58 units/kg) compared with teenagers (21–41 units/kg). Those switching from an evening dialysis schedule to nocturnal schedule require on average a 50% increase in their dalteparin dose. The anti-Xa level 1 h after dosing ranged from 0.13–0.6 and predialysis anti-Xa levels suggests no bioaccumulation of dalteparin.

Citrate is a small molecule and is dialyzable, with an extraction coefficient similar to that of urea, and any citrate that escapes into the systemic circulation is rapidly cleared by the tricarboxylic acid pathway, primarily in the liver and skeletal muscle. Citrate exerts its anticoagulant effect by chelating ionized calcium ions, preventing activation of calcium-dependent procoagulants. Regional anticoagulation of the extra-corporeal circuit without systemic effects is achieved by infusing citrate solution through the arterial limb of the circuit, removal of citrate through dialysis and then neutralizing its anticoagulant effect by infusion of calcium into the venous limb of the circuit, making it a very attractive option for patients with a bleeding risk despite a lack of supportive data in children on maintenance dialysis.

Each method of anticoagulation is associated with specific side-effects. Heparin-induced thrombocytopenia is mediated by heparin-dependent IgG antibodies that bind to platelets, causing platelet activation and subsequent risk of thromboembolic events, characterized by markedly increased thrombin levels. Several alternatives to heparin are commercially available, but only danaparoid sodium use has been documented in pediatric HD, reporting stabilization of both thrombocytopenia and thromboembolic risk [104]. However, it has 30% cross-reactivity with platelet-heparin antibodies [105]. The direct thrombin inhibitor, hirudin, is efficacious but its half-life is prolonged in renal failure and it is

associated with anaphylactic reactions [106, 107]. Argatroban, a synthetic direct thrombin inhibitor shows the greatest promise owing to its rapid onset of action, a half-life ranging from 39–51 min, hepatic metabolism, and the fact that it can be used in dialysis patients with no dose adjustment as only a 20% systemic clearance is seen even with high-flux dialyzers. Complications reported with citrate dialysis include hypocalcemia resulting in arrhythmias and paresthesias, hypernatremia, volume expansion, and metabolic alkalosis (one molecule of trisodium citrate is metabolized to 3 molecules of bicarbonate). Citrate toxicity with metabolic acidosis can occur from citrate accumulation due to ineffective dialysis clearance or poor metabolism secondary to impaired synthetic liver function. It is diagnosed biochemically by an increased anion gap acidosis and high total plasma calcium combined with low or normal plasma ionized calcium (so-called citrate lock).

Additionally, evidence suggests a role of citrate in attenuating the chronic inflammatory response to HD, which is linked to atherosclerosis, arteriosclerosis and malnutrition [108]. The use of citrate in pediatrics is growing through its application in plasmapharesis and continuous renal replacement therapy and because its actions are easily neutralized with calcium. These factors make it an attractive option, but until protocols are simplified and validated in children, it cannot presently be recommended as an alternative to heparin for routine dialysis therapy.

Commonly Encountered Hemodialysis Complications

Dialysis Disequilibrium Syndrome (DDS)

Dialysis disequilibrium occurs as a result of changes in osmolarity inducing water shifts from the extracellular to the intracellular compartment across the highly permeable blood brain membrane. It manifests during or immediately after HD as a self-limiting entity, but recovery can take several days. Symptoms typically include nausea, vomiting, headache, blurred vision, muscular twitching, disorientation, hypertension, tremors, seizures and coma, but others such as muscular cramps, anorexia, restlessness, and dizziness have been reported. The diagnosis is often one of exclusion.

The exact pathophysiology of disequilibrium remains unknown, although two mechanisms have been proposed. Both mechanisms support that rapid changes in brain volume disrupt the blood brain barrier and cerebral autoregulation. The reverse urea effect postulates that urea is cleared from plasma more rapidly than from brain tissue, resulting in a transient osmotic gradient and cerebral oedema. The second theory is based on the observation of a paradoxical acidaemia of the cerebral spinal fluid and cerebral cortical grey matter in patients and animals treated with rapid HD. This is accompanied by increased brain osmole activity due to displacement of sodium and potassium ions and enhanced organic acid production. The increased intracellular osmolarity induces fluid shifts with subsequent cytotoxic oedema.

The dialysis prescription can be adjusted to reduce the rate of plasma urea clearance by using a smaller dialyser, decreasing the blood or dialysate flow rate, or switching to more frequent, shorter, treatments. Intradialytic osmotic shifts can be minimized with the use of sodium profiles or higher dialysate sodium concentrations, the substitution of bicarbonate for acetate in the dialysate, or if the patient is grossly fluid overloaded, sequential HD in which an initial period of UF alone is followed by conventional dialysis. Mannitol is an osmotically active solute that artificially increases plasma osmolarity at the time of rapid urea clearance. It rapidly lowers intracranial pressure within minutes of administration and has a peak effect at 20–40 min. A maximal intradialytic dose of 1 g/kg is recommended once a week in high risk patients. If more frequent dosing is required, a smaller dose of 0.5 g/kg is advised, as mannitol accumulates in renal failure (half-life: 36 h) and can cause a rebound rise in the intracranial pressure, especially in the face of acidosis. Other adverse effects include nausea, vomiting, lower limb oedema, thrombophlebitis, headache and chest pain. An alternative to man-

nitol is infusion of 3–5% sodium chloride or the use of higher dialysate sodium baths. Concurrent antiepileptic therapy is required with both therapies if the patient is seizing.

Intradialytic Hypotension

The major barrier to achieving optimal UF is the development of hemodynamic instability, manifesting as intradialytic hypotension. Hypotension occurs in about 20–30% of treatments, can result in underdialysis because of treatment interruptions, and may leave the patient volume overloaded. Frequent hypotensive episodes may accelerate a decline in residual renal function and potentially lead to serious vascular complications such as cerebral, cardiac, and mesenteric ischaemia. In children, the UF goal is often higher because of nutritional supplements or poor adherence to fluid restrictions.

As fluid is removed, plasma refilling, passive venoconstriction and active increases in heart rate, heart contractility and arterial tone are working simultaneously to preserve the effective plasma volume. As a result, even with a UF volume equal to the entire plasma volume, the measured blood volume only changes by 10–20%. Impaired compensatory responses cause hypotension in the face of total body water expansion. Most of the plasma volume resides in the veins, with a marked difference in the venous capacitance between organs. During fluid removal, the ability to mobilize blood from the splanchnic venous pool is vital for preserving the central blood volume. Venous tone is affected by vasoactive hormones, the sympathetic nervous system, and upstream filling pressures. The De-Jager Krogh phenomenon refers to the transmission of upstream arterial pressure through the capillaries to the veins causing venous distension and altered venous capacitance. During arteriolar constriction the distending pressure to the vein is reduced and blood is extruded centrally towards the heart to maintain cardiac refilling. Conversely, factors that cause arterial dilatation, such as antihypertensive medications, increase venous capacitance, reduce cardiac filling pressures and, through transmission of increased hydrostatic pressure to the capillary bed, inhibit vascular refilling. Adenosine is thought to augment splanchnic blood pooling through an inhibitory effect on norepinephrine release and by causing regional vasodilatation. It is hypothesized that during a sudden, but not gradual intradialytic hypotensive episode, ischaemia leads to increased metabolism of adenosine triphosphate and generation of adenosine [109].

The sympathetic nervous system is the principal control mechanism of arteriolar tone and therefore of central BP. Patients with ESKD show increased basal level of peripheral sympathetic activity [110]. In HD patients prone to hypotension, a paradoxical decrease in sympathetic activity is seen at the time of a hypotensive episode [110], which results in a rapid decline in the peripheral vascular resistance and increased vascular bed capacitance. Problems with sympathetic end-organ responsiveness and the efferent parasympathetic baroreceptor pathway have also been reported, but the underlying mechanism remains unexplained. Some believe this may be a heightened manifestation of the Bezold-Jarisch reflex, a cardiodepressor reflex resulting in a sudden loss of sympathetic tone causing abrupt severe hypotension accompanied by bradycardia. It is postulated that conditions associated with reduced cardiac refilling pressures such as LVH, diastolic dysfunction, or structural heart defects stimulate cardiac stretch receptors and thus is a maladaptive variant of the Bezold-Jarisch reflex resulting in hypotension.

The final and interconnecting component relating to intradialytic hypotension is plasma refilling, the movement of fluid from the extravascular to the vascular compartment under the influences of hydraulic, osmotic, and oncotic pressure gradients at the capillary wall. If UF rates exceed refilling rates the intravascular volume will fall. Arterial vasoconstriction decreases hydrostatic pressures in the capillary bed, facilitating vascular refilling. The oncotic pressure, which is effectively the plasma protein concentration, promotes refilling. Plasma sodium and glucose mobilizes fluid from the intracellular space as a result of increased plasma tonicity

[111]. Finally, refilling is facilitated by greater tissue hydration and occurs at a faster rate when the interstitial space is overloaded. Hypovolaemia within the uremic milieu can augment ineffective venoconstriction, inadequate cardiac refilling, reduced plasma refilling and activation of the Bezold-Jarish reflex leading to sudden hypotension.

Haemodynamic stability during dialysis is improved by withholding antihypertensive medications on dialysis days, avoiding food during dialysis, cooling dialysate, using bicarbonate buffers, high sodium dialysate, and treating intradialytic hypocalcaemia. In some patients, the intradialytic BP can be artificially maintained by pharmacological measures. One study demonstrated that prophylactic caffeine administration, an adenosine antagonist, reduced the occurrence of sudden intradialytic hypotensive episodes [112]. A more widely used alternative is midodrine, a prodrug of a specific α-1 adrenergic receptor agonist, desglymidodrine. It maintains intradialytic BP by mediating constriction of both arterial and venous capacitance vessels and preventing venous pooling while increasing the central BP. Administered orally route, it achieves peak levels at 1 h, and has a half-life of 3 h. We have used it in children successfully, starting with doses of 2.5 mg, incrementally increased to 10 mg. A systematic review of 9 trials, using midodrine doses of 2.5–10 mg given 15–30 min before dialysis, reported a benefit in 6 trials with attenuation of the drop in BP during dialysis, and a decrease in number of hypovolaemia related symptoms. No serious adverse events were described, but minor reactions such as scalp paraesthesia, heartburn, flushing, headache, weakness and neck soreness were reported [113].

Modifying the UF rate throughout dialysis to allow adequate vascular refilling may optimize fluid removal. This is the rationale behind UF profiles. The plasma refilling capacity increases proportionately with interstitial volume expansion. Decreasing stepwise or linear profiles start with high UF rates at the time of maximal tissue hydration, progressively reducing the rate in line with decreasing interstitial hydration in the hope of maintaining the crucial balance between fluid removal and vascular refilling. Intermittent profiles aim to provide periods of active mobilization of interstitial fluid into the vascular space when UF rates are low, making it amenable to removal during periods of high UF rates (Fig. 66.6).

Donauer et al. reported less symptomatic hypotension with the decreasing profiles, but the intermittent profile was associated with an increased incidence of symptomatic hypotension and postdialysis fatigue [114]. The incidence of intradialytic hypotension was highest with UF rates greater than 1.5 times the average. Ronco et al. observed hypotension at a rate of 6.7/100 treatments when the UF rate was 0.3 ml/min/kg, increasing to 15.8 at an UF rate of 0.4 ml/min/kg, 25.6 at a rate of 0.5 ml/min/kg, and 67.4 at a rate of 0.6 ml/min/kg [115]. In children, application of these figures would suggest that hypotension

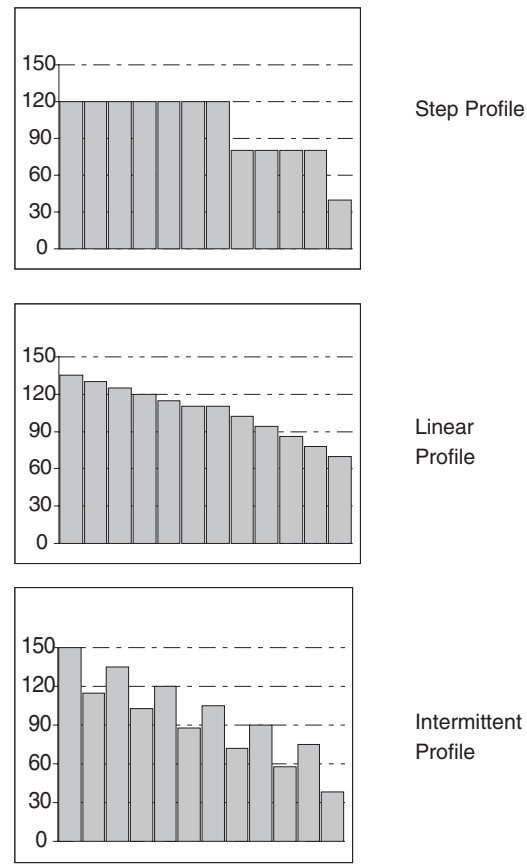

Fig. 66.6 Ultrafiltration profiles

may occur in 25.6 of 100 treatments if a UF rate of 30 ml/kg/h (300 ml/h in a 10 kg child) is exceeded. This data has not been validated in children, but in our experience increasing the UF rate increases the likelihood of intradialytic morbidity. UF profiles will inevitably result in higher UF rate for part of the treatment; the maximal UF rate has to be factored in when considering the most appropriate profile for a patient.

Combining UF profiles with sodium profiles can induce plasma hypertonicity through utilization of a high UF rate during a high-sodium period, and thus provide a greater driving force for plasma refilling. It has been shown to be superior to either sodium or UF profiles alone in attenuating intradialytic symptoms and cardiovascular instability. Finally, the supportive measures for managing hemodynamic instability in high risk patients have a ceiling effect and in resistant cases patients may need to be switched to alternative dialysis programs. HDF, short daily HD, and home nocturnal HD can all potentially be of benefit in these situations.

Myocardial Stunning

Acutely, intradialytic hypotension requires immediate action to stop or reduce the severity of symptoms that may precede or follow the drop in BP. These include a temporary suspension of UF, a 5 ml/kg fluid bolus, and in resistant cases, premature discontinuation of the dialysis treatment. Such measures, although necessary, have an adverse impact on dialysis outcomes by reducing UF goals and adequacy of solute removal. Of greater concern, however, is the evidence linking repeated episodes of intradialytic hypotension with a more severe effect on morbidity and mortality. Several observational studies in adult patients with essential hypertension have described a "J" shaped curve between BP and mortality [116]. The same trend has been described in adult dialysis patients, with a suggestion that hypertension is associated with morbidity, but mortality is more closely associated with hypotension [117]. Zager et al. reported a fourfold increase in the relative risk of cardiac-related death in adults patients with predialysis systolic BP less than 110 mmHg compared with a systolic BP between 140 to 149 mmHg, and a 2.8-fold increase in relative risk for a cardiac-related death with postdialysis systolic BP less than 110 mm Hg compared with systolic BP 140 to 149 mmHg [118].

Frequent intradialytic hypotensive episodes have been implicated in accelerating the decline in residual renal function and precipitating serious vascular complications. There is growing evidence from isotopic, electrocardiographic, biochemical and echocardiographic studies implicating HD as a source of recurrent ischemic injury. Silent intradialytic ST depression [119, 120] associated with acute changes in serum cardiac troponin levels both in adults [121, 122] and children [123] have been demonstrated. Using single photon emission computed tomography, McIntyre et al. demonstrated an acute reduction in global and segmental myocardial blood flow in adults during dialysis with matched reductions in segmental contractile function, even in patients without angiographically proven epicardial coronary artery disease [124]. A direct correlation was seen between the degree of myocardial dysfunction and intradialytic BP changes and UF volume [125]. Such transient myocardial ischemia with resultant reversible regional left ventricular dysfunction is known as myocardial stunning [126]. In the model of coronary heart disease, repeated stunning is progressive and leads to myocardial hibernation, defined as ischaemic, and non-infarcted myocardium that exists in a state of contractile dysfunction [127]. In dialysis patients, myocardial stunning also appears to be progressive. In a 12 month follow-up of adult HD patients, the presence of acute HD induced regional myocardial dysfunction negatively influenced survival, increased the likelihood of cardiac arrhythmias [128], and resulted in regional fixed systolic dysfunction and a reduction in global systolic function [125, 129] with resultant congestive heart failure. Records from the USRDS have shown that HD associated de novo and recurrent congestive heart failure is highly relevant as it is associated with a 2-year mortality as high as 51% [130]. The left atrial volume is commonly driven

by intravascular volume overload and progressive diastolic dysfunction. In a single observational study, the strongest predictor of left atrial volume indexed to height (LAVI) was the presence of stunning. LAVI was a better predictor of mortality than left ventricular (LV) mass index, but both factors became statistically insignificant during a multi-confounder analysis with the addition of myocardial stunning [131].

Of greater concern, perhaps, has been the demonstration of dialysis induced acute regional myocardial dysfunction in 15 children aged 2–17 years. This was associated with varying degrees of compensatory hyperkinesis in unaffected segments and thus the global LV function was maintained throughout HD. In children, intradialytic systolic BP reduction was significantly associated with segmental left ventricular dysfunction, but no correlation was seen with actual intradialytic systolic BP or dialysis vintage [132, 133]. Interestingly, patients on peritoneal dialysis do not appear to have an increased risk of myocardial stunning, despite changes in systemic hemodynamics [134].

We know HD poses a significant hemodynamic challenge. It is conceivable that other vulnerable vascular beds with defective vasoregulation may also be susceptible to significant episodic dialysis-related ischemia. The gut for example is also a high-flow vascular bed. Translocation of endotoxin across the gut wall causes endotoxinaemia and becomes a profoundly pro-inflammatory stimulus. In both children and adults with chronic kidney disease (CKD), circulating endotoxin levels were 1000 times greater than in patients without CKD and almost quadrupled from predialysis levels after initiating HD [135]. Serum endotoxin levels correlated with intradialytic instability, systemic inflammation and dialysis-induced myocardial stunning [135]. One group have even postulated that postdialysis fatigue is a clinical manifestation of cardiac ischemia and cardiac fatigue [136]. The acute cardiac injury that occurs as a direct effect of the HD procedure may be attenuated by altering the dialysis prescription. Cooling, biofeedback and frequent dialysis have all been demonstrated in adults to lower the risk of myocardial stunning [137–139].

Intradialytic or Paradoxical Hypertension

Hypertension is endemic in HD patients and is most often due to salt and volume overload, which responds to UF. The prevalence of hypertension in children receiving HD is reported at 65–69% in studies conducted in Europe and the United States [140, 141]. Intradialytic or paradoxical hypertension is less well-characterized but nonetheless important. Estimates of its frequency are hampered by the lack of a standardized definition. Suggested definitions include an increase in mean arterial pressure of more than 15 mmHg during or immediately after dialysis or an increase in BP that is resistant to fluid removal. Estimates of the incidence in adults range from 5–15%, with no pediatric data available [142].

The pathogenesis of intradialytic hypertension is complex and poorly understood. There may be an iatrogenic aetiology with mobilization of extracellular fluid or in response to osmotic agents such as sodium, mannitol, or concentrated albumin solutions or dialysis induced hypokalemia. Dolson et al. demonstrated significant rebound hypertension at 1 h postdialysis in patients dialyzed against lower potassium baths [143]. In these instances, the hypertension is frequently transient and improves with UF.

Sustained hypertension is commonly due to failure to achieve an appropriate dry weight [144]. However, some patients manifest refractory intradialytic hypertension despite appropriate UF. It is speculated that overzealous UF activates the renin-angiotensin system with resultant vasoconstriction. In support of this theory is the lower incidence of hypertension in anephric HD patients.

Sympathetic nervous system overactivity is well documented in CKD secondary to a number of mediators, including angiotensin II, afferent renal nerve stimulation, impaired brain nitric oxide synthesis and increased production of catecholamines [145–149]. Studies have shown enhanced endothelin I production during dialysis in hypertensive patients, especially those exhibiting paradoxical hypertension [150–152]. This raises the possibility that paradoxical hyperten-

sion is secondary to an imbalance of nitric oxide and endothelin I production [153]. Pearl et al. suggested a role for a new pressor protein a 30-kD extra-renal enzyme related to the coagulation factor β-FXIIa that exhibits cardiotonic and pressor activity in rats. The serum of three anephric children produced characteristic pressor responses, suggesting in vivo activation of this protein as a contributory factor in their hypertension [154].

Finally, a number of antihypertensive drugs are removed by dialysis and this conceivably may result in paradoxical hypertension. As a general rule, the beta blockers (atenolol, nadolol, metoprolol), angiotensin converting enzyme (ACE) inhibitors (captopril, enalapril, lisinopril, ramipril) and vasodilators such as minoxidil, nitroprusside and diazoxide are removed, by a variable degree, during dialysis. Calcium channel blockers such as amlodipine and angiotensin receptor blockers (ARBs), such as losartan, are generally not cleared during HD. No data exists for α-blockers such as doxazosin. The management of intradialytic hypertension should start with an assessment of the dry weight and salt and fluid intake. Treatment options include further salt and water restrictions, and where feasible, augmentation of urine output with loop diuretics. The dialysate composition should be examined for the sodium, potassium and calcium content to ensure that the dialysis procedure does not result in acute hypokalemia or a net transfer of sodium and calcium load. Consideration should be given to replacing conventional HD prescriptions with intensive HD or HDF. If hypertension persists despite appropriate salt and water control, blockade of the renin-angiotensin system with ACE inhibitors or ARBs have been shown to improve BP control and reduce sympathetic tone in HD patients. If this produces insufficient BP control, the addition of α-blockers, β-blockers or centrally acting antihypertensive such as methyldopa is physiologically logical. Attention should be paid to the timing of BP medications to ensure they do not contribute to intradialytic hypotension. Similarly, if drug removal by HD is contributing to suboptimal BP control, consideration should be given to switching to an agent that is not significantly removed by dialysis such as calcium channel blockers. Finally, the incidence of hypertension in dialysis patients appears to have increased in the post-erythropoiesis stimulating agent (ESA) era. This may relate to increased viscosity, increased peripheral vascular resistance or a direct effect of ESAs on the vascular endothelium. While there are no published studies showing a direct relationship between hemoglobin and hypertension, effort should be made to avoid excessive hemoglobin values in patients with intradialytic hypertension.

Left Ventricular Hypertrophy

LVH is common in dialysis patients. At the initiation of dialysis, 69–82% of children show evidence of LVH [155] and during maintenance dialysis 40–75% of children have LVH [156]. Several factors increase the risk of developing cardiac hypertrophy, including chronic hypervolemia, a hyperdynamic circulation secondary to arteriovenous fistulae or anemia, increasing arterial stiffness and elevated parathyroid hormone (PTH) levels [157]. There is emerging evidence from established animal models of CKD implicating a klotho-independent, causal role for FGF23 in the pathogenesis of LVH. This raises the possibility of FGF23 being directly involved in the high rates of LVH and mortality in HD patients [158]. Somewhat surprisingly, both adult data and now pediatric data from the ESCAPE trial have failed to demonstrate any relationship between office BP or ambulatory BP and left ventricular mass [159].

Cardiac hypertrophy in combination with continued mechanical stress triggers pathways that result in myocardial remodeling characterized by decreased capillary density and reduced coronary flow reserve predisposing the heart and other organs to ischemic injury. Fortunately, cardiac hypertrophy in HD patients is amenable to treatment, with evidence of resolution in patients on intensified dialysis programmes and HDF.

Endothelial Dysfunction

Endothelial dysfunction is thought to be the initiating step in atherosclerosis and arteriosclerosis. It starts early in renal failure, progressing in dial-

ysis as a number of pathophysiological pathways contribute. HD is pro-inflammatory as a consequence of an immune mediated response to bio-incompatible membranes, blood contact with non-sterile dialysate solution and "back-leaking" of dialysate across the membrane. UF changes endothelial cell dynamics through its effects on blood viscosity and laminar shear stress [160]. Intradialytic hypotension and resultant ischemia causes apoptosis of the vascular endothelium. Finally, reduced clearance of asymmetric dimethylarginine, decreased bioavailability of endothelial nitric oxide, activation of angiotensin II, hyperhomocystinemia and hyperlipidemia are postulated mechanisms for endothelial dysfunction. Compounding these effects, uremia is also associated with reduced hematopoiesis and capacity for repair. In adults, endothelial progenitor cells are reduced, with pronounced functional impairment [161, 162], and HD depletes this source further. In contrast, the pool of smooth muscle cell progenitor cells are preserved and with it the potential for adverse remodeling [163]. Little is known about circulating endothelial progenitor cells in children, but there is clinical evidence of endothelial dysfunction with loss of flow-mediated dilatation and increased aortic pulse wave velocity in children on dialysis [164, 165]. Encouragingly, the degree of endothelial injury is attenuated by switching adults HD patients to either HDF or home nocturnal HD [166, 167].

Sudden Cardiac Death

Sudden cardiac death is a common phenomenon in dialysis patients that appears to be temporally related to the HD procedure. In adults, the risk of sudden death is 1.7 times higher in the 12 h period starting with the dialysis procedure and 3 times higher in the 12 h before HD at the end of the weekend interval [168]. Cardiac arrests are 50% higher for HD patients 3 months after dialysis initiation. The risk remains higher in HD compared with peritoneal dialysis for up to 2 years on maintenance dialysis, but then the trend reverses at 3 years of maintenance dialysis. The most vulnerable patients are infants aged 0–4 years, with a five- to ten-fold increase risk of cardiac arrest compared to other age groups [169].

The precise aetiology of sudden cardiac death remains elusive but a number of dialysis specific and uremic factors have been implicated. Myocardial interstitial fibrosis, LVH, endothelial dysfunction, cardiac and vascular calcification, microvascular disease with decreased perfusion reserve and diminished ischemia tolerance are all prevalent in dialysis patients and increase the vulnerability of the heart. This, in combination with dialysis related acute fluid shifts, acid-base disturbances, rapid electrolyte shifts and autonomic imbalance with abnormal sympathetic activity, places patients at risk of sudden cardiac death.

Clinically the only modifiable risk factor for fatal cardiac events is manipulation of the dialysate potassium. Patients who suffered a cardiac arrest at the time of dialysis were twice as likely to be dialyzed against a 0 or 1.0 mEq/L potassium dialysate compared to controls, despite no difference in predialysis potassium levels [170]. Kovesdy et al. found that serum potassium between 4.6 and 5.3 mEq/L was associated with the best survival, but levels below 4.0 mEq/L or higher than 5.6 mEq/L were associated with increased mortality [171]. As a result, there is a growing consensus of nephrologists advising against zero potassium dialysate baths.

Atherosclerosis, Arteriosclerosis, and Calcification

Calcification of the cardiovascular system is accelerated in dialysis patients. Studies of young adults who developed ESKD during childhood found a high prevalence of abnormal carotid intima-media thickness (cIMT), diminished arterial wall compliance and coronary artery calcification [172, 173]. Such vascular and cardiac aberrations were also demonstrated in children on dialysis [174]. The vascular abnormalities positively correlated with serum phosphorus levels, while cIMT and cardiac calcification scores also correlated with intact PTH levels and dosage of vitamin D. Patients with mean intact PTH levels greater than twice the upper limit of normal

demonstrated stiffer vessels and increased cIMT and cardiac calcification scores. In contrast, 1,25-dihydroxy vitamin D levels showed a U-shaped distribution, with a significantly greater cIMT and calcification score in patients with low and high 1,25-dihydroxy vitamin D levels compared with patients with normal levels. Calcification was most frequently observed in patients with the lowest 1,25-dihydroxy vitamin D and the highest high-sensitivity C-reactive protein (CRP) [175]. Litwin et al. reported vascular abnormalities in children with CKD, but again found the most marked changes in the dialysis patients. The degree of arteriopathy correlated with conventional cardiovascular disease risk factors such as hypertension and dyslipidemia in predialysis CKD and with hyperphosphatemia, hyperparathyroidism and treatment with calcium-containing phosphate binders in dialysis patients [176]. In contrast, in a study examining the effects of dialysate calcium concentrations on changes in arterial stiffness, increased pulse wave velocity was seen even in the group dialysing using the lowest dialysate calcium [177]. Therefore, it is highly probably that factors other than simple net calcium influx and efflux during dialysis are involved in the pathogenesis of accelerated vascular calcification in HD patients.

Inflammation

Inflammation predicts mortality in dialysis patients and may contribute to cardiovascular risk. CRP, an acute phase protein, is a recognized marker of inflammation, but is also reported to be predictive of mortality, structural heart changes such as LVH, and higher coronary calcification scores. Data has also implicated CRP in the pathogenesis of vascular inflammation and atherosclerosis [178]. Plasma CRP levels increase with declining kidney function and then continue to rise after initiation of HD, with levels correlating with the length of the dialysis session [179]. It has been postulated that an interaction of circulating monocytes with bio-incompatible membranes, blood contact with non-sterile dialysate solution and "back-leaking" of dialysate across the membrane results in a chronic inflammatory state. However because there is a high incidence of pre-dialytic inflammation [180], the dialysis procedure is unlikely to be the only factor associated with inflammation [181]. Changes in CRP may also represent an acute inflammatory stimulus [182]. Additionally, vitamin D deficiency has been correlated with inflammatory cytokine levels (IL-10 and SIL-2R) in children receiving HD [183].

The dialysis prescription can be modified to become less inflammatory by using ultrapure dialysate and synthetic biocompatible membranes. Both ACE inhibitors and statins, more commonly recognized for their respective roles in treating hypertension and hypercholesterolemia, have been reported to have anti-inflammatory actions [184, 185]. Finally, lifestyle and dietary changes may be associated with decreasing inflammation and uremic toxins (p-cresyl sulfate and indoxyl sulfate), although data is limited in children [186].

Malnutrition

Protein malnutrition and growth delay commonly occurs in underdialyzed patients and may be associated with mortality in children [187, 188]. Measurement of the nPCR has become an indirect measure of daily protein intake in stable dialysis patients. Measurement of nPCR has traditionally relied on the availability of formal urea kinetic modeling and is included with the web-based programs (www.hdcn.com, and www.Kt-v.net) alluded to above. Goldstein, however, has demonstrated strong agreement between nPCR calculated from urea kinetic modeling and the formula [nPCR = 5.43 × G/V + 0.17] [187]. This calculation requires a blood urea nitrogen (BUN) level 30 s after a mid-week dialysis session, documentation of the time until the next dialysis session, and a BUN value prior to the second dialysis session. In this formula the urea generation rate (G) is calculated as G (mg/min) = (predialysis BUN2 × predialysis V) – (postdialysis BUN1 × postdialysis V)/T, where V is total body water estimated from 0.58 × body weight, and T

is time in minutes from the end of the mid-week dialysis treatment to the beginning of the next dialysis treatment. Validation of this formula has eliminated the need for complicated computer modeling in order to measure nPCR and estimate daily protein intake. A subsequent report, which compared the values of nPCR calculated as above with a simplified formula using only pre- and postdialysis BUN specimens from the same mid-week session has found there is a significant and variable difference between the two methods and invalidates the simplified formula [189].

Although nPCR values are a useful guide to protein intake, because nPCR values may be influenced by factors other than nutrient intake, these values should be interpreted in combination with a review of weight gain and the dietary history. Goldstein et al [190] demonstrated a substantial increase in nPCR associated with improvement in nutritional status of three adolescents treated with intradialytic total parenteral nutrition. However, Van Hoek et al. in a comparison of protein intake from dietary records kept by children, with an estimate of nPCR calculated using an on-line urea monitor, showed significant variation, and PCR significantly underestimated the prescribed and recorded protein intake [33]. These authors concluded that use of their online urea kinetic monitor is therefore not recommended for estimation of nPCR. Also, as reported by Grupe et al. [191], nPCR may be significantly affected by factors other than nutrient intake in as many as 25% of patients.

The safety of enteral intake during dialysis should be assessed on a patient-by-patient basis as blood is diverted to the splanchnic circulation, potentially increasing the risk of intradialytic hypotension. In patients able to tolerate enteral intake during dialysis, it offers an opportunity to provide nutritional supplements. Intradialytic parenteral nutrition is an alternative method of providing calories and protein to undernourished patients during HD. While this increases the amount of fluid needed to remove, utilizing a constant UF to parallel the infusion can minimize excessive UF rates. Use of recombinant growth hormone is another important means of maximizing growth in children on HD, assuming their nutritional status has first been optimized [192].

Dialysis-Related Carnitine Disorder

Levocarnitine (L-carnitine) facilitates the transport of fatty acids across the inner mitochondrial membrane and is thus a critical co-factor for normal energy production in cardiac and skeletal muscle. There is evidence of reduced plasma free carnitine levels in HD patients, with an inverse relationship between muscle carnitine and duration on dialysis [193]. Within a single dialysis session, clearance is 30 times greater than would be expected in a healthy individual [194] and HD results in an abnormal acylcarnitine:free carnitine ratio (normal <0.25).

Low carnitine may be associated with anaemia that is hyporesponsive to ESAs, intradialytic hypotension, cardiac dysfunction, fatigue, muscle cramping, and reduced exercise tolerance [195]. The National Kidney Foundation Interdisciplinary Consensus Panel recommends L-carnitine [196] supplementation for those patients with these clinical findings even in the absence of a low plasma carnitine levels. As measuring skeletal muscle L-carnitine concentrations is not feasible, some advocate a trial of therapy with discontinuation at 9–12 months if no benefits are observed [196]. Repeated doses of 20 mg/kg given intravenously at the end of dialysis appear to be the most beneficial, as oral carnitine is not recommended in ESKD due to the toxicity of metabolites which accumulate in kidney failure.

Hyperhomocysteinaemia

Homocysteine is a non-protein forming amino acid that results from methionine metabolism. Only 1–2% of total homocysteine circulates freely in the blood in a reduced form, 70–90% is protein bound, and the rest exists in oxidized forms. Studies have shown that plasma homocysteine concentrations start rising in CKD and are inversely related to glomerular filtration rate.

ESKD results in hyperhomocysteinaemia from altered metabolism and impaired clearance. There is conflicting evidence on the impact of hyperhomocysteinemia on outcomes. A meta-analysis reported a positive association between hyperhomocysteinemia and atherosclerosis, ischemic heart disease, stroke, and thrombosis [197]. Conversely, others have found no significant or even an inverse association between plasma homocysteine levels, cardiovascular events, and mortality in ESKD patients [198]. In addition to being a potential marker of inflammation, homocysteine, like β2-microglobulin, can also be a marker of middle molecule clearance in patients receiving more frequent, longer duration, or convective treatment modalities (detailed below).

Treatment options for hyperhomocystinaemia have included folate and vitamin B12 supplementation to achieve supranormal plasma levels and intravenous N-acetylcysteine [199]. An alternative therapeutic strategy involves using high-flux dialysers to achieve greater clearance, but this has not impacted predialysis plasma concentrations [200]. With these therapeutic options, plasma homocysteine levels improve, but normalization is uncommon.

Future Directions

Hemodiafiltration

Conventional HD is based on diffusive transport of solutes across a semipermeable membrane and is effective in removing small uremic retention solutes, such as urea, and correcting electrolyte and fluid imbalances. However, as clearance is inversely proportional to the molecular weight of the toxin (and also depends on its protein binding and tissue distribution), conventional HD does not clear large or protein-bound toxins effectively, and fails to adequately correct the uremic milieu [201, 202]. In HDF, solute clearance involves a combination of diffusion and convection. HDF optimizes the removal of middle (up to 300–500 Dalton (Da) molecular weight) and larger molecules (greater than 15–50 kilo Da).

Definition of HDF Therapy

The European Dialysis Working Group (EUDIAL) has defined HDF as a blood purification therapy that combines diffusive and convective solute removal by UF of 20% or more of the blood volume processed through a high-flux dialyzer and maintenance of fluid balance by sterile replacement fluid infused directly into the patient's blood [202, 203]. Sterile replacement fluid is obtained in large amounts by on-line filtration of standard dialysate through a series of bacteria- and endotoxin-retaining filters [203]. A high-flux membrane is defined as one that has an UF coefficient greater than 20 mL/h/mmHg transmembrane pressure/m^2 and a sieving coefficient for β2-microglobulin of greater than 0.612.

Importance of Convective Volume

A high convective volume is a fundamental requirement for HDF [202, 204]. The convective volume is the sum of the net UF volume (i.e., the amount of fluid removed during a dialysis session based on the interdialytic weight gain) and the amount of substitution fluid (i.e., the sterile replacement fluid given as replenishment for the removal of extra fluid during HDF).

The effectiveness of a membrane to UF fluid is described by the UF coefficient (KUF):

$$KUF = QUF/\Delta P$$

(volume of UF per unit time, divided by the pressure gradient across the membrane, also called the transmembrane pressure gradient [TMP])

Theoretically, the convective volume should be prescribed as a proportion of plasma water volume processed rather than blood volume processed, but blood volume processed is displayed on the machine control panel, and hence this term is commonly used. An UF volume equivalent to 20% of the total blood volume processed for the treatment was chosen by the EUDIAL group because randomized controlled trials in adults [205–207] and a pooled individual participant data analysis [208] suggest that any improved

survival associated with HDF occurs when the convective volume exceeds 20 L/session.

Depending on where the replacement volume is infused in the dialysis circuit, there are different modalities of HDF: pre-, post-, mid- or mixed-dilution HDF. Post-dilution, where the replacement fluid is infused downstream of the dialyser, usually into the venous bubble-trap, is the most commonly performed.

The phenomenon of 'backfiltration,' which commonly occurs during high-flux HD [209] (but not in low-flux HD), can achieve some convective clearance, but this is low and unreliable, and called the 'poor man's HDF'! Backfiltration occurs because the hydrostatic pressure of both blood and dialysis fluid decrease as they pass through the dialysis filter. Since blood and dialysis fluid pass through the filter in counter-current directions, the resulting TMP may become negative at the venous side, especially when the venous blood pressure is low. In adults it has been shown that the convective volume achieved by back-filtration is no more than 1–10 L per session depending on the dialyser type, and can vary throughout the dialysis session depending on TMP. Importantly, given the phenomenon of backfiltration, it has been suggested that dialysis fluid used for high-flux HD should also be sterile and pyrogen-free.

Essential Requirements for Performing HDF

High Flux Membranes

Only highly permeable membranes, defined as membranes characterized by an UF coefficient (KUF) greater than 20 mL/h/mmHg transmembrane pressure/m^2 and a sieving coefficient (S) for β2-microglobulin of greater than 0.6 [202, 203, 210], are suitable for HDF. A high-flux membrane allows a larger and pre-defined convective flow as required for HDF. In practice, the KUF should be high enough to allow 50 mL/m/m^2 BSA (equivalent to 2 mL/min/kg body weight) convective flow in post-dilution HDF. The albumin loss through a high-flux membrane should be <0.5 g in a 4-h HD session [210–212].

As with conventional HD, the dialyser surface area must be equal to (or slightly higher) than the BSA for maintenance dialysis, so that the internal volume of the dialyser and blood lines is less than the safe extracorporeal blood volume permissible (i.e. less than 10 ml/kg body weight). For HDF a biocompatible dialyzer must be selected; biocompatibility is assessed by complement activation, thrombogenicity, contact activation and cytokine generation [63, 211]. European recommendations state that ultrapure dialysate must be used with synthetic high-flux membranes [63].

Ultrapure Water for HDF

Sterile, non-pyrogenic fluid that is used to maintain fluid balance is called replacement fluid or substitution fluid. This can be provided as a sterilized, packaged solution (like intravenous fluid) or as an on-line prepared solution. In all modern HDF machines, the replacement fluid is generated on-line by filtering dialysis fluid through bacteria- and endotoxin-retentive filters to prepare a sterile and pyrogen-free solution that is immediately infused into the patient. Strict safety standards and regulatory oversight are required as large volumes of fluid are removed from, and added to, blood during on-line therapies. Water purification systems are discussed separately in this chapter. Bacteria- and endotoxin-retentive filters installed on the inlet dialysis fluid circuit are the key components of the on-line HDF safety system. Those filters are disinfected after each dialysis treatment according to manufacturer's recommendations and replaced periodically to ensure proper operation of the cold sterilization process.

Dialysis Machines with Accurate Ultrafiltration Control

Today almost all new dialysis machines allow for both HD and HDF. These machines are suit-

able for children from 10–17 kg body weight, and require a pediatric circuit with low extracorporeal volumes. These systems can be used in a pressure-control mode (fixed TMP and variable substitution flow rate) or a volume-control mode (the target substitution volume or the substitution flow rate are set). Some machines offer an automatic substitution mode, in which the substitution rate is automatically regulated in response to variations in patient- and treatment-related parameters throughout the session.

Writing a HDF Prescription

The following points need to be considered when writing an HDF prescription:

1. A high-flux membrane with surface area equal to the child's BSA is used.
2. Double needle circuits—HDF is rarely ever performed with single-needle circuits given the high convective volume goals.
3. The total extracorporeal circuit should be less than 10 ml/kg body weight. Pediatric blood lines (36–105 ml volume) with or without the possibility to do on-line HDF and to monitor blood volume variation are available.
4. Blood flow: HDF requires an optimal arterial blood flow of 5–8 mL/min/kg body weight or 150 to 250 mL/m^2 BSA per minute. An optimal blood flow can be achieved through either a fistula or a central venous line, although in most cases a fistula allows a higher blood flow rate. Both the diffusive clearance of molecules with a high K0A and the substitution volume in post-dilution HDF depend on the blood flow rate.
5. Dialysate flow of twice the blood flow is sufficient to optimize the diffusive blood purification process using highly permeable membranes for HDF.
6. Replacement fluid that is generated on-line from the dialysate must be ultrapure (<0.1 CFU/ml and <0.03 endotoxin unit/ml, as per European guideline Dialysate purity 2002, European Pharmacopoeia 2009) [63] as discussed earlier. The microbiologic purity (bacterial count and endotoxin level) should be determined at intervals of 1–3 months.
7. Convective flow is equal to total UF flow, that is, the sum of the desired UF volume and the replacement fluid. The convective flow needs to be maximal, but is limited by the risk of the filter clotting. Modern dialysis machines automatically adjust the convective volume throughout the session in order to optimize this convective flow without increasing the coagulation risk. In pre-dilution HDF, the convective flow is set at 100% of blood flow. The actual substitution volume obtained per session has to be monitored regularly in order to ensure that the goal of 23 L/1.73 m^2 per session in post-dilution and 75–100% of blood volume treated in pre-dilution is achieved.
8. The dialysate and substitution fluid are produced 'on-line' by the dialysis machine by dilution of acid concentrate and bicarbonate powder with dialysis water produced by the water treatment system. Dialysate composition is similar to that used in HD, but careful attention to dialysate sodium concentration is important in order to maintain sodium balance and for hemodynamic tolerance of the session [63]. To avoid the risk of positive sodium balance, the dialysate sodium concentration required is lower than in conventional HD, particularly when high convective volumes are infused, as with pre-dilution HDF. Sodium is predominantly drained in ultrafiltered water by convection [213]. A low dialysate sodium enables additional sodium removal by diffusion, but it may be associated with a risk of intradialytic hypotension and disequilibrium syndrome. Conversely, a high dialysate sodium increases hemodynamic tolerance, but causes sodium and water overload that leads to hypertension and increased thirst post-session.
9. Anticoagulation to prevent filter clotting can be achieved with a single dose of LMWH that is effective for a 4-h session. Alternatively, a continuous heparin infusion may be used.

To attain a high convective volume, one needs a high blood flow rate because filtration fraction depends on blood flow and cannot be higher than 35%; optimization of substitution volume by automated programs in new dialysis machines; and careful monitoring of the dialysis prescription and blood results to ensure that all dialysis related parameters are achieved [214–216]. In the HDF, Hearts and Height (3H) study (described in detail below), median convection volumes of 13.4 L/m^2 were achieved in children [217], which is comparable to the 23 L per 1.73 m^2 per session that proved beneficial in the pooled adult studies [208]. Importantly, the convection volume was independent of patient related factors such as age, gender, access type or dialyser used, but strongly correlated with the blood flow rate [217], implying that convection volume is a modifiable factor that can be manipulated and optimized by the dialysis team.

Potential Advantages of HDF Over Conventional HD

HDF is thought to be superior to conventional HD in the following key areas:

1. Clearance of toxins across a wide molecular weight range: HDF has been shown to clear 70–80% of β2-microglobulin compared to HD [218], and increase removal of inflammatory cytokines, with reduction in inflammation and oxidative stress [219].
2. Improved hemodynamic stability: HDF increases UF and improves intradialytic hemodynamic stability [220], leading to less intradialytic hypotension [221], reduced incidence of strokes [221] and faster recovery time postdialysis, even in children [222].
3. Biocompatibility and reduced inflammation: The use of ultrapure dialysate and increased removal of inflammatory cytokines reduces inflammation and oxidative stress [219].

Clinical Studies

A Cochrane review suggests that there is no clear benefit of HDF over HD, but these meta-analyses combine outcomes of both hemofiltration as well as HDF studies as 'convective therapies' and do not interpret outcomes based on convective volumes [223]. The Estudio de Supervivencia de Hemodiafiltración On-line (ESHOL) randomized controlled trial (RCT) comparing HDF vs. high-flux HD in adults, and achieving convective volumes of 23 L/session, has shown that patients on high volume HDF have a survival benefit compared to those on high-flux HD [206]. Pooled data [208] from the three RCTs (including the Turkish [207] and CONTRAST [205] studies) has indicated a critical dose-response relationship between the magnitude of the convective volume and survival, with a goal of at least 23 L per session.

In children, Fischbach et al. showed improved growth, with children achieving a normal height at or above their target mid-parental height [224, 225], reduced inflammation [225], regression of LVH [226, 227], improved anaemia control [214] and reduced postdialysis recovery time [222, 224] in a small number of children undergoing daily HDF. However, this study utilised 6 days per week HDF in the pre-dilution mode, making it difficult to ascertain the benefits of HDF vs. longer dialysis therapy times. A small single-centre study also suggests that switching children from nocturnal in-centre HD to nocturnal in-centre HDF may significantly improve BP, phosphate and PTH control [228]. Further single-centre studies have shown improvements in left ventricular function within a short period of HDF therapy [229]. Studies have shown that when HD patients are switched to HDF, keeping all other dialysis related parameters constant, a significant improvement in inflammation, antioxidant capacity and endothelial risk profile is achieved within 3 months [219], suggesting that even in children who have a short anticipated time on dialysis, HDF is superior to conventional HD. A report from the Italian Registry suggests that HDF use in Italy has been limited to approxi-

mately a quarter of patients on extracorporeal dialysis, in particular to those with high dialysis vintage, younger age or a long expected waiting time to renal transplantation [230].

The International Paediatric Hemodialysis Network (IPHN) has performed a multicenter observational study to test the hypothesis that HDF improves the cardiovascular risk profile, growth, nutritional status and health-related quality of life in children compared to conventional HD—the HDF, Hearts and Height (3H) study [217, 222]. The 3H study suggests that HDF halts the progression of increasing carotid intima-media thickness, is associated with an increase in height standard deviation score and improves patient-related outcomes compared to HD [222]. Children on HDF had improved blood pressure and hemodynamic stability, reduced inflammatory markers and lower β2-microglobulin compared to children on HD [222]. The annualised change in vascular measures correlated with improved BP control and clearances on HDF. In a post-hoc analysis of the 3H data, it was shown that children on HD had a significant and sustained increase in BP over 1 year compared to a stable BP in those on HDF, despite an equivalent dialysis dose.Improved fluid removal as well as clearance of middle molecular weight uraemic toxins by HDF were strongly correlated with improved vascular outcomes in HDF [231]. Although mechanisms of improved growth in HDF are not clear, the 3H study showed an inverse correlation between height SDS increase and serum β2-microglobulin, suggesting that clearance of middle-molecular weight compounds may partly alleviate growth hormone resistance in dialysis patients. In the HDF cohort, patient related outcome measures that are primarily associated with fluid status, such as the postdialysis recovery time, headaches, dizziness and cramps, were less frequent and severe compared to HD patients, leading to an improvement in school attendance as well as physical activity [222]. Importantly, HDF is a safe treatment, with no reduction in serum albumin levels, and no difference in the rate of change of residual renal function [222] compared to conventional HD.

Intensified Hemodialysis Options

Our current standard of three times weekly hemodialysis evolved during the 1960s from one 24 h treatment per week, to twice weekly treatments of 16–24 h each, and finally three weekly sessions of 8–10 h performed at home. By the time United States Medicare program adopted its ESKD program in 1973, the three times per weekly schedule offered the optimal balance between patient outcome, quality of life, and cost. Remarkably, the first patient treated with these prolonged sessions lived for more than a decade. More than 50 years later, similar outcomes have become difficult to achieve [232].

To improve the health of patients receiving three times weekly in-center hemodialysis, researchers have trialed alternative, intensified dialysis regimens. These methods include short daily hemodialysis, nocturnal home hemodialysis, and in-center nocturnal hemodialysis. In 2010, the Frequent Hemodialysis Network, sponsored by the National Institutes of Health, published their findings in 245 adults randomized to three times versus six times per week in-center hemodialysis. After 1 year of treatment, those in the more frequent group averaged about 5 sessions per week and demonstrated improvements in mortality, LVH, reported physical health, hypertension, and hyperphosphatemia. However, they were also more likely to receive vascular access interventions compared to the three times per week treatment group [11]. A treatment benefit was not observed among adults randomized to 6 times per week home nocturnal hemodialysis [233].

Smaller, uncontrolled studies in children have also supported the potential benefits of intensified dialysis regimens. As mentioned above, Fischbach et al. reported their experience treating 12 children (median age 7 years) in France with 5–6 times per week in center HDF. After a median follow-up of 11 months, dietary restrictions could be lifted and BP and phosphate control improved [234]. Observations by the same group have also noted decreased LVH and postdialysis fatigue with six times per week treatments [225]. In children, weekly treatment times of 15–18 h have

been associated with improved growth [224, 227, 235, 236].

The success noted by the investigators in France has prompted others to trial alternative dialysis regimens in children. Four children treated at The Hospital for Sick Children had improved dietary intake, BP control, and school attendance with home nocturnal hemodialysis [237]. Calcium and phosphate metabolism improved, even requiring dialysate phosphate supplementation during therapy [83]. These experiences have been expanded to a larger group of children in Europe, resulting in no postdialysis recovery time and improved energy and quality of life. Patient selection criteria for home therapy have included weight >20 kg, adequate family support and supervision, and the necessary technology to operate the dialysis equipment [237, 238]. While the therapy may represent a 30% cost savings compared with in-center treatment, the requirements for staffing and family resources are high [237]. Future technology may allow home HD to be performed in infants.

In the United States, a published report of four children treated with 6 times per week, in-center or home hemodialysis used the NxStage™, which provides sterile dialysis fluid without the requirement for home modifications for a reverse osmosis water treatment system [239, 240]. After a 16 week pilot study, these children no longer needed antihypertensive therapy and had improved BP control as measured by 24 h ambulatory monitoring, without reporting treatment-related complications [240].In London, we have dialysed 15 children aged from 3 to 17 years on the NxStage™ system. We are currently using 3 circuits: the standard CAR172 circuit, CAR124 for those developing intradialytic thrombocytopenia and CAR125 for children weighing less than 18 kg. Routinely children start on 5 h of dialysis 4 evenings/week (except infants) [241]. From 2 months onwards, we discuss the possibility of switching to nocturnal HD where appropriate. All children report reduced or no postdialysis recovery times, greater energy and improved quality of life scores and vastly improved school attendance, social and family lives. All the children on 20 h or more of dialysis/week are free of diet and fluid restrictions; appetites have improved with better growth. Cardiac echocardiograms were normal at baseline in 6/11, in the 5 remaining signs of LVH and/or fluid overload had regressed within 6 months. PTH levels were successfully maintained within twice upper limit of normal in all except 2 teenagers who became calcium deficient on 1.5 mmol/l calcium dialysate baths [241]. In our experience, one advantage that intensified home HD holds over all other dialysis treatment is in the management of patients with ESKD and cardiac failure, where intensified dialysis prescriptions have resulted in the complete resolution of the cardiac failure [242].

Combining the benefits of more frequent treatments, the quality of life advantages offered by nocturnal therapy, and the safety and convenience of monitoring available in the clinic, investigators in Germany have reported their experience in 16 children and adolescents treated with in-center, nocturnal hemodialysis. Children were prospectively enrolled over an almost 5 year period and there were over 2000 treatments provided. Participants were treated 8 h per session for 3 days per week and each treatment was monitored by a pediatric nephrologist and 2 dialysis nurses. During the study, quality of life improved and children missed fewer days of school. Nutrition indices improved, phosphate levels decreased, and the number of antihypertensive medications decreased compared to matched control patients [243].

While pediatric studies have been mostly uncontrolled and have included a small number of subjects, most demonstrate that intensified hemodialysis is associated with improved quality of life, biochemical markers (especially phosphate clearance), growth, nutrition, and BP control [244]. Nevertheless, significant barriers exist, precluding the more wide-spread adoption of these potentially beneficial options for children with ESKD. These include financial hurdles related to treatment costs, transportation, missed work for parents, and equipment and supplies for those choosing home therapy [244]. Missed time from school and other social activities is a concern for children treated with non-nocturnal,

more frequent in-center hemodialysis. We must also be mindful of patient, caregiver, and provider burnout in a patient population already struggling with the management of a very significant chronic disease [237, 239, 241, 243].

It is clear that change is needed to improve the health and survival of children with ESKD. Health-related quality of life remains poor in children with CKD, especially in those receiving HD, in whom daily life activities are greatly limited [245]. While the data suggest that the current treatment is not optimal and more intensified treatment may offer benefit, we must also not minimize the subjective input of our patients. To this end, a 16 year old female, after being switched to three times per week long nocturnal hemodialysis, noted, "regular dialysis was hell and I never want to go back to it again." [243]

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Immunosuppression in Pediatric Kidney Transplantation

67

Burkhard Tönshoff, Anette Melk, and Britta Höcker

Introduction

Ideally, a recipient (host) would accept a kidney transplant without induction of an antigen-specific response. However, it is not currently possible to induce specific immunologic tolerance, and transplantation requires immunosuppressive therapies. The goal is to use immunosuppressive agents that are not only potent and selective, but allow reversibility of their action, can be reliably delivered and display long-term safety. Most therapies alter immune response mechanisms but are not immunologically specific, and a careful balance is required to find the dose that prevents rejection of the graft, while minimizing the risks of over-immunosuppression leading to infection and cancer.

Current immunosuppressive agents reduce acute rejection, but do not induce tolerance. A few patients with organ transplants can successfully withdraw their immunosuppressive therapy without rejecting their grafts for long periods of time. However, these are rare exceptions, and such patients may eventually reject, even after years as this operational state of prope tolerance is unstable and poorly understood. The hunt for biomarkers to harness this "operational" tolerance state has remained elusive [1]. Even though antigen-specific T cells with reactivity to the foreign antigen persist in the host indefinitely, some graft and host adaptation must occur, since the level of immunosuppression required long-term is very low compared to the levels required within the first weeks post-transplant. This adaptation makes long-term immunosuppression possible; however, the long-term risk of cancer in the immunosuppressed patient remains increased. Thus, the distinction between immunosuppression and tolerance induction is partly artificial: any immunosuppression involves some apparent antigen-specific adaptation, i.e. down-regulation of the host response to the graft; and many tolerance protocols involve some non-specific immunosuppressive therapies.

The goal of immunosuppressive therapy is to prevent acute rejection while minimizing drug side effects. In children who undergo renal transplantation, immunosuppression is divided into 3 categories: (i) Induction therapy, i.e. intense immunosuppression administered during the perioperative period to prevent acute rejection, (ii) maintenance therapy, i.e. immunosuppressive treatment to prevent acute rejection after the peri-

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operative period, and (iii) immunosuppressive therapy to treat graft rejection. In general, immunosuppression should be most intense during the first 3 months after transplantation when the risk of acute rejection and allograft loss is greatest. Immunosuppression is tapered slowly to a maintenance level by 6–12 months post-transplant. The goal remains to find the best combination of immunosuppressive agents that optimizes allograft survival by preventing rejection episodes while limiting drug toxicities. Although data from adult renal transplantation trials are used to help guide management decisions in pediatric patients, immunosuppression must often be modified because of the unique clinical effects of some of these agents in children, including their impact on longitudinal growth and development.

Allograft survival rates vary among the various immunosuppressive agents due to patient-specific clinical characteristics, such as age, ethnicity, obesity, hyperlipidemia, hypertension, proteinuria, delayed allograft function and some donor-related factors such as living-related versus deceased kidney donation. Immunosuppressive agents should therefore be chosen in part based on patient characteristics. Other issues to be considered are related to the immunologic history of the patient, such as ABO compatibility and the degree of HLA matching, pre-sensitization, re-transplantation, history of acute rejection episodes and the risk of recurrent disease.

The common immunosuppressive agents used in pediatric renal transplantation include glucocorticoids (steroids), azathioprine (AZA), mycophenolate mofetil (MMF), the calcineurin inhibitors tacrolimus (TAC) and ciclosporin (CSA), the mammalian target of rapamycin (mTOR) inhibitors sirolimus (SRL) and everolimus (EVR), antibodies to cell surface antigens on lymphocytes, including anti-thymocyte globulin (ATG), anti-CD25 antibodies (anti-interleukin-2 [IL-2] receptor antibodies), alemtuzumab (a humanized anti-CD52 pan-lymphocytic monoclonal antibody) and belatacept, a co-stimulation blocker of T cells. The structures of some immunosuppressives are shown in Fig. 67.1 [2]. Our discussion will focus on these agents and how they inhibit the immune response.

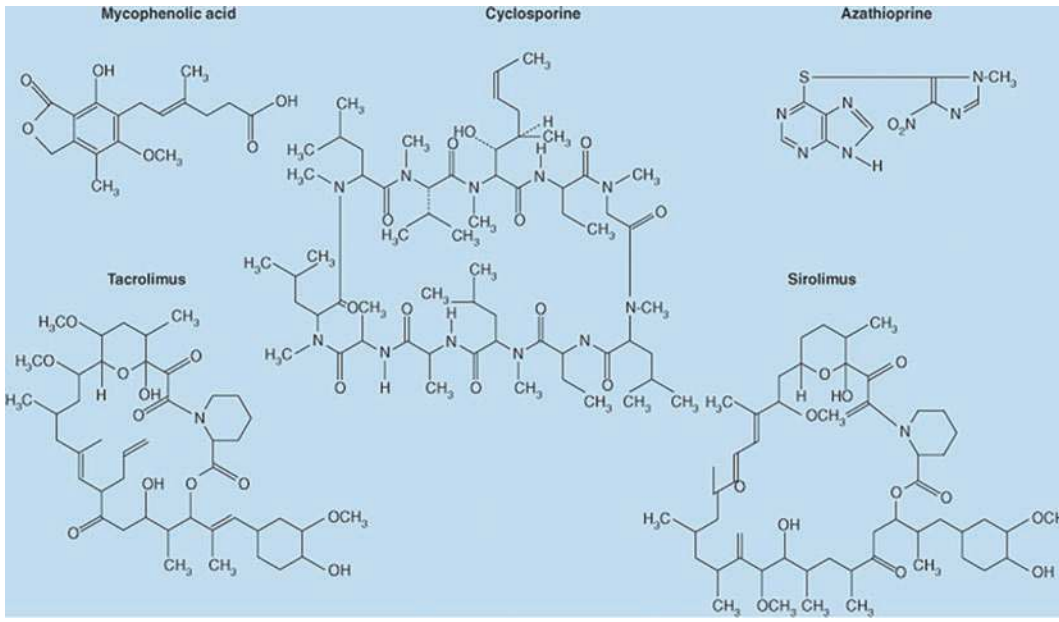

Fig. 67.1 Structure of mycophenolic acid, ciclosporin, azathioprine, tacrolimus, and sirolimus. These are all small molecules with molecular weights of 320, 1203, 277, 804, and 914, respectively. (Used with permission from Johnson RJ, Feehally J (eds): Comprehensive Clinical Nephrology, 2nd ed. Philadelphia: Elsevier; 2003) [2]

The Immune Response

By the time transplant surgery is completed, the graft has undergone acute injury leading to an increased expression of major histocompatibility complex (MHC) molecules by cells within the graft that are either constitutively expressed (class I, HLA-A and HLA-B) or inducible (class II, HLA-DR) antigens. Injury recruits lymphocytes and antigen-presenting cells (APCs), typically monocytes, macrophages, and dendritic cells from the host. These injury-related events may influence the probability of rejection and thereby contribute to the superior outcome in transplants from live donors (with less injury) vs. those from deceased donors.

Allorecognition of donor MHC molecules may occur either by the direct route (host T cells recognize donor MHC on donor cells) or indirectly (host T cells recognize donor MHC as peptides in the MHC groove of host APCs). T cell receptors (TCR) engaging MHC–peptide complexes provide signal 1. Co-stimulatory signals from the APC engaging receptors on the T cells provide signal 2 (Fig. 67.2) [3]. The major co-stimulatory molecules of the APC are B7–1/B7–2, which bind

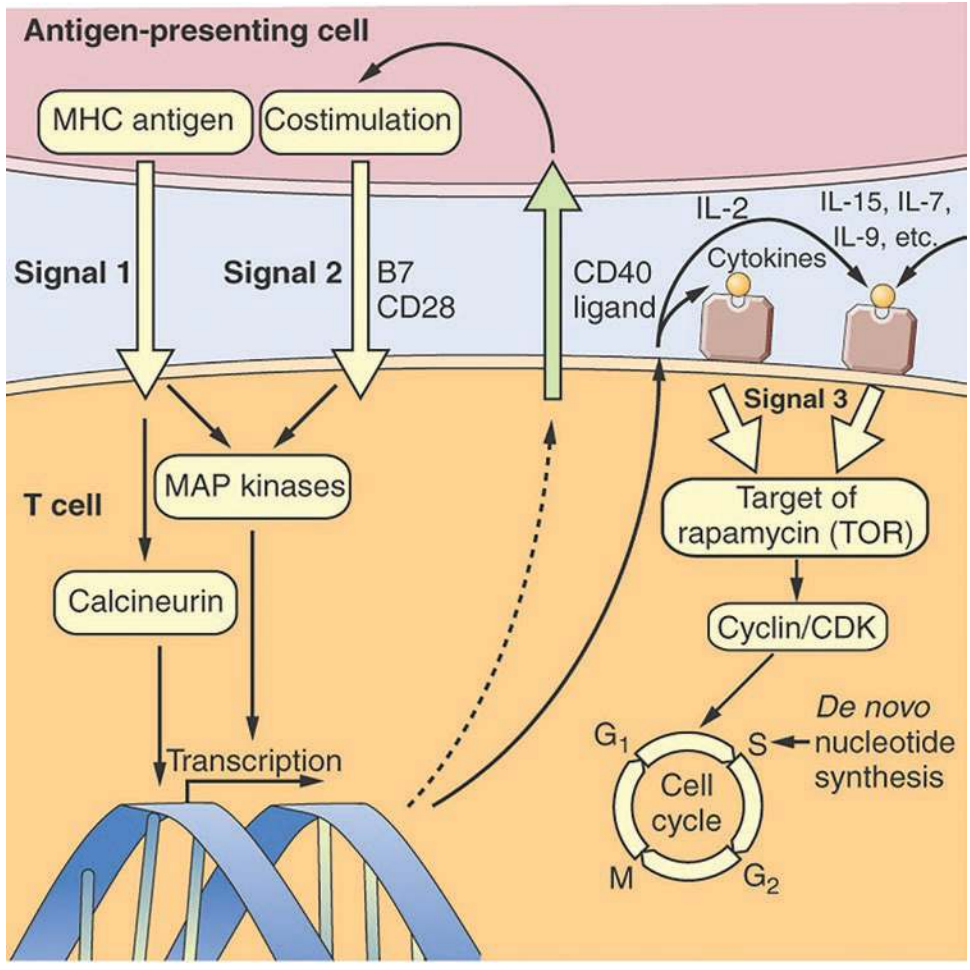

Fig. 67.2 The 3 events in T cell activation. Engagement of the T cell receptor with the antigenic peptide in the context of self–MHC class II molecule leads to the activation of the calcineurin pathway and results in the induction of cytokine genes (e.g., IL–2) (signal 1). Signal 2, the co-stimulatory signal, involves the engagement of CD28 with members of the B7 family. This synergizes with signal 1 to induce cytokine production. Interaction between cytokine production and its corresponding receptor leads to induction of cell division, probably through the target of rapamycin (TOR) pathway. This constitutes signal 3. (Used with permission from Feehally J, Floege J, Johnson RJ (eds.): Comprehensive Clinical Nephrology, 3rd ed. Philadelphia: Mosby, 2007) [3]

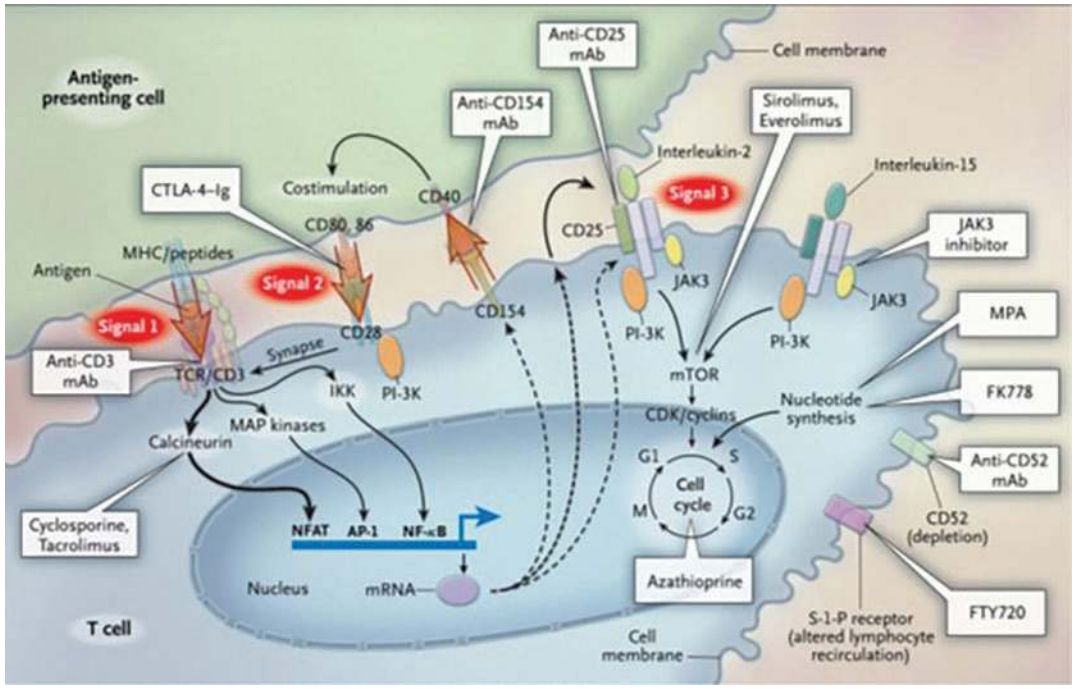

Fig. 67.3 Individual immunosuppressive drugs and sites of action in the 3-signal model. Anti-CD154 antibody has been withdrawn from clinical trials but remains of interest. FTY720 engagement of sphingosine-1-phosphate (S-1-P) receptors triggers and internalizes the receptors and alters lymphocyte recirculation, causing lymphopenia. Antagonists of chemokine receptors (not shown) are also being developed in preclinical models. MPA denotes mycophenolic acid. (Reproduced with permission from [4])

CD28 on the T cells. Activated T cells express CD40L that can activate the APC by engaging CD40 on the APC, and Fas ligand (FasL), which binds Fas on other lymphocytes or other cells to induce apoptosis in the Fas-bearing cell. Activation of signals 1 and 2 is followed by T cell activation with production of many cytokines. Cytokines, such as IL–2, engage specific receptors on the T cells to provide signal 3, the signal for cell division and clonal expansion. The engagement of CD40 by CD40L and the cytokines and growth factors from T cells regulate the T cell response, recruit and activate inflammatory cells, and alter adhesion molecules to cause mononuclear cells to accumulate in the graft. Depending on the type and degree of signaling, full activation of the T cell may occur, or T cells may undergo partial activation, apoptosis, anergy, or neglect (ignoring the antigen). T cells also bind *via* CD40L to CD40 on B cells, thereby directing the switch from IgM to IgG production by B cells and promoting the maturation of IgG-producing B cells.

Chemotactic factors (chemokines) and expression of adhesion proteins and foreign (MHC) antigens mediate localization (homing) of CD4 and CD8 T cells to the graft endothelium. Lymphocyte recirculation depends on the ability to enter and leave lymphoid tissue. CD8 T cells that recognize peptide in the groove of class I MHC become cytotoxic T cells (CTL). Graft rejection is associated with infiltration by cytotoxic CD8 lymphocytes. Delayed type hypersensitivity may also be involved in T cell-mediated damage. Antibody-mediated injury may also occur and causes damage to endothelium. A summary of the effects of immunosuppressive drugs is presented in Fig. 67.3 [3].

Classification of Immunosuppressive Agents

Immunosuppressive and immunomodulatory drugs can be pharmacologically categorized on the basis of their mechanism of action. The 3-signal

model of T cell activation and proliferation is helpful in understanding the molecular mechanisms and site of action of various immunosuppressive drugs. Figure 67.3 depicts a schematic representation of the 3-signal model along with the site of action of common immunosuppressive drugs [4]. Signal 1 features APCs (macrophages and dendritic cells) presenting the foreign antigen to the T lymphocyte, activating the TCR, which further relays the signal through the transduction apparatus known as the CD3 complex. Signal 2 is a non-antigen-specific co-stimulatory signal which occurs as a result of binding of the B7 molecule on the APC to CD28 on the T cell. Both signal 1 and signal 2 activate signal transduction pathways: the calcium–calcineurin pathway, mitogen-activated protein (MAP) pathway, and the nuclear factor-κB (NF-κB) pathway. This in turn leads to increased expression of IL-2, which through its receptor (IL-2R) activates the cell cycle (signal 3). Signal 3 activation requires the enzyme mTOR for translation of mRNA and cell proliferation. Thus, various drugs act on different cellular signals and achieve immunosuppression by a number of mechanisms: depleting lymphocytes, diverting lymphocyte traffic, or blocking lymphocyte response pathways.

Induction Immunosuppressive Therapy

Induction refers to the administration of an intensive immunosuppressive regimen during the perioperative period. The rationale behind this approach is that the risk of acute rejection is greatest in the first weeks and months after transplantation. Induction therapies often involve the use of polyclonal or monoclonal antibodies to achieve rapid and profound early immunosuppression. Polyclonal antibodies used for this purpose include those against thymocytes (e.g., commercially available rabbit or equine preparations); monoclonal antibodies include basiliximab (a chimeric human–murine anti-CD25 or anti–IL-2R antibody) and alemtuzumab (an anti-CD52 antibody targeting both B and T cells), which is not always readily available.

A number of trials have been and are being conducted in adult and pediatric renal transplant recipients to look into the effects of prophylactic antibody induction therapies. Evaluation of any induction protocol requires consideration of the following factors: (i) Incidence and severity of delayed allograft function or primary non-function, including post-transplant dialysis requirements; (ii) incidence of acute rejection; (iii) incidence, type, and severity of associated infections; (iv) long-term allograft survival and function; (v) mortality and morbidity, including length of hospitalization, (vi) cost, and (vii) incidence and type of malignancy during long-term follow-up. Several studies from single centers and registries, as well as meta-analyses, have found that induction with antibodies may be superior to non-antibody-based regimens, especially in high-risk groups [5]. Unfortunately, most if not all published studies have addressed only some of the above issues. As a result, although each protocol may have specific advantages and disadvantages in a particular patient population, none is yet proven to be superior when all the above factors are considered. The optimal prophylactic induction immunosuppressive therapy to prevent renal transplant rejection remains therefore controversial. Figure 67.4 presents the induction antibody use from 2007–2018, as reported in the Scientific Registry of Renal Transplant Recipients (SRTR) 2018 annual report, indicating that administration of ATG has increased while that of anti-IL-2R and no induction have decreased over time. This is in contrast to reports from the Cooperative European Paediatric Renal Transplant Initiative (CERTAIN) Registry showing that 60% of pediatric kidney transplant recipients in Europe do not receive any induction therapy, 35% receive basiliximab and 5% ATG [6]. Hence, the frequency and type of the chosen immunosuppressive anti-lymphocyte regimens for induction therapy vary markedly between North America and Europe and probably reflect differences in patient characteristics and estimated immunological risk, but is unfortunately not based on comprehensive clinical trials.

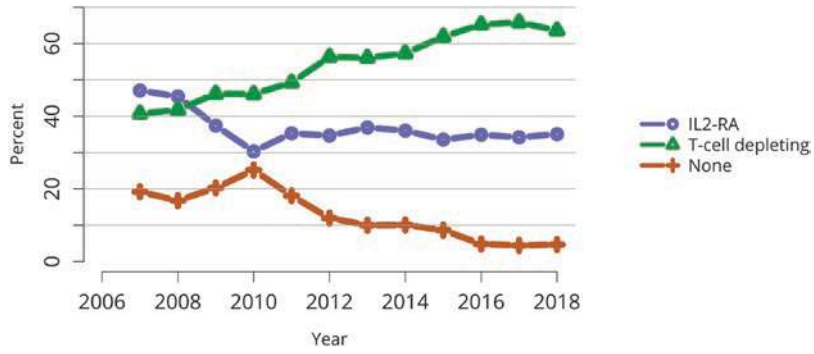

Fig. 67.4 Induction agent use in pediatric kidney transplant recipients in the United States of America. Immunosuppression at transplant reported to the Organ Procurement and Transplantation Network (OPTN) (https://pubmed.ncbi.nlm.nih.gov/31898417). IL2-RA, interleukin-2 receptor antagonist

In fact, induction therapy produces the greatest benefits in groups at high risk of allograft rejection. These high-risk groups include African-Americans, recipients of kidneys with prolonged cold ischemia time, and those at high immunologic risk, particularly individuals who are presensitized. The sequential induction regimen of thymoglobulin followed by a combination of TAC and MMF with or without steroids is recommended in these high-risk groups.

Lymphocyte Depleting Antibodies

Polyclonal Antibodies

Because of the redundancy of the immune system, polyclonal antibodies, which have a broad specificity, should theoretically be more effective in induction therapy than monoclonal anti-lymphocyte agents. Anti-thymocyte globulin (ATGAM) is a purified gamma globulin solution obtained by immunization of horses with human thymocytes. It contains antibodies to a wide variety of human T cell surface antigens, including the major MHC antigens. Thymoglobulin is a rabbit-derived polyclonal antibody preparation approved for the treatment of rejection and induction therapy by the US Federal Drug Administration (FDA). As for ATGAM, thymoglobulin contains antibodies to a wide variety of T cell antigens and MHC antigens.

Polyclonal antibodies act in 3 ways: by activating or altering the function of lymphocytes, by lysing lymphoid cells, and by altering the traffic of lymphoid cells and sequestering them. These antibodies are potently immunosuppressive, but often produce side effects. By triggering T cells, they generate significant first-dose effects, with the release of tumor necrosis factor alpha (TNFα), interferon γ (IFN-γ), and other cytokines, causing a first-dose reaction (flu-like syndrome, fever, and chills).

Dosage of Thymoglobulin

Thymoglobulin induction is usually dosed from 1 to 6 mg/kg per dose, and the duration may range from 1 to 10 days, although a more typical regimen is 1.5 mg/kg per dose for 3–5 days [7–10]. In animal models, higher initial doses of shorter duration approximating a human-equivalent dose of 6 mg/kg were associated with more peripheral and central lymphocyte depletion and better allograft survival [11]. Based on these models, the optimal induction dose is felt to total 6 mg/kg [9, 12]. Total doses of 5.7 mg/kg, on average given as 1.5 mg/kg per day, have been shown to produce similar outcomes as higher doses in high-risk recipients who received an average of 10.3 mg/kg [10]. Higher

doses and prolonged duration of induction agents are thought to be associated with an increased risk of infection and the potential development of lymphoma, whereas cumulative doses of less than 3 mg/kg may not effectively prevent acute rejection [13].

Efficacy and Safety

Few studies have compared the relative efficacy of thymoglobulin and ATGAM for induction therapy. In one study in adult renal transplant recipients, 72 patients were randomly assigned in a double-blind 2:1 fashion to receive intravenous doses of thymoglobulin at 1.5 mg/kg or ATGAM at 15 mg/kg intra-operatively, then daily for at least 6 days [14]. The delayed graft function rate was only 1 percent for both groups. At 1 year, the thymoglobulin group had a significantly lower acute rejection rate (4% vs. 25%, respectively) and higher allograft survival (98% vs. 83%). The lower rejection rate was thought to be due in part to a more sustained lymphopenia with thymoglobulin, while the exceptionally low delayed graft function rate seen in both groups may have been due to the intra-operative use of the ATGs. Both antibodies have the ability to block a number of adhesion molecules, cytokines, chemokines, and their receptors, which may contribute to ischemia reperfusion injury and delayed graft function. At 5 years, allograft survival was significantly better in the thymoglobulin arm (77% vs. 57%, respectively) [15]. Two cases of post-transplant lymphoproliferative disorder (PTLD) developed with ATGAM, while none were observed with thymoglobulin. The mean 5-year serum creatinine concentration was similar in both groups.

In pediatric renal transplant recipients, a historical cohort study compared the rates of survival, rejection, and infection in patients who received induction therapy with ATGAM (n = 127) or thymoglobulin (n = 71) [16]. Maintenance immunosuppression included CSA, AZA or MMF, and prednisone. Mean follow-up was 90 ± 25 months for ATGAM recipients and 32 ± 15 months for thymoglobulin recipients. Overall, the incidence of acute rejection was lower in thymoglobulin recipients vs. ATGAM recipients (33% vs. 50%, P = 0.02). Epstein-Barr virus (EBV) infection was higher in thymoglobulin recipients versus ATGAM recipients (8% vs. 3%, P = 0.002). But the two groups did not significantly differ in patient and graft survival rates, incidence of chronic rejection, EBV lymphoma, or other infections. The authors concluded that thymoglobulin induction was associated with a reduced incidence of acute rejection and an increased incidence of EBV infection in pediatric renal transplant recipients.

IL-2 Receptor Antibodies

Full T cell activation leads to the calcineurin-mediated stimulation of the transcription, translation, and secretion of IL-2, a key autocrine growth factor that induces T cell proliferation. Thus, an attractive therapeutic option is abrogation of IL-2 activity via the administration of anti-IL-2R antibodies. Currently, only basiliximab, a chimeric monoclonal antibody, is commercially available and has been approved by the FDA for use in renal transplantation in adults and pediatric patients (Fig. 67.5). The IL-2R consists of 3 transmembrane protein chains: CD25, CD122, and CD132. CD25 is present on nearly all activated T cells, but not on resting T cells. IL-2 induces clonal expansion of activated T cells. Although CD25 does not transduce the signal, it is responsible for the association of IL-2 with the β- and γ-chains, which triggers the activated T cell to undergo rapid proliferation. This antibody binds to activated T cells and render them resistant to IL-2 by blocking, shedding or internalizing the receptor; it may also deplete and sequester some activated T cells. However, IL-2R functions are partially redundant because other cytokine receptors have overlapping functions, e.g., IL-15R. Therefore, saturating IL-2R produces stable, but relatively mild immunosuppression, and is only effective in combination with other immunosuppressants.

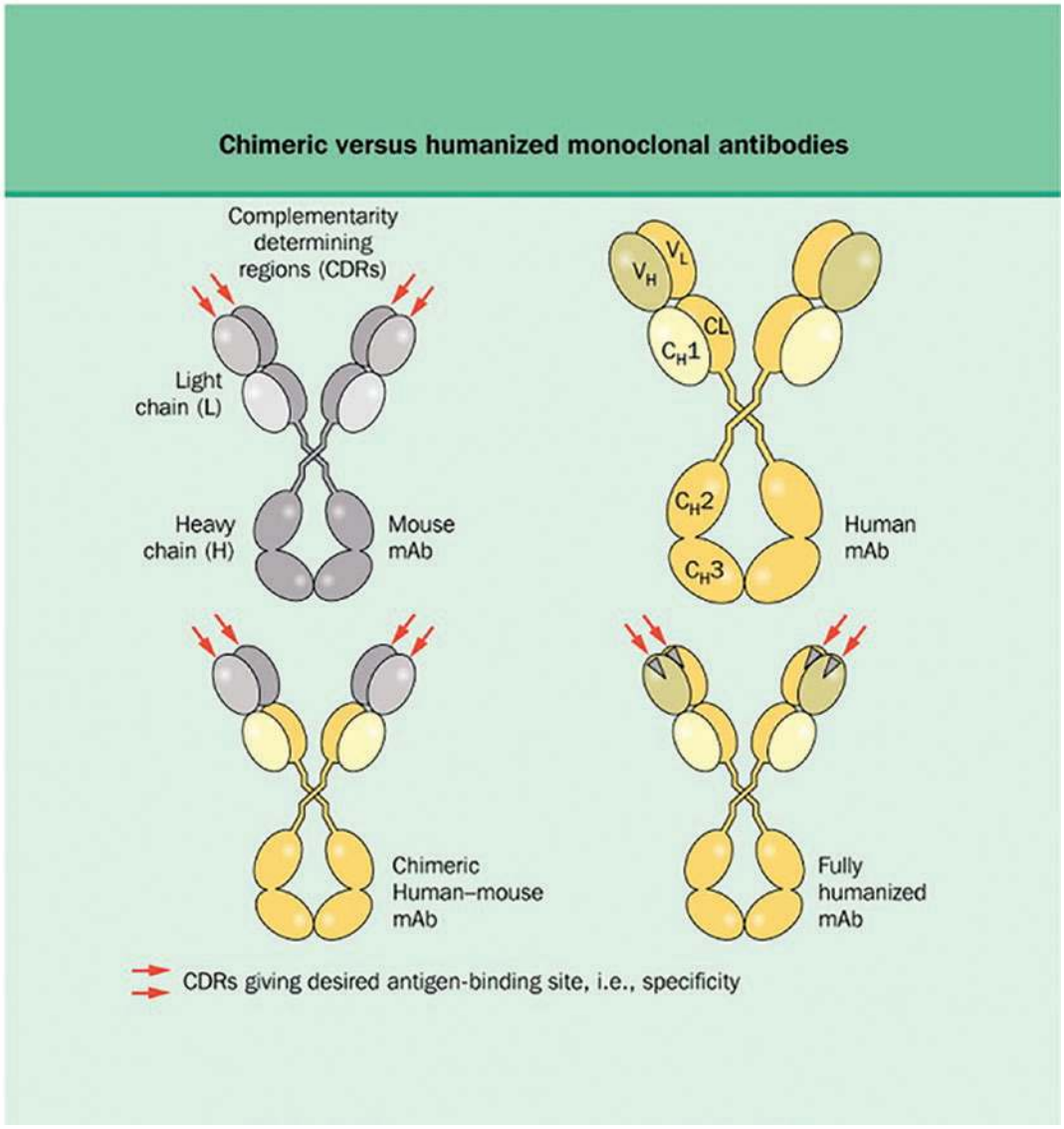

Fig. 67.5 Chimeric vs. humanized monoclonal antibodies. (Used with permission from Feehally J, Floege J, Johnson RJ (eds.): Comprehensive Clinical Nephrology, 3rd ed. Philadelphia: Mosby, 2007) [3]

Dosage of Basiliximab

The dosing schedule for basiliximab is the following: intravenous administration of two 10 mg doses to children <35 kg in weight and two 20 mg doses to children ≥35 kg in weight, with the first dose given within 2 h prior to surgery, and the second on post-transplant day 4. In the 14 patients who were evaluated for the pharmacokinetics and pharmacodynamics of basiliximab and received concomitant immunosuppression with CSA and AZA, the mean duration of IL-2R saturation was 42 ± 16 days [17]. In a larger study of 82 patients who received basiliximab in combination with MMF, MMF reduced basiliximab clearance and thereby prolonged CD25 saturation from 5 to 10 weeks [18].

Efficacy and Safety

The effectiveness of IL-2R antibody therapy was best reported in a meta-analysis involving 38 tri-

als that enrolled nearly 5000 patients that assessed the impact of therapy on allograft loss and rejection [19]. Data were derived from published trials and abstracts of completed and ongoing trials. From these 38 trials, 14 trials enrolling 2410 patients compared IL-2R antagonists with placebo for at least one outcome. Compared with placebo, IL-2R antagonists reduced acute rejection rates at 6 months (relative risk [RR 0.66], CI 0.59–0.74) and 1 year (RR 0.67, CI 0.60–0.75), but the incidence of graft loss was the same.

In pediatric renal transplant recipients, two large prospective randomized controlled trials showed that induction therapy with basiliximab in patients with low to medium immunological risk on maintenance therapy (TAC in conjunction with AZA, or CSA in conjunction with MMF) did not lead to a statistically significant reduction in the incidence of acute rejection episodes [20, 21]. As a result, there is presently no consensus amongst pediatric renal transplantation centers regarding the use of and regimen for immunosuppressive induction therapy. Considerations in choosing the appropriate agent include the efficacy in the patient population (e.g., recipients with high or low risk of graft loss), the side effect profile, and the concomitant immunosuppressive therapy (steroid avoidance, early steroid withdrawal, or conventional steroid therapy).

Comparison of Basiliximab with Thymoglobulin

Few studies have compared the use of different induction immunosuppressive regimens. In adult patients at increased risk of acute rejection, thymoglobulin is more effective than basiliximab in preventing rejection [22–25]. A multicenter, international, randomized, prospective study of 278 first deceased-donor kidney transplant recipients compared the safety and efficacy of a 5-day course of thymoglobulin (n = 141) or 2 doses of basiliximab (n = 137) [26]. Recipients and donors were chosen based upon characteristics that would predict an increased risk of rejection or delayed graft function. Patients in both arms were administered CSA, MMF, and prednisone for maintenance immunosuppression, and received antiviral prophylaxis with ganciclovir. The primary endpoint was a composite of acute rejection, delayed allograft function, transplant loss, and death. At 1 year, there was no difference between thymoglobulin and basiliximab in the incidence of the composite endpoint. However, thymoglobulin was associated with a significantly lower acute rejection rate (16% vs. 26%), and incidence of acute rejection that required antibody treatment (1.4% vs. 8%). Although overall adverse event and serious adverse event rates were similar, thymoglobulin was associated with a higher incidence of infection (86% vs. 75%), but lower incidence of cytomegalovirus (CMV) disease (8% vs. 18%).

At 5-year follow-up, the incidence of acute rejection and need for antibody treatment of acute rejection remained lower among those treated with thymoglobulin, compared with basiliximab (16% vs. 30% and 3% vs. 12%, respectively) [22]. Patients treated with thymoglobulin also had a significantly lower composite endpoint of acute rejection, graft loss, and death at 5 years (39% vs. 52%) and incidence of treated CMV infection (7% vs. 17%); however, the incidence of malignancy did not differ. Hence, the relative benefits of thymoglobulin were sustained over a 5-year period.

Alemtuzumab

Alemtuzumab (Campath-1H, MabCampath) is a humanized IgG1 monoclonal antibody directed against CD52, a glycoprotein expressed on mononuclear cells, including T and B lymphocytes, monocytes, and natural killer cells [23]. Its efficacy for induction and maintenance immunosuppression with low-dose calcineurin inhibitors (CNIs) was first introduced by Calne et al. [24] and later supported by the Pittsburgh group [25] in adults and children. Alemtuzumab induction has been associated with lower rates of acute rejection than basiliximab and daclizumab in low immunological risk patients and was associated with similar efficacy as compared with rabbit anti-thymocyte globulin in high-risk patients [26]. Although most of these studies have involved adult kidney transplant recipients, there is also pediatric experience that supports this efficacy claim, especially in highly sensitized, high-risk children [27, 28]. A significant

reduction of white blood cell and absolute lymphocyte counts, up to 1 year post-transplant, has been observed in children receiving alemtuzumab treatment [29].There is no currently recommended dose for children, but 0.3 mg/kg per dose has been most frequently used in pediatric studies [30]. The most common number of doses administered was 2 doses, with a range from 1 to 4 doses during the first week post-transplant. Due to infusion-related reactions, pre-medication with methylprednisolone, acetaminophen, and diphenhydramine is recommended in addition to administration of anti-emetics to avoid nausea and vomiting. The major side effect that limits the use of alemtuzumab is profound lymphopenia, which may contribute to significant adverse events, including infections, autoimmune complications, and malignancies [30]. The Cooperative Clinical Trials in Pediatric Transplantation program of the National Institute of Allergy and Infectious Disease has completed a multicenter pilot trial of alemtuzumab induction in pediatric kidney transplant recipients with initial maintenance immunosuppression of TAC and MMF, but the results have yet to be published.

Recommendations

There is currently no consensus for immunosuppressive induction therapy following kidney transplantation in children. At the present time, no consistent evidence exists that induction therapy is beneficial or cost-effective in low-risk patients on triple therapy with CNIs in conventional doses, MMF and steroids. According to 2 prospective, randomized, controlled trials, induction therapy with basiliximab in pediatric patients with low or normal immunological risk on maintenance therapy with either TAC in conjunction with AZA or CSA in combination with MMF did not lead to a statistically significant reduction in the incidence of acute rejection episodes [20, 21]. In contrast, the Kidney Disease: Improving Global Outcomes clinical practice guidelines recommend induction therapy for all adult patients, with an IL-2R blocker as first line for those not at high immunological risk [31]. There is some evidence favoring the use of IL-2R blockers over no induction in adult renal transplantation [32]. However, it has been pointed out in the more recent literature that these studies mainly used outdated maintenance regimens [33]. No large randomized trial has examined the effect of IL-2R antibodies or rabbit ATG induction vs. no induction in patients receiving TAC, mycophenolic acid (MPA) and steroids. With this triple maintenance therapy, the addition of induction may achieve an absolute risk reduction for acute rejection of only 1–4% in standard-risk patients without improving renal allograft or patient survival. In contrast, rabbit ATG induction lowers the relative risk of acute rejection by almost 50% vs. IL-2R antibodies in patients with high immunological risk. These recent data raise questions about the need for IL-2R antibodies in kidney transplantation, as it may no longer be beneficial in standard-risk transplantation. Although augmentation of immunosuppression by IL-2R antibody induction may allow steroid minimization, it may be inferior to rabbit ATG in high-risk situations. Updated evidence-based guidelines are necessary to support clinicians deciding whether and what induction therapy is required for their transplant patients today [33]. Studies in the US and Europe have investigated the potential of IL-2R antibodies or thymoglobulin in replacement of steroids with promising results (see section in this chapter on “Steroid Withdrawal or Avoidance”). Another potential application is delayed graft function when the use of CNIs should be avoided.

Maintenance Immunosuppressive Therapy

Maintenance immunosuppressive therapy is administered to kidney transplant recipients for prevention of acute rejection. Although an adequate level of immunosuppression is required to dampen the immune response to the allograft, the level of chronic immunosuppression is slowly decreased over time to help lower the overall risk of infection and malignancy. The type of immunosuppression may also be modified to decrease the risk of developing chronic antibody-mediated rejection, the most common underlying long-term cause of allograft loss.

Conventional maintenance regimens consist of a combination of immunosuppressive agents that differ in their mechanism of action. This strategy minimizes morbidity and mortality associated with each class of agent while maximizing overall effectiveness. Such regimens vary by transplant center and geographic area. There are a number of important issues to consider when deciding upon the immunosuppressive protocol to administer in a particular patient. First, the risk of acute rejection and allograft loss is highest in the first 3 months post-transplant. As a result, immunosuppression should be at its highest during this period (see section in this chapter on "Induction Immunosuppressive Therapy"). Second, the occurrence of the most serious side effects of immunosuppressive therapy, infections and malignancy, correlate with the total amount of immunosuppression. It is therefore essential that immunosuppression is gradually tapered to a maintenance level by 6–12 months post-transplant.

Allograft survival rates vary among the various immunosuppressive agents due to patient-specific clinical characteristics and co-morbidities, such as age, ethnicity, obesity, hyperlipidemia, hypertension, proteinuria, and/or delayed allograft function. The choice of immunosuppression should consider these factors, but also take into account the "immunologic" history of the patient. Transplant physicians must reflect the following questions: Is the patient sensitized? Is this the first kidney transplant or a re-transplant? How many acute rejection episodes has the patient had? What is the degree of HLA matching?

The optimal maintenance immunosuppressive therapy in pediatric kidney transplantation is not established. The major immunosuppressive agents currently used in various combination regimens are TAC, CSA (in standard form or microemulsion), MMF, EVR, SRL, AZA and steroids (primarily oral prednisone or methylprednisolone). We and most transplant centers currently utilize a maintenance regimen consisting of triple immunosuppression therapy with a CNI (TAC or CSA), an anti-metabolite (MMF or AZA), and methylprednisolone. EVR or SRL are also used by some transplant centers in triple therapy regimens, often in place of the CNI or the antimetabolite. Within the North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS) registry, marked changes in the type of maintenance immunosuppression and dosing strategies have been observed over time [34]. These are substantially caused by the introduction of newer drugs such as MMF and TAC. In the transplant era 2008–2017, the most popular immunosuppressive regimen at post-transplant day 30, utilized in 48.9% of patients, was triple therapy with TAC, MMF and prednisone (Table 67.1).

Table 67.1 Observed drug utilization rates in North American pediatric renal transplant recipients among transplanted grafts with ≥30 days function that have occurred since 1996. (Data are from [34])

	Transplant era 1996–2001				Transplant era 2002–2007				Transplant era 2008–2017			
	30 days	1 year	3 years	5 years	30 days	1 year	3 years	5 years	30 days	1 year	3 years	5 years
Prednisone/ CSA/MMF	35.4	38.1	30.6	22.4	9.7	8.6	7.9	7.5	1.7	1.9	0.5	0.7
Prednisone/ CSA/AZA	23.1	17.7	14.2	8.9	0.8	0.8	0.6	0.7	0.1	0.2	0.3	0.4
Prednisone/ CSA	10.7	4.4	3.8	3.5	1.5	0.8	0.3	0.8	0.4	0.3	0.2	0.0
Prednisone/ TAC/MMF	14.3	19.6	24.4	30.1	51.3	49.6	44.2	42.1	48.9	41.7	38.6	33.1
Prednisone/ TAC/AZA	2.3	4.9	6.5	6.9	1.7	2.4	2.7	3.9	2.0	2.3	4.3	6.7
Prednisone/ TAC	4.2	5.0	6.7	6.9	4.1	5.8	6.7	6.2	2.9	8.2	8.0	6.7
TAC/MMF	0.4	1.1	1.7	2.5	10.7	9.4	11.5	13.1	33.8	27.3	26.5	27.5
Other combination	9.5	9.2	12.0	17.3	20.1	22.7	26.0	25.8	10.1	18.1	21.6	25.3

Glucocorticoids

Glucocorticoids, developed in the early 1950s, represent one of the principal agents used for both maintenance immunosuppression and treatment of acute rejection.

Mechanism of Action

Steroids have both anti-inflammatory and immunosuppressive actions [35]. Lymphopenia and monocytopenia occur with the inhibition of lymphocyte proliferation, survival, activation, homing, and effector functions. Steroids suppress production of MHC molecules and numerous cytokines and vasoactive substances, including IL-1, TNFα, IL-2, chemokines, prostaglandins (*via* inhibition of phospholipase A2), and proteases. Steroids also cause neutrophilia (often with a left shift), but neutrophil chemotaxis and adhesion are inhibited. They also affect non-hematopoietic cells.

Steroids exert their effect by binding to glucocorticoid receptors (GR), which belong to a family of ligand-regulated transcription factors called nuclear receptors. GR are normally present in the cytoplasm in an inactive complex with heat shock proteins (hsp90, hsp70, and hsp56). The binding of steroids to the GR dissociates hsp from the GR and forms the active steroid–GR complex, which migrates to the nucleus and dimerizes on palindromic DNA sequences, called the glucocorticoid response element (GRE), in many genes. The binding of GR in the promoter region of the target genes can lead to either induction or suppression of gene transcription (e.g., of cytokines). GR also exert effects by interacting directly with other transcription factors independent of DNA binding. One principal effect of steroids on immune and inflammatory responses may be attributable to their ability to affect gene transcription by regulating key transcription factors involved in immune regulation: activator protein-1 (AP-1) and NF-κB. The regulation of NF-κB by steroids may be via induction of IkB, the inhibitor of NF-κB. Other effects of steroids are mediated through the release of a regulatory protein, lipocortin, which inhibits phospholipase A2, thereby inhibiting the production of leukotrienes and prostaglandins. The total immunosuppressive effect of steroids is complex, reflecting effects on cytokines, adhesion molecules, apoptosis, and activation of inflammatory cells.

Pharmacokinetics and Drug Interactions

The major steroids used are prednisone or prednisolone (given orally with comparable efficacy) and methylprednisolone (given orally or intravenously with 25% more potency). These agents are rapidly absorbed and have short plasma half-lives (60–180 min), but long biological half-lives (18–36 h). The effect of prednisone (dose per body weight) is greater in the setting of renal failure or hypalbuminemia, in women, and in the elderly, but less prednisone effect is observed in children. Certain drugs can decrease steroid efficacy by increasing metabolism: rifampicin, phenytoin, phenobarbital, and carbamazepine. In contrast, increased steroid effects may be observed in patients receiving oral contraceptives, estrogens, ketoconazole, and erythromycin.

Administration

In many transplant centers, the initial dose of steroids is usually administered during surgery as intravenous methylprednisolone, at doses between 2 and 10 mg/kg body weight. The oral dose of steroids used for maintenance therapy varies between 15 and 60 mg/m^2 per day (0.5–2 mg/kg body weight per day), which is gradually tapered over time to approximately 3–5 mg prednisone per m^2 body surface area, usually taken as a single morning dose. Alternate-day dosing is often administered 6–12 months post-transplant to minimize the effect of steroids on growth.

Side Effects

Steroids have multiple side effects in children, including growth impairment, susceptibility to infections, cushingoid appearance, body disfigurement, acne, cardiovascular complications, hypertension, hyperglycemia, aseptic bone necrosis, osteopenia, cataracts, poor wound healing, and psychological effects (Table 67.2). The

Table 67.2 Semi-quantitative comparison of safety profiles of current primary immunosuppressive compounds

	Tacrolimus	Ciclosporin	Mycophenolate mofetil	Sirolimus/ everolimus	Glucocorticoids
Nephrotoxicity[a]	++(+)	+++	–	+	–
Hyperlipidemia	+(+)	++	–	+++	++
Hypertension	++	+++	–	–	+
Neurotoxicity	+++	+++	–	–	+
Post-transplant diabetes mellitus	+++	++	–	–	++
Bone marrow suppression	–	–	++	++	–
Gastrointestinal adverse effects[b]	+	+	+++	+	–
Hepatotoxicity	+	+	–	+	–
Esthetical changes	+	++	–	–	++
Wound healing problems[c]	–	–	+	++	+
Pulmonary toxicity	–	–	–	+	–
Fetal toxicity	+	+	++	NA	–
Osteoporosis	+	+	–	?	++
Inhibition of longitudinal growth	–	–	–	+	+++

– indicates the drug has no effect on this adverse effect, + indicates mild, ++ indicates moderate, +++ indicates severe, ? indicates clinical data available but insufficient to provide conclusions, *NA* no information available

[a] Sirolimus without calcineurin inhibitor

[b] Gastrointestinal disorders: diarrhoea, abdominal pain, nausea and vomiting, ileus, rectal disorders, mucosal ulcerations

[c] Wound healing problems including lymphocele formation

negative impact that steroids have on appearance may play a role for poor adherence, especially in the body image conscious adolescent. The risk for infection is excessive if high-dose pulse therapy is prolonged (typically >3 g per 1.73 m^2). Steroids dosage should therefore be decreased gradually during rejection treatment even if serum creatinine fails to improve. Interestingly, steroids are not associated with an increased risk of malignancy. One of the most important reasons for stopping steroids or switching to alternate-day therapy is statural growth impairment, which is frequently observed in those on continuous treatment.

Steroid Withdrawal or Avoidance

Because of the multiple adverse effects of maintenance steroid therapy, attempts have been made to withdraw or minimize steroid therapy in children with a renal allograft [36–40]. There are two major strategies in steroid minimization in pediatric kidney transplantation: (i) *Late steroid withdrawal* (>1 year post-transplantation) and (ii) either *complete steroid avoidance* or *early steroid withdrawal* (<7 days post-transplantation). In the *late steroid withdrawal* approach, the patients suitable for minimization are identified by a stable post-transplant clinical course and renal function. In *late steroid withdrawal*, there is no need for an antibody induction in the perioperative period [41]. In *early withdrawal or complete avoidance* protocols, the criteria of suitability are predefined before transplantation (e.g., low immunological risk), and antibody induction is used in all patients [42–47]. There is also an *intermediate* approach, combining elements from *early and late withdrawal* protocols, in which antibody induction is used; however, the decision of *steroid withdrawal* is delayed until 6–9 months post-transplant, when stable renal graft function (sometimes combined with a normal protocol biopsy) allows identification of suitable candidates (as in the late withdrawal approach) [48]. *Steroid withdrawal* has the advantage over *steroid avoidance* that immunologically high-risk patients and those with unstable graft function

can easily be identified beforehand and be excluded from steroid-free immunosuppression.

Late steroid withdrawal without induction therapy was investigated in a trial of 42 patients with low immunologic risk who were maintained on CSA, MMF, and steroids. At 1 year post-transplant, patients were randomly assigned to either steroid continuation or withdrawal over a 3-month period [40]. At 1 year follow-up, the steroid withdrawal group had increased catch-up growth, lower arterial blood pressure, and a better carbohydrate and lipid profile than those on continuous steroid therapy. In a subsequent follow-up report, longitudinal growth in the steroid withdrawal group continued to be superior to controls; catch-up growth was especially pronounced in pre-pubertal patients off steroids (Fig. 67.6) [41]. Although the relative height gain after steroid withdrawal was less pronounced in pubertal patients, they still benefited from cessation of steroid treatment. Also, the prevalence of the metabolic syndrome declined in the withdrawal group from 39% at baseline to 6% 2 years after discontinuing steroid therapy [41]. Allograft function remained stable in the withdrawal group compared with controls, and the incidence of acute rejections was similar in the steroid withdrawal and control groups (4% vs. 11%, respectively). An earlier retrospective case–control study had reported a beneficial effect of *late ste-*

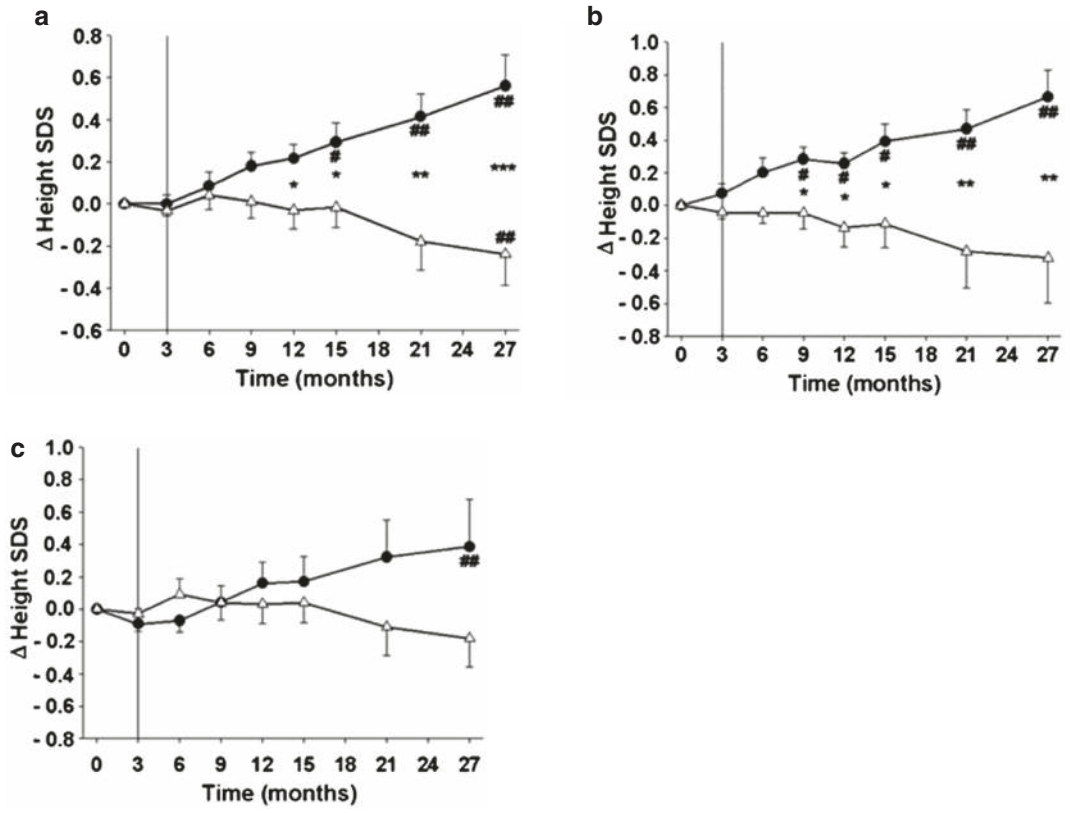

Fig. 67.6 Randomized controlled trial on late steroid withdrawal: Change of height SDS (mean ± SEM) in the steroid withdrawal group (filled circles) and the control group (open triangles) during the 27-month study period. Panel A, all patients; panel B, pre-pubertal patients; panel C, pubertal patients. ∗P < 0.05 vs. control; ∗∗P < 0.01 vs. control; ∗∗∗P < 0.001 vs. control; #P < 0.05 vs. baseline; ##P < 0.01 vs. baseline. (Used with permission of Oxford University Press from Höcker B, Weber L, Feneberg R, Drube J, John U, Fehrenbach H, et al. Improved growth and cardiovascular risk after late steroid withdrawal: 2-year results of a prospective, randomized trial in paediatric renal transplantation. Nephrol Dial Transplant 2010; 25:617–624) [41]

roid withdrawal (mean time 1.5 ± 1.3 years post-transplant) on growth in similarly treated (pre-pubertal) children. The patients in this study who had steroids withdrawn also had better blood pressure control with a lower requirement for antihypertensive therapy [49].

More recently, a single-center study has reported on efficacy and safety of a different regimen combining the *intermediate withdrawal of steroids* (>6 months and <1 year) with minimization of exposure to CSA. The protocol initially included a 2-dose basiliximab induction, standard exposure to CSA (C_0 150–200 μg/L) and prednisolone. This was followed by adding EVR at 2 weeks (C_0 4–6 μg/L), reducing CSA exposure by half (C_0 75–100 μg/L), and then steroid withdrawal at 9 months in patients with a normal protocol biopsy. Results of the open-label uncontrolled trial have been promising, without acute rejection and a 100% 3-year graft survival [48, 50–52], while another study on *intermediate steroid withdrawal* (2 doses of basiliximab and sequential replacement of tapered steroids with MMF at 6 months post-transplant under protocol biopsy) reported an acute rejection rate of 13% [53]. Another *intermediate steroid withdrawal* protocol was used in a US multicenter trial, during which patients received 2 doses of basiliximab, combined with SRL, TAC or CSA, and steroids. After 6 months and a protocol biopsy without signs of rejection, patients were randomized to withdraw or maintain steroids [37]. It should be noted that during the first phase of the trial (prior to randomization) 6.9% of the patients developed PTLD. This was mainly seen in young EBV-naïve children, receiving an EBV-seropositive renal allograft; however, this complication should not be regarded as directly related to steroid minimization but rather to initial over-immunosuppression [53].

Early steroid withdrawal or s*teroid avoidance* may eventually be found to provide the best overall risk-to-benefit ratio with maintenance immunosuppressive therapy in renal transplantation. *Early steroid withdrawal* or *steroid avoidance* protocols have been used successfully and have undergone extensive evaluation both in the US and in Europe. However, many of these protocols have chosen low-risk individuals and utilized intensive induction therapy with extended daclizumab induction therapy or thymoglobulin, TAC, and MMF [42]. A randomized, controlled study in 196 pediatric kidney transplant recipients investigated the efficacy and safety of *early steroid withdrawal*. Two doses of daclizumab in patients treated with a regimen of TAC and MMF allowed *early steroid withdrawal* on day 5 post-transplant [45]. There was a comparable rate of biopsy-proven acute rejection after 6 months in patients off steroids compared with controls (10.2% vs. 7.1%). In addition, pre-pubertal patients with *early steroid withdrawal* showed better growth and lipid and glucose metabolism profiles compared to controls, without increases in graft loss. These favorable effects were confirmed in a follow-up study over a 2-year observation period [54]. The *steroid avoidance* strategy was examined in a North American, randomized, controlled, multicenter study [55]. One hundred thirty children receiving primary kidney transplants were randomized to steroid-free or steroid-based immunosuppression, with concomitant TAC, MMF and standard dose daclizumab (steroid-based group) or extended dose daclizumab (steroid-free group). Follow-up was 3 years post-transplant. Recipients under 5 years of age showed improved linear growth under a steroid-free regimen compared to controls on steroids. No differences in the rates of biopsy-proven acute rejection were observed at 3 years post-transplant (16.7% in steroid-free vs.17.1% in steroid-based; P = 0.94). Patient survival was 100% in both arms; graft survival was 95% in the steroid-free and 90% in the steroid-based arms (P = 0.30) at 3 years follow-up. Over the 3-year follow-up period, the steroid-free group had lower systolic blood pressure (P = 0.017) and cholesterol levels (P = 0.034) (Fig. 67.7). The authors concluded that *complete steroid avoidance* is safe and effective in unsensitized children receiving primary kidney transplants [55].

Despite these encouraging results, *steroid withdrawal* or *avoidance* following kidney transplantation remains a controversial issue. Although the benefits of using steroid-free protocols in pediatric patients are obvious, they may increase

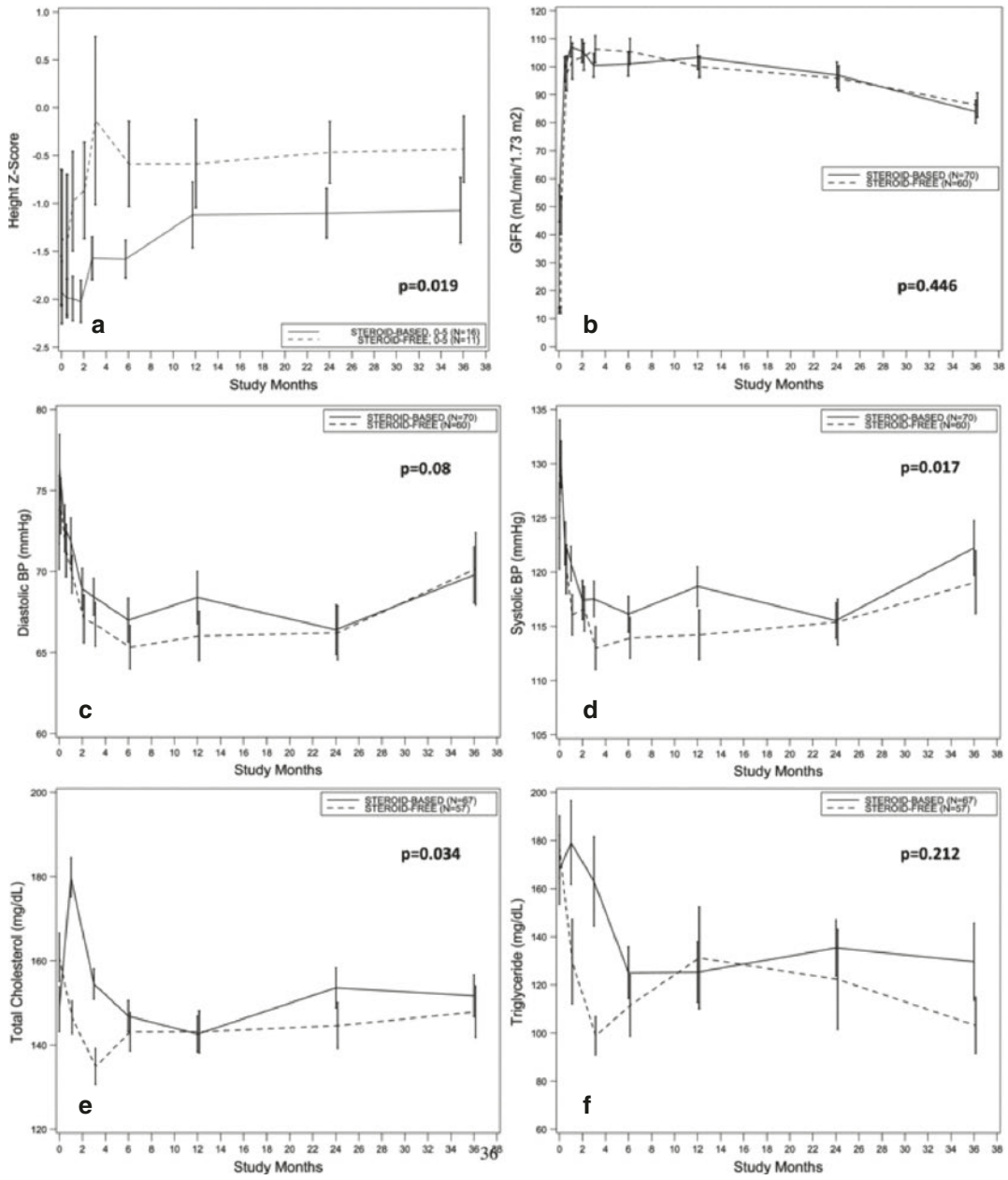

Fig. 67.7 North American randomized controlled multicenter study on steroid avoidance: Estimated group mean standardized change in growth (Z-score) among infants and young children (**a**), mean eGFR level (by Schwartz method) (**b**), mean diastolic (**c**) and systolic (**d**) blood pressure levels and serum cholesterol (**e**) and triglyceride (**f**) levels from transplantation up to 3 years. (Used with permission of John Wiley and Sons from Sarwal MM, Ettenger RB, Dharnidharka V, Benfield M, Mathias R, Portale A, et al. Complete corticosteroid avoidance is effective and safe in children with renal transplants: a multicentre randomized trial with three years follow-up. Am J Transpl 2012; 12: 2719–29) [55]

risk in patients with certain immunological constellations. Further studies need to identify new monitoring tools to assess the immunologic safety to allow successful and safe conversion of patients to steroid-free immunosuppression at later times after transplantation, even after having

initially started on a steroid-based maintenance immunosuppression protocol.

Recurrent Kidney Disease

Because recurrent disease is the fourth most common cause of graft loss in children undergoing kidney transplantation, there have been concerns that steroid withdrawal may be associated with an increased risk of graft loss. Although information is limited, a study demonstrated no difference in graft survival due to recurrent disease in children (4–18 years of age) treated with a rapid prednisone discontinuation protocol compared with historical controls who received steroid therapy [56]. However, more data are needed to ensure there is not an untoward increased risk of recurrent disease, for example lupus erythematosus-associated nephritis, following steroid withdrawal.

Calcineurin Inhibitors

CSA, a lipophilic cyclic peptide of 11 amino acid residues, and TAC, a macrolide antibiotic, are drugs with similar mechanisms of action that have become major maintenance immunosuppressive agents used in transplantation.

Mechanism of Action

CSA and TAC act by inhibiting the calcium-dependent serine phosphatase calcineurin, which normally is rate-limiting in T cell activation (Fig. 67.8). Calcineurin is activated by the engagement of the T cell receptor, followed by activation of tyrosine kinases and phospholipase C-γ1, release of inositol triphosphate, release of calcium stored in the endoplasmic reticulum, and opening of membrane calcium channels. Calcineurin provides an essential step for transducing signal 1 to permit cytokine and CD40L

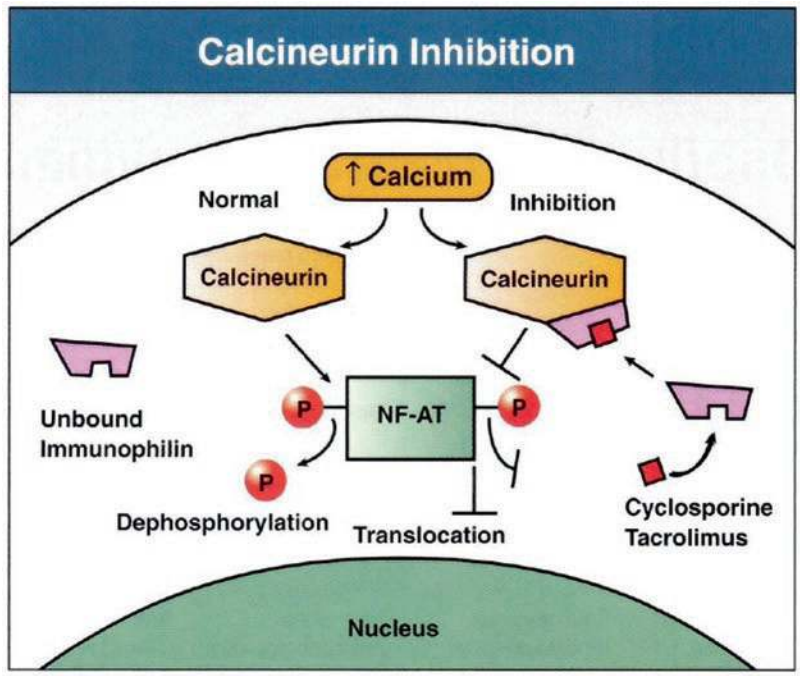

Fig. 67.8 Calcineurin inhibition. During normal T cell activation, calcium release activates calcineurin's phosphatase activity, causing dephosphorylation of the transcription factor nuclear factor of activated T cells (NF-AT) and subsequent translocation to the nucleus. Ciclosporin and tacrolimus form a complex with immunophilins (cyclophilin or FK-binding protein 12, respectively), which bind calcineurin and sterically inhibit the phosphatase activity, preventing dephosphorylation and nuclear translocation of NF-AT. (Used with permission from Floege J, Johnson RJ, Feehally J, (eds.): Comprehensive Clinical Nephrology, 4th ed. St. Louis: Elsevier, 2010)

transcription. A high cytoplasmic calcium concentration activates calcineurin, which then dephosphorylates regulatory sites in key transcription factors, the 'nuclear factors of activated T lymphocytes' ($NFAT_p$ and $NFAT_c$). This causes the NFAT proteins to translocate (with calcineurin) into the nucleus and bind to their DNA target sequences in the promoters of cytokine genes. Calcineurin has been implicated in the dephosphorylation of transcription factor Elk-1, and indirectly in the activation of Jun/AP-1 and NF-κB.

CSA and TAC cross cell membranes freely and bind to immunophilins (cyclophilin and FK-binding protein 12 [FKBP12], respectively), which are ubiquitous and abundant intracellular proteins with isomerase activity. The active complex then binds to a site on calcineurin and blocks its interactions with key substrates. The inactivation of calcineurin bound to CSA–cyclophilin or TAC–FKBP12 is the key to the immunosuppressive effect and some of the toxic effects of these drugs. While inhibition of calcineurin has many effects on the T cell, the best studied is the blocking of the translocation of the NFAT proteins from the cytoplasm into the nucleus.

CSA and TAC partially inhibit the calcineurin pathway at therapeutic blood levels (e.g., trough levels of 200 µg/L CSA or 5–20 µg/L TAC) [57]. However, even partial inhibition of calcineurin reduces the transcription of many genes associated with T cell activation (e.g., IL-2, IFN-γ, granulocyte–macrophage colony-stimulating factor (GM-CSF), TNF-α, IL-4, CD40L). Therefore, the functional consequence of partial calcineurin inhibition is probably a quantitative limitation in cytokine production, CD40L expression, and lymphocyte proliferation. The effect of CSA and TAC on calcineurin *in vivo* is rapidly reversible, emphasizing the importance of patient compliance, drug monitoring, and reliable formulations for delivery. The effects on non-T cells could also be clinically significant.

Pharmacokinetics and Drug Interactions

CSA and TAC are both variably absorbed and are metabolized extensively by the liver via the cytochrome P450 system. CSA is excreted primarily by the biliary system. The absorption of some CSA preparations may be bile-dependent, and therefore may be reduced in the presence of cholestatic liver disease. The absorption of the microemulsion formulations of CSA or TAC is bile-independent. Neither CSA nor TAC pharmacokinetics are affected by alterations in renal function. Both CSA and TAC bind to cells and to plasma components (primarily lipoproteins for CSA and albumin for TAC) in the blood; consequently, they must be assayed in whole-blood. Many drugs and agents can affect CSA and TAC levels through effects on their absorption or metabolism (see Table 67.3).

Since the absorption of CSA is decreased and its metabolism increased in children compared to adults, relatively higher dosages are required in pediatric patients. CSA is usually administered initially as 8–15 mg/kg daily in divided doses (or intravenously using one third the oral dose over a 24-h period) during the induction phase, with target trough blood levels of 150–300 µg/L for the first 3–6 months post-transplant. Doses are reduced after 3–6 months (typically 4–6 mg/kg daily); long-term target trough blood levels of 75–125 µg/L appear to provide comparable patient and graft survival as higher blood levels, but with less risk of malignancy [58]. A microemulsion form of CSA (Neoral™, Novartis, East Hanover, NJ) gives more reliable and slightly higher absorption, and may allow a slightly lower dose. Generic forms of CSA are available, but oral formulations of CSA may not be equivalent and readily interchangeable. Knowledge of the characteristics of the oral formulations is necessary before switching between them.

TAC is 20- to 30-fold more potent than CSA, and is therefore administered at a 20-fold lower dose. Initial dosing is usually 0.2–0.3 mg/kg daily in two divided doses orally (or 0.05–0.1 mg/kg daily intravenously over 24 h), and target trough levels are 5–15 µg/L. Since TAC is more water-soluble than CSA, it is not as dependent upon bile salts for absorption. However, food intake can reduce the absorption of TAC by up to 40 percent; thus, it is recommended that this agent be taken on an empty

Table 67.3 Examples of common drug interactions of immunosuppressants used in solid-organ transplantation: ciclosporin, tacrolimus, sirolimus, and everolimus

Common types of drug interactions	Examples of interacting drugs
Co-administration of drugs that inhibit CYP3A metabolism and/or P-gp efflux can increase immunosuppressant whole blood concentrations, leading to significant toxicities	Amiodarone ART-boosting agents (e.g., ritonavir, cobicistat) Azole antifungals (e.g., fluconazole, posaconazole, voriconazole) HIV protease inhibitors (e.g., atazanavir, nelfinavir, saquinavir) Macrolide antibiotics (except azithromycin) Non-dihydropyridine calcium-channel blockers Ombitasvir-paritaprevir-ritonavir with or without dasabuvir (an HCV, direct-acting antiviral regimen) Grapefruit juice
Co-administration of drugs that induce CYP3A metabolism and/or P-gp efflux pumping can decrease immunosuppressant whole blood concentrations, increasing the risk of organ rejection	CYP3A-inducing anti-seizure drugs (e.g., carbamazepine, fosphenytoin, oxcarbazepine, phenobarbital, phenytoin, primidone) Enzalutamide Nafcillin Rifamycins (e.g., rifabutin, rifampin, rifapentine) St. John's wort
Co-administration of nephrotoxic drugs with cyclosporine or tacrolimus can cause additive or synergistic kidney injury	Aminoglycosides Amphotericin B Colchicine Nonsteroidal anti-inflammatory drugs (NSAIDs)
Co-administration of drugs that increase serum potassium with cyclosporine or tacrolimus may cause severe hyperkalemia	ACE inhibitors/ARBs Amiloride Spironolactone Triamterene Trimethoprim, trimethoprim-sulfamethoxazole (cotrimoxazole)
Co-administration of statin drugs with cyclosporine can increase statin levels and risk of myotoxicity	Atorvastatin Lovastatin Pitavastatin Rosuvastatin Simvastatin

CYP cytochrome P450 metabolism, *P-gp* P-glycoprotein drug efflux pump, *ART* HIV antiretroviral therapy, *HIV* human immunodeficiency virus, *HCV* hepatitis C virus, *ACE* angiotensin-converting enzyme, *ARB* angiotensin II receptor blocker

stomach [59]. In addition, TAC is best absorbed in the morning. There is also an extended release formulation of TAC available that is designed to be given once per day with similar efficacy and safety.

Genetic polymorphisms in genes encoding TAC-metabolizing enzymes partly explain the inter-patient variability in TAC pharmacokinetics [60]. The key enzymes involved in the metabolism of TAC are CYP3A4 and CYP3A5 [61]. Individuals are considered expressers of CYP3A5 if they carry at least one CYP3A5*1 allele, whereas individuals homozygous for the CYP3A5*3 allele are classified as CYP3A5 non-expressers. In addition to CYP3A5*3, the CYP3A5*6 and CYP3A5*7 variant alleles can also lead to non-functional CYP3A5 protein [62]. There are ethnic distribution differences of CYP3A5 variant alleles, with expressers (carriers of the CYP3A5*1 variant allele) being more frequently found among non-Caucasian populations. Approximately 10–40% of Caucasians are CYP3A5 expressers, 33% of Asians and 55% of African-Americans [63]. CYP3A5 expressers require a TAC dose that is approximately 1.5–2-fold higher than non-expressers to reach the same exposure [64, 65]. This implies that, following a standard, bodyweight-based TAC dose,

CYP3A5 expressers are prone to have sub-therapeutic TAC concentrations whereas non-expressers are expected to have supra-therapeutic TAC concentrations [66].

Efficacy: Comparison of Ciclosporin and Tacrolimus

To help assess the relative efficacy of TAC and CSA, a 2005 meta-analysis and meta-regression was performed based upon 30 trials consisting of 4102 adult patients [67]. Despite a certain variability between studies, the overall conclusion from data in adults is that TAC-based immunosuppression is associated with decreased acute rejection rates, superior long-term renal function and more favorable cardiovascular risk profile than CSA-based immunosuppression, which translates into improved long-term renal allograft survival. This statement is supported by the results of the SYMPHONY trial, which compared standard immunosuppression vs. 3 regimens with low-dose or no CNI in *de novo* single-organ renal transplant patients over 1 year [68]. In this prospective, randomized, open-label study with 4 parallel arms, 1645 adult patients in 15 countries were randomized to standard immunosuppression with normal-dose CSA (target trough level 150-300 µg/L for 3 months, 100-200 µg/L thereafter), MMF (1 g bid) and steroids, or to one of three regimens consisting of daclizumab induction, MMF (1 g bid) and steroids potentiated by a low-dose of either CSA (50–100 µg/L), TAC (3-7 µg/L) or SRL (4-8 µg/L). The low-dose TAC group was significantly superior to all other groups with respect to glomerular filtration rate (GFR) and biopsy-proven acute rejection ($p < 0.01$) and to normal-dose CSA and low-dose SRL for graft survival (pair-wise $p < 0.05$). The authors concluded that immunosuppression consisting of daclizumab induction, MMF, low-dose TAC and corticosteroids provides the most optimal balance between efficacy (control of acute rejection) and toxicity (preserving graft function and graft survival) [68].

In pediatric patients, the efficacy and safety of TAC and CSA were compared in one multicenter trial in 196 patients, who were randomly assigned to receive either TAC or CSA microemulsion administered concomitantly with AZA and steroids [69]. TAC therapy resulted in a significantly lower incidence of acute rejection (36.9% vs. 5.91% with CSA therapy (59.1%); P = 0.003) and of steroid-resistant rejection (7.8% vs. 25.8%, P = 0.001) compared with the CSA group. The difference was also significant for biopsy-confirmed acute rejection (16.5% vs. 39.8%, P < 0.001). At 1 year post-transplant, patient survival was similar (96.1% vs. 96.6%); ten grafts were lost in the TAC group compared with 17 in the CSA group (P = 0.06). The TAC group had a significantly better eGFR. A follow-up study at 4 years showed that patient survival was similar (94% vs. 92%, P = 0.86), but graft survival significantly favored TAC (86% vs. 69%; P = 0.025) [70]. The mean eGFR was superior in TAC-treated patients vs. those on CSA (Fig. 67.9). Cholesterol remained significantly higher with CSA throughout follow-up. Three patients in each arm developed PTLD. Incidence of insulin-dependent diabetes mellitus did not differ. From these studies, the authors concluded that TAC was significantly more effective than CSA in preventing acute rejection and preserving renal function in pediatric renal recipients.

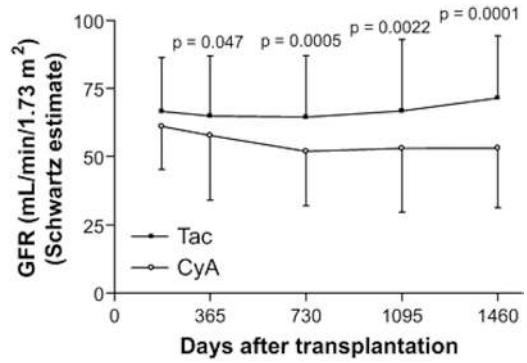

Fig. 67.9 Mean glomerular filtration rate (±1 SD) over 4 years post-transplant. Multicenter trial in 196 pediatric patients, who were randomly assigned to receive either tacrolimus or ciclosporin administered concomitantly with azathioprine and corticosteroids. (Used with permission of John Wiley and Sons from Filler G, Webb NJ, Milford DV, Watson AR, Gellermann J, Tyden G, et al. Four-year data after pediatric renal transplantation: a randomized trial of tacrolimus vs. cyclosporin microemulsion. Pediatr Transplant. 2005; 9:498–503) [70]

A retrospective study of the NAPRTCS database of 986 pediatric renal transplant recipients who were treated either with CSA, MMF and steroids (n = 766) or TAC, MMF and steroids (n = 220) revealed that TAC and CSA, in combination with MMF and steroids, produce similar rejection rates and graft survival in pediatric renal transplant recipients [71]. However, TAC was associated with improved graft function at 1 and 2 years post-transplant (Fig. 67.10). There was no difference in time to first rejection, as well as in risk of rejection or graft failure at 1 or 2 years post-transplant. TAC-treated patients were significantly less likely to require antihypertensive medication at 1 and 2 years post-transplant. TAC-treated patients had a higher mean eGFR at both 1 year (99 vs. 78 mL/min/1.73 m^2, P = 0.0003) and 2 years post-transplant (97 vs. 73 mL/min/1.73 m^2, P < 0.0001). Hence, there is evidence that TAC is superior to CSA (conventional or microemulsion form) in preventing acute rejection after kidney transplantation in adult and pediatric populations. It also seems more effective in improving long-term graft survival in adults.

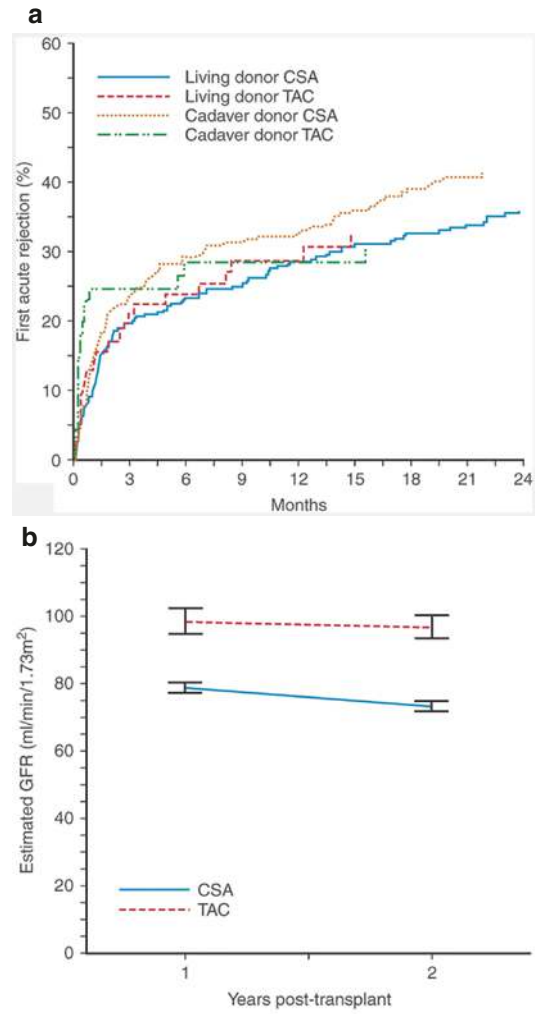

Fig. 67.10 (**a**) Kaplan–Meier estimates of the percentage of patients experiencing a first acute rejection in the first 2 years post-transplant, by treatment group: tacrolimus (TAC), mycophenolate mofetil (MMF) and steroids vs. ciclosporin (CSA), MMF and steroids and donor source. Patients were included in this analysis if they were transplanted between 1997 and 1999 and had a 2-year follow-up in the database. (**b**) Mean eGFR as calculated by the Schwartz formula at 1 and 2 year post-transplant in patients treated with TAC, MMF and steroids or CSA, MMF and prednisone. (Used with permission of John Wiley and Sons from Neu AM, Ho PL, Fine RN, Furth SL, Fivush BA. Tacrolimus vs. cyclosporine A as primary immunosuppression in pediatric renal transplantation: a NAPRTCS Study. Pediatr Transplant. 2003; 7:217–22) [71]

Side Effects

CSA and TAC have similarities and differences in their toxicity profiles (Table 67.2). Both can cause nephrotoxicity, hyperkalemia, hyperuricemia with occasional gouty attacks, hypomagnesemia secondary to urinary loss, hypertension, diabetes mellitus, and neurotoxicity, especially tremor. In the European pediatric study, the incidence of hypomagnesaemia was significantly higher in the TAC-treated group (34%) compared with the CSA-treated group (12.9%) [69]. Similarly, diarrhea was more frequent in TAC-treated patients (13.6% vs. 3.2%). Hypertrichosis, gum hyperplasia and flu syndrome were reported only in CSA-treated patients, and tremor was reported only in TAC-treated patients [69]. These results are similar to that found in adults, where tremor is consistently more common with TAC, and hirsutism and gum disease more common with CSA [72]. Also, hypertension and hyperlipidemia are more commonly observed with CSA. Interestingly, higher CSA doses are more likely to induce higher blood pressure in older girls compared to boys [73]. In the NAPRTCS study, during which CNIs were used in combination with MMF and steroids, TAC-treated patients

were significantly less likely to require antihypertensive medications at 1 and 2 years post-transplant [71]. This is similar to adults, where a lower systemic blood pressure was reported in TAC-treated patients in several studies [72]. In the European pediatric study, the mean total cholesterol levels were reported to decline in the TAC group and increase in the CSA group at the end of 6 months [69]. In the multicenter analysis from the European CERTAIN Registry, the prevalence of dyslipidemia was 95% before transplantation, and 88% at 1 year post-transplant [74]; the use of TAC and MMF was associated with significantly lower concentrations of all lipid parameters compared to regimens containing CSA and mTOR inhibitors. Regimens consisting of CSA, MPA, and corticosteroids as well as of CSA, mTOR inhibitors, and steroids were associated with a 3- and 25-fold increased risk of having more than one pathologic lipid parameter as compared to the use of TAC, MMF, and steroids [74]. Similarly, several studies in adults have shown remarkably lower lipid levels in TAC-treated patients than in those receiving CSA [72]. The improved lipid profiles on TAC may contribute to a better long-term outcome with less cardiovascular morbidity in adult patients.

On the other hand, tremor and glucose intolerance are more common with TAC. In the pediatric multicenter European study, there was no difference in the incidence of new onset insulin-dependent diabetes mellitus between TAC- (3%) and CSA-treated patients (2.2%) [69, 70]. Although in early clinical trials of TAC, a significantly higher incidence of diabetes mellitus was reported in TAC-treated adult patients than in recipients on CSA, the incidence of diabetes mellitus under TAC immunosuppression has become less frequent in recent randomized trials comparing these two CNIs [75]. Post-transplant diabetes regresses after dose reduction in some but not all patients. Both the reduction of steroid dosage and low target trough TAC concentrations contribute to this marked reduction of the incidence of diabetes mellitus under TAC immunosuppression in both adults and children [69, 72]. CSA may also be associated with coarsening of facial features, especially in children. Bone pain that is responsive to calcium-channel blockers may also occur with CSA use and sometimes may require changing to TAC.

The most common serious problem with CNIs is nephrotoxicity, with both a reversible vasomotor component and an irreversible component. Both CSA and TAC can cause acute elevations in serum creatinine that reverse with reduction of the dose, apparently caused by renal vasoconstriction, which may be mediated by calcineurin inhibition. Chronically, CSA and TAC can induce interstitial fibrosis and tubular atrophy with characteristic hyalinosis of the afferent arteriole [76]. This lesion appears to result from long-standing renal vasoconstriction, perhaps mediated in part by an increase in local vasoconstrictor tone (increased angiotensin II, endothelin-1, thromboxane, and sympathetic nerve transmitters) and an inadequate vasodilatory response (impaired nitric oxide formation). The importance of this lesion is apparent from studies in cardiac and liver transplant recipients, in whom CSA or TAC use is associated with chronic kidney disease progressing to end-stage kidney disease (ESKD) in a significant fraction of patients [77]. This problem was more relevant at a time when higher doses of CSA were administered for longer periods. Fortunately, currently, CSA and TAC toxicity is associated with only mild to moderate declines in renal function. However, as the number of patients with long-standing non-renal transplants rises, there is increasing concern about future ESKD in this population. In these cases, it is important to establish the diagnosis of CNI toxicity by renal biopsy and reduce or stop calcineurin inhibition whenever possible [75, 78, 79]. Experimentally, CSA nephropathy is exacerbated in the presence of salt restriction/volume depletion and is lessened by treatment with angiotensin-converting enzyme (ACE) inhibitors, calcium-channel blockers, vasodilators (hydralazine), and steroids.

CSA and TAC treatment can cause hemolytic uremic syndrome, probably through endothelial dysfunction. This complication, which is usually associated with elevated drug levels, may respond to temporary withdrawal of CSA or TAC, plasma

exchange, switching to another CNI, or conversion to another immunosuppressive drug class.

There is no difference in the incidence of PTLD between TAC- and CSA-treated recipients when used in combination with AZA/steroids [1% (1/103) vs. 2.1% (2/93)] [69] or when given in conjunction with MMF/steroids (1.4% vs. 2%) [71]. This is similar to adults, in whom recent large, randomized studies showed no differences in the incidence of malignancy between patients treated with TAC or CSA [72].

Therapeutic Drug Monitoring

CSA is a drug with a narrow therapeutic index and broad intra-individual and inter-individual pharmacokinetic variability. Serious clinical consequences may occur because of underdosing or overdosing. Hence, individualization of CSA dosage by therapeutic drug monitoring is required. The traditional monitoring strategy for CSA is based on pre-dose trough level measurements (C_0). However, C_0 shows a relatively poor correlation with CSA exposure (area under the concentration-time curve [AUC]) and with clinical outcome [80]. Studies on the pharmacokinetic and pharmacodynamic relationship of CSA have shown that CSA induces a partial inhibition of calcineurin activity, the rate-limiting step in the activation of primary human T lymphocytes and the target of the CSA/cyclophilin complex [81]. The greatest calcineurin inhibition and the maximum inhibition of IL-2 production occur in the first 1–2 h after dosing. Calcineurin is only partially inhibited in patients, which can result in rejection even when CSA blood concentrations are in the putative therapeutic range. From these observations, it was hypothesized that the CSA $AUC_{0–4}$(absorption profile) or the C_2concentration (sample 2 h after oral intake) may be a better predictor of immunosuppressive efficacy than the CSA $AUC_{0–12}$. However, a prospective, randomized study in adult renal transplant recipients did not show any advantage of C_2 monitoring strategy in the early post-transplant period compared to a C_0 monitoring strategy, but led to significantly higher CSA doses and blood levels than C_0 monitoring [82]. In a large study in pediatric renal transplant recipients, CSA absorption profiles predicted the risk of acute rejection, while the single pharmacokinetic parameters C_0 or C_2 did not [83]. A disadvantage of C_2 monitoring is the fact that it requires a timed blood sample within a narrow time window (±15 min) that necessitates further organizational requirements for physicians and nursing staff, which may be judged differently between transplant centers. In our center, we routinely monitor CSA therapy by 12-h pre-dose trough concentrations. We aim for the following trough levels in combination with MMF therapy and prednisone: 120–200 μg/L during months 0–3 post-transplant and 80–160 μg/L thereafter. We feel that a monitoring strategy based on CSA C_2 concentrations in the stable period post-transplant is an additional tool in preventing chronic CSA-induced nephrotoxicity. In patients with low or normal immunological risk, who are on additional maintenance therapy with MMF, we aim for CSA C_2 concentrations between 300–600 μg/L beyond the first year post-transplant; C_2 concentrations are monitored every 3–6 months.

When TAC is utilized, a monitoring strategy based on trough levels is in general sufficient because trough levels are good indicators of systemic exposure. In most transplant centers, doses are adjusted to attain target whole-blood trough concentrations of 8–12 μg/L during the first 3 months post-transplant, between 5 and 10 μg/L during months 4–12, and 4–8 μg/L thereafter [84]. It must be emphasized that these target ranges are dependent on the concomitant immunosuppressive therapy. In the SYMPHONY trial, for example, low tacrolimus exposure (trough levels between 3 and 7 μg/L) in the first year post-transplant, in conjunction with MMF, prednisone and daclizumab induction, was associated with excellent efficacy and little TAC-associated toxicity [68]. However, although TAC trough level goals in the low-dose TAC group of the Symphony study were protocol specified at 3–7 μg/L, the achieved levels were 6.4 and 6.5 μg/L at 12 and 36 months post-transplant, respectively. Hence, a more appropriate interpretation of the SYMPHONY trial is that, in combination with MMF, TAC trough level goals of 5–8 μg/L

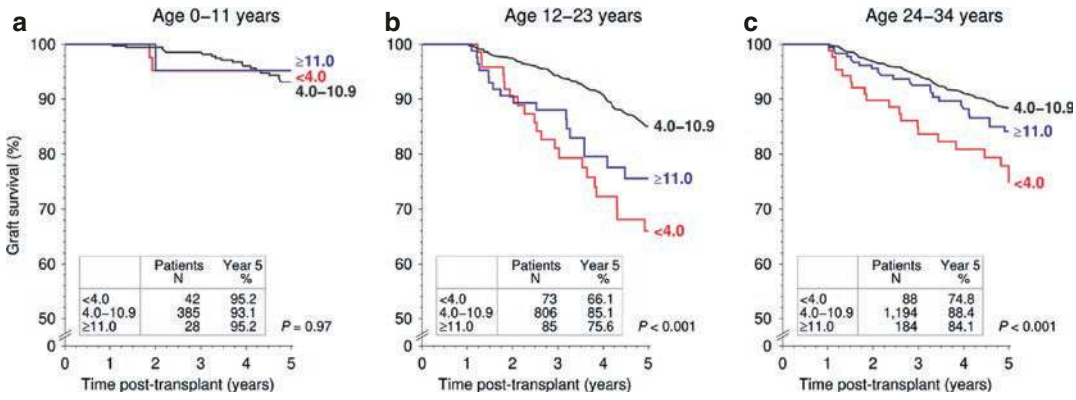

Fig. 67.11 Association of 1-year tacrolimus trough level (ng/mL) with graft survival during post-transplant years 2–5 for the age groups (**a**) 0–11, (**b**) 12–23, and (**c**) 24–34 years at the time of transplantation. Log rank P values of Kaplan–Meier analysis are shown. (Reproduced from [86], with permission)

should be considered as standard of care in adult patients. In addition, recent studies in adult kidney allograft recipients lend support to maintain TAC trough levels above 5 µg/L in order to reduce the risk of *de novo* donor-specific antibodies [85].

Recent registry data indicate that that a more consistent and less variable exposure to the main immunosuppressant TAC later than 1 year post-transplant is associated with a better 5-year graft survival, especially in adolescents and young adults (Fig. 67.11) [86]. In adolescent and young adult patients, the risk of premature graft loss associated with a low 1-year TAC trough level <4.0 ng/mL was 2.38-fold higher compared to a trough level of 4.0–10.9 ng/mL, whereas the risk was not significantly increased in recipients aged 0–11 years. In 24–34-year-old adult patients, the risk of premature graft loss due to a low 1-year TAC trough level <4.0 ng/mL was 1.94-fold increased, but still lower than in adolescent and young adult patients. Importantly, trough levels in the range of 4.0–10.9 ng/mL resulted in a good 5-year graft survival of 85% in the group of 12–23-year-old recipients, comparable to the 88% survival rate observed in 24–34-year-old adult patients (Fig. 67.11). Hence, it appears that optimal TAC exposure can at least partially counteract the enhanced immunoreactivity in this high-risk age group.

Antimetabolic Agents: Azathioprine

AZA, developed by Nobel Prize laureates Elion and Hitchings in the1950s, has been widely used in renal transplantation for 4 decades. AZA is a purine analog derived from 6-mercaptopurine (6-MP).

Mechanism of Action

AZA is metabolized in the liver to 6-MP and further converted to the active metabolite thioinosinic acid (TIMP) by the enzyme hypoxanthine–guanine phosphoribosyltransferase. Some but not all of the immunosuppressive activity of AZA is attributable to 6-MP. AZA acts mainly as an antiproliferative agent by interfering with normal purine pathways, by inhibiting DNA synthesis, and by being incorporated into DNA, thereby affecting the synthesis of DNA and RNA [64]. By inhibiting the synthesis of DNA and RNA, AZA suppresses the proliferation of activated B and T lymphocytes. In addition, AZA has been shown to reduce the number of circulating monocytes by arresting the cell cycle of promyelocytes in the bone marrow. The anti-proliferative action of AZA probably explains much of its observed effects on the immune system and its toxicity. AZA shows some selectivity in its effects with certain cell types and different kinds of immune reactions [87]. For instance, it has been shown that primary immune responses are more susceptible to AZA

than secondary responses despite the fact that there is a more rapid proliferation of lymphocytes during a secondary response.

Pharmacokinetics and Drug Interactions

AZA is administered orally at 1.5 mg/kg per day in conjunction with CNIs and 2.5 mg/kg per day when used without CNIs. Higher initial doses (5 mg/kg per day) combined with monitoring of 6-thioguanine nucleotide levels in red blood cells are associated with an approximately 20 percent reduction in the acute rejection rate as compared to lower doses [88]. It is metabolized in the liver to 6-MP and further converted to the active metabolite TIMP. Because 6-MP is degraded by xanthine oxidase, allopurinol, a xanthine oxidase inhibitor, will increase the levels of TIMP. Severe leukopenia can occur if allopurinol, used for the treatment of hyperuricemia and gout, is given with AZA. Thus, allopurinol should generally be avoided in patients treated with AZA. If, however, the patient has severe gout and allopurinol must be used, AZA doses must be reduced by about two-thirds, and the white blood cell count must be carefully monitored. AZA eventually has to be discontinued in many such patients. A possible alternative is switching from AZA to MMF, which does not interact with allopurinol.

Side Effects

The major side effect of AZA is bone marrow suppression. All 3 hematopoietic cell lines can be affected, leading to leukopenia, thrombocytopenia, and anemia. The hematologic side effects are dose-related and can occur late in the course of therapy. They are usually reversible upon dose reduction or temporary discontinuation of the drug. AZA should be temporarily withheld if the white cell count falls below 3000/mm^3 or if the count drops by 50 percent between blood draws. Recovery usually occurs within 1–2 weeks. The drug can then be restarted at a lower dose and increased gradually to the usual maintenance dose while monitoring the white cell count. Occasionally, AZA has to be discontinued because of recurrent or persistent leukopenia. The mean cell volume is commonly increased in patients on full-dose AZA, and red cell aplasia can eventually result. Interactions between AZA and ACE inhibitors have been reported, causing anemia and leukopenia.

Another potentially serious side effect of AZA, which requires decreasing the dose or even stopping the drug, is hepatotoxicity. This complication is manifested by abnormal liver function tests, usually showing a cholestatic picture. The diagnosis of AZA-induced liver disease is one of exclusion, and the patient should be evaluated for other more serious causes of hepatic dysfunction. AZA has also been linked to the development of skin cancer, the most common malignancy in renal transplant patients. As a result, patients taking AZA for a prolonged period should be instructed to avoid direct exposure to sunlight or to use heavy sun screens when exposed. Other side effects include increased susceptibility to infection and hair loss.

Antimetabolic Agents: Mycophenolate Mofetil

MMF impairs lymphocyte function by blocking purine biosynthesis via inhibition of the enzyme inosine monophosphate dehydrogenase (IMPDH). MMF was developed as a replacement for AZA for maintenance immunosuppression. It is not nephrotoxic and has less bone marrow toxicity than AZA. However, gastrointestinal toxicity can occur, usually manifested by gastritis and diarrhea. MMF is currently available in intravenous, capsule, and liquid formulations.

Mechanism of Action

MPA, the active ingredient of the prodrug MMF, acts by blocking *de novo* purine synthesis in lymphocytes. Purines can be generated either by *de novo* synthesis or by recycling (salvage pathway). Lymphocytes preferentially use *de novo* purine synthesis, whereas other tissues such as brain use the salvage pathway. MPA uncompetitively inhibits IMPDH, which is the rate-limiting enzyme in the *de novo* synthesis of guanosine monophosphate (GMP) (Fig. 67.12). Inhibition of IMPDH creates a relative deficiency of GMP and a relative excess of adenosine monophosphate (AMP). GMP and AMP levels act as a con-

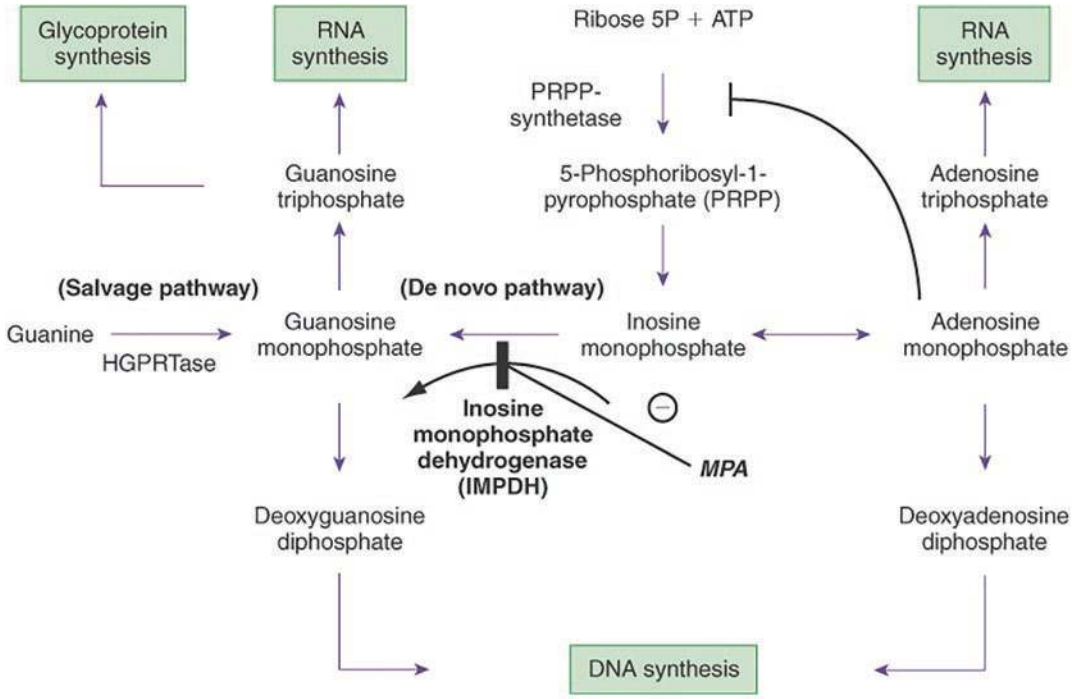

Fig. 67.12 Immunosuppressive mechanism of mycophenolic acid. By inhibiting inosine monophosphate dehydrogenase (IMPDH), mycophenolic acid (MPA) antagonizes the *de novo* pathway of purine synthesis on which lymphocytes particularly depend. Accumulation of adenosine monophosphate inhibits 5-phosphoribosyl-1-pyrophosphate (PRPP) activity thereby diminishing the substrate of IMPDH. Depletion of guanosine phosphates inhibits DNA and RNA synthesis. Lymphocytes lack the salvage pathway of purine synthesis, which depends on the activity of the enzyme hypoxanthine guanosine phosphoribosyl transferase (HGPRTase)

trol on *de novo* purine biosynthesis, which is essential for T and B lymphocyte proliferation, but not for division in other cells. Therefore, MMF, by blocking IMPDH, creates a block in *de novo* purine synthesis that selectively interferes with proliferative responses of T and B lymphocytes, inhibiting clonal expansion, and thus inhibiting antibody production, the generation of cytotoxic T cells, and the development of delayed type hypersensitivity. Furthermore, MPA impairs the ability of dendritic cells to present antigen, suppresses the recruitment of monocyte lineage cells, suppresses the glycosylation of adhesion molecules, inhibits vascular smooth muscle proliferation, improves endothelial function, and inhibits mononuclear cell recruitment into allografts and nephritic kidneys [89]. MPA also decreases cytokine-induced nitric oxide synthesis and prevents the formation of reactive species such as peroxynitrite. Furthermore, MPA exhibits antioxidant effects in experimental nephropathies. These properties of MPA likely augment its immunosuppressive properties by limiting fibrosis and vascular sclerosis after immunological injury [90].

Dosage and Pharmacokinetics

MMF, a semi-synthetic ethyl ester of MPA, is rapidly and completely absorbed and hydrolyzed by esterases to yield the active drug MPA. The recommended dose in pediatric patients in conjunction with CSA is 1200 mg/m^2 per day in two divided doses, the recommended MMF dose in conjunction with TAC is 800 mg/m^2 per day in two divided doses. However, recent data from a large, prospective, randomized study in both pediatric and adult renal transplant recipients on fixed dose MMF vs. a concentration-controlled

regimen, the FDCC study, indicate that a higher initial MMF dose, for example 1800 mg MMF/m^2 per day in conjunction with CSA and 1200 mg MMF/m^2 per day in conjunction with TAC for the first 2–4 weeks post-transplant, is required to achieve adequate MPA exposure in the majority of patients [91, 92]. The MMF dose should be reduced with active CMV infection. When MMF is associated with diarrhea (a side effect of MMF, see below), dividing the daily dosing to 3–4 doses per day may be effective in controlling the diarrhea.

The difference in MMF dosing depending on the concomitant CNI is explained by a pharmacokinetic interaction of CSA with the main MPA metabolite 7-O-MPA glucuronide (7-O-MPAG). CSA inhibits the multidrug resistance protein 2-mediated transport of 7-O-MPAG into the bile. MPAG is subject to enzymatic and non-enzymatic hydrolysis in bile and more importantly in the intestine, thereby liberating the unconjugated drug MPA, which is then reabsorbed into the systemic circulation. This enterohepatic circulation is responsible for a secondary MPA peak occurring 6–12 h after administration. The impact of the enterohepatic cycle on the MPA plasma concentration varies within and between individuals due to factors such as meal times or co-medication of drugs that interrupt the enterohepatic circulation (e.g., bile acid sequestrants, antibiotics). These factors should be considered when evaluating MPA concentrations (particularly pre-dose concentrations) in clinical practice. Furthermore, genetic differences and disease can affect enterohepatic cycling and thus the bioavailability of MPA [93]. If CSA doses are tapered, the pre-dose concentrations of MPA significantly increase, and after complete discontinuation of CSA they can reach about twice the values seen in patients still on CSA co-therapy. When using MMF in combination with TAC or SRL, lower MMF doses can be used to achieve comparable MPA exposure, guided by therapeutic drug monitoring, to that seen with CSA [93]. However, an uncritical approach used by some centers to reduce the MMF dose generally by 50% when co-administered with TAC or SRL is not advisable.

Table 67.4 Drug-Drug-Interactions between mycophenolate mofetil and frequently used co-medications

Drug	Effect	Site of interaction
Antacids	MPA AUC ↓	Absorption
Cholestyramine	MPA AUC ↓ MPAG AUC ↓	Absorption
Corticosteroids	MPA trough ↓ MPA AUC ↓ MPAG ↑	Glucuronidation
Ciclosporin	MPA trough ↓ MPA AUC ↓	Enterohepatic cycling
Metronidazole	MPA AUC ↓ MPAG AUC ↓	Enterohepatic cycling, suppression of anaerobic bacterial glucuronidase
Norfloxacin	MPA AUC ↓ MPAG AUC ↓	Enterohepatic cycling, suppression of anaerobic bacterial glucuronidase
Phosphate binder	MPA AUC ↓ Cmax ↓	Absorption

AUC area under the concentration time-curve, *Cmax* maximal (peak) plasma concentration, *MPA* mycophenolic acid, *MPAG* mycophenolic acid-glucuronide

The metabolism of MPA due to glucuronidation can also be affected by drug induction. Steroids are known inducers of UDP-glucuronosyltransferases *in vitro*, and there is evidence that this may hold true *in vivo*. In one study, for example, the effect of steroid withdrawal on MPA bioavailability was studied in 26 kidney transplant recipients [94]. When steroids were completely withdrawn 12 months post-transplant, a 33% increase in the mean dose-normalized MPA pre-dose concentrations and MPA-AUCs was observed compared with concentrations at 6 months, when the patients were still receiving maintenance doses of steroids. The relevant drug-drug interactions are summarized in Table 67.4.

An important pharmacokinetic property of MPA is its extensive and tight protein binding particularly to serum albumin. The free MPA fraction in individuals with conserved renal function ranges from 1% to 3%. Based on *in vitro* studies, the free MPA fraction is responsible for the pharmacological activity of the drug. Furthermore, it is an important determinant of the

MPA clearance. Of the factors evaluated for their effect on MPA protein binding, serum albumin was the most important. In patients with delayed graft function or renal impairment, there are many factors which can affect MPA protein binding. These may lead to substantially elevated free MPA concentrations despite total MPA levels similar to those found in patients with relatively preserved renal function [95, 96].

Efficacy

Following the success of the early MMF studies in adults, MMF was investigated in pediatric renal transplant recipients in open-label studies with historical controls, since randomized, controlled trials were quite difficult to carry out due to the relatively small numbers of pediatric kidney transplants performed each year. Since studies in adult renal transplant recipients had previously established the superiority of MMF over AZA or placebo in reducing the risk of acute rejection, it was important for the pediatric transplant community to have prompt access to open-label studies. Data from 3 large multicenter studies [97–101] and one smaller study [102] provided support for the safety and efficacy of MMF in the pediatric renal transplant population when used with CSA and prednisone. Induction therapy was optional in one study [97] and not used in the other two studies [99, 102]. The incidence of acute rejection within the first 6 months to 1 year for patients receiving MMF in these studies ranged from 28% to 37% [97, 99, 101]. Those studies comparing MMF patient groups to historical controls reported significant reductions in the incidence of acute rejection with MMF vs. AZA [99, 101]. There was also a significant improvement in the incidence of acute rejection between patients receiving MMF and those receiving AZA at 3 years in a follow-up report to one study [100]. In one large study [97], no differences in the incidence of acute rejection were observed when results were stratified by age. Long-term (3-year) graft and patient survival were excellent, with a 30% incidence of acute rejection [98]. MMF has a role in the prevention and/or treatment of chronic rejection. Among children with chronic rejection, some evidence suggests that substituting MMF for AZA may improve renal function [103, 104].

Side Effects

The major toxicity of MMF is gastrointestinal, mainly diarrhea, possibly as a result of the high concentrations of acyl-MPAG in the gut. MMF is devoid of intrinsic renal, cardiovascular or metabolic toxicities, but can increase the risk for CMV infections, leukopenia, and perhaps, mild anemia (Table 67.2). MPA has been associated with protection from *Pneumocystis jirovecii* pneumonia (PJP) and may actually have some anti-PJP activity because *Pneumocystis jirovecii* has IMPDH activity. MMF should not be used in pregnant transplant patients since its safety in pregnancy has not yet been established.

In the MMF suspension trial in pediatric renal transplant recipients, MMF safety was evaluated based on the occurrence of adverse events, including the development of opportunistic infections and malignancies. The most frequently noted adverse events were hematological problems such as leukopenia and gastrointestinal disorders like diarrhea, which occurred in 25% and 16% of all patients, respectively, and were observed more often in the youngest age group. In general, the risk of developing side effects declined with increasing age.

Therapeutic Drug Monitoring

Patients on standard-dose MMF therapy show considerable between-patient variability in pharmacokinetic parameters. This variability is attributable to factors that influence exposure to MMF, such as renal function, serum albumin levels, concomitant medications such as CSA that inhibit enterohepatic recirculation of the active metabolite of MMF, MPA, (Table 67.4) and genetic polymorphisms of MPA-metabolizing enzymes. This variability is clinically relevant, as higher plasma concentrations of MPA are correlated with reduced risk of acute rejection after kidney transplantation [96, 105]. These findings have suggested that individualizing the dose regimen of MMF may further improve clinical outcomes compared with a standard-dose regimen.

There has been considerable debate regarding the utility of measuring MPA levels. Advocation for MPA monitoring is based on the premise that monitoring will result in avoiding both underdosing, which prevents rejection, and overdosing, which increases the risk of adverse reactions [105]. One study in adults, for example, showed significantly fewer treatment failures and acute rejection episodes in the monitoring arm compared with the fixed dose arm with no significant difference in side effects [106]. Within this study, MPA exposure and MMF dosing were higher in the monitoring arm based on 3 levels measured over the first 3 h post-dose. Awareness of the potential for a more personalized dosing has led to development of methods to estimate MPA AUC based on the measurement of drug concentrations in only a few samples. This approach is feasible clinically, and has proven successful in terms of correlation with outcome [107]. An MPA-AUC > 40 mg x L/12 h has been recommended for sufficient MPA exposure for the prevention of acute rejection episodes [92]. In general, monitoring of MPA exposure by MPA pre-dose plasma levels is more popular in clinical practice than monitoring of the MPA-AUC by a limited sampling strategy, but less precise. Therefore, some transplant centers monitor MPA pre-dose and target levels between 1.5 and 4 mg/L. In addition, they use levels as a measure of adherence.

Target of Rapamycin (TOR) Inhibitors

SRL (sirolimus, rapamycin) is a macrocyclic triene antibiotic that is produced by the actinomycete *Streptomyces hygroscopicus*. SRL was approved in September 1999 by the US FDA and in December 2000 by the European Medicines Agency for use in adult renal transplant recipients. EVR is an analog of SRL that has similar effectiveness and side effect profile.

Mechanism of Action

SRL displays a novel mechanism of immunosuppressive action. Interaction with at least two intracellular proteins is required to elicit its antiproliferative activity. SRL first binds to the cytosolic immunophilin FK-binding protein 12 (FKBP12). In contrast to the TAC-FKBP12 complex, the complex of SRL with FKBP12 does not inhibit calcineurin activity. Instead, this complex binds to and inhibits the activation of mTOR, a key regulatory kinase. This inhibition suppresses cytokine-mediated T-cell proliferation, inhibiting the progression from the G1 to the S-phase of the cell cycle. Thus, SRL acts at a later stage in the cell cycle than do the CNIs CSA and TAC. SRL can, therefore, be used in combination with the CNIs to produce a synergistic effect [108].

Dosage and Pharmacokinetics

EVR is available as tablets and dispersible tablets for administration in water. The current evidence from pediatric renal transplantation suggests that EVR should be administered at an initial dose of 0.8 mg/m^2 body surface area twice daily when given in combination with CSA therapy, adjusted to target a trough concentration of 3–8 μg/L [109, 110]. There is a well-documented drug-drug interaction between mTOR inhibitors and CSA [111], arising from a shared metabolic pathway via the cytochrome P450 CYP3A4 isoenzyme system, and the fact that both are substrates for the drug transporter P-glycoprotein. EVR exposure is increased by up to three-fold in patients receiving concomitant CSA [112, 113], while TAC exerts only a minimal effect [113, 114]. In patients receiving EVR with concomitant TAC, a dose of 2 mg/m^2 body surface area twice daily is therefore appropriate [112]. Similarly, SRL bioavailability is higher in the presence of CSA than TAC [115, 116].

SRL is available as tablets form and an oral solution. Data on target doses and blood concentrations for SRL in pediatric transplant recipients are more limited than for EVR. The half-life of SRL increases with age in children [117]. Twice-daily dosing and daily dosing is therefore recommended in young children and older recipients, respectively. However, there is no clear guidance regarding the age or body weight when the switch should be considered. One trial used 13 years of age as a cutoff point [118]. In a pharmacokinetic study of 13 children receiving SRL in a CNI-free regimen, with a median age of 15.5 years, the

authors concluded that twice-daily dosing was required in this setting due to more rapid metabolism of SRL in the absence of concomitant CNI therapy [119]. High SRL trough concentrations (>10 μg/L) either with or without concomitant CNI appear inadvisable in children in view of the high risk of toxicity and discontinuation. One small prospective study (n = 19) converted pediatric kidney transplant patients to a CNI-free regimen of SRL with MMF using a single SRL loading dose of 5–7 mg/m^2 body surface area, then a daily dose of 2–4 mg/m^2 body surface area adjusted to target a SRL trough concentration of 5–10 μg/L, and achieved a low rate of rejection with a good renal response [118].

EVR and SRL are both macrolide derivatives and share many pharmacokinetic features, including a close correlation between total exposure and trough concentration, low absorption that varies between and within patients, and differences in absorption between adults and children [111, 120]. However, EVR is the 40-O-(2-hydroxyethyl) derivative of SRL, a modification that results in some important pharmacokinetic differences between the two drugs. EVR is more hydrophilic than SRL and is absorbed more rapidly from the gut with more systemic clearance than SRL [121]. As a result, the elimination half-life of EVR is shorter than for SRL (mean 28 h vs. 62 h) [122, 123]. The clinical effect is that no loading dose is required for EVR whereas a loading dose of 3 times the maintenance dose has been recommended for starting SRL in adults to accelerate achievement of steady-state concentration [124]. EVR is administered twice a day in pediatric and adult patients whereas once daily dosing is appropriate for SRL in older children and adult patients (see above for considerations regarding SRL dosing in younger children).

Efficacy

The most frequent reason to include an mTOR inhibitor in the immunosuppressive regimen is to facilitate a reduction in CNI exposure, or to eliminate CNI therapy entirely. The current evidence suggests that *de novo* administration of EVR with low-exposure CNI therapy in children undergoing renal transplantation is efficacious and safe. The recently published 36-month, multicenter, open-label randomized study investigated EVR with reduced-dose TAC and steroid elimination from month 5 post-transplant compared with a standard-dose TAC regimen with MMF and steroids (control) [125]. The incidence of composite efficacy failure (biopsy-proven acute rejection [BPAR], renal allograft loss, or death) at month 36 was 9.8% vs. 9.6% for EVR with reduced-dose TAC and MMF with standard-dose TAC, respectively, which was driven by BPARs. Kidney allograft loss was low (2.1% vs. 3.8%) with no deaths. Mean eGFR rate at 3 years post-transplant was comparable between groups. Growth in pre-pubertal patients on EVR with reduced-dose TAC without steroids was better (P = 0.05) vs. MMF with standard-dose TAC and steroids. The overall incidence of adverse events and serious adverse events was comparable between groups. Rejection was the leading adverse event for study drug discontinuation in the EVR with reduced-dose TAC group. The authors concluded that, although adverse events-related study drug discontinuation was higher, an EVR with reduced-dose TAC regimen represents an alternative treatment option that enables steroid withdrawal as well as CNI reduction in pediatric kidney transplant recipients [125].

Use of *de novo* EVR with complete CNI avoidance has not been explored in large trials in pediatric transplant recipients, but is unlikely to be preferable to concomitant reduced-exposure CNI. Primarily mTOR-based, CNI-free immunosuppression is associated with a significantly increased risk of the development of DSA [126]. Switching maintenance patients to an mTOR inhibitor to facilitate CNI minimization can improve renal allograft function or avoid further functional deterioration, particularly when undertaken before irreversible damage has developed. Late switch below an eGFR of 40 mL/min/1.73 m^2, however, may be associated with an increase in pre-existing proteinuria, favoring early conversion. It remains unresolved whether CNI therapy should be reduced or, indeed, eliminated in maintenance patients regardless of whether renal dysfunction is believed to be due to CNI-related nephrotoxicity. Currently, many

transplant centers use mTOR inhibitors as part of a maintenance immunosuppressive regimen only in the following patient subsets in which this drug class may have particular utility: (i) Patients, who have histologically proven CNI nephrotoxicity despite low levels and doses of the CNI; (ii) patients with malignancy (e.g., skin cancers and Kaposi sarcoma), either in remission or being actively treated; (iii) patients after treatment of B cell PTLD; (iv) patients with recurrent CMV viremia, because EVR has anti-CMV activity *in vitro* and is associated with less CMV replication and disease *in vivo* compared to MMF [127, 128]. Notably, the incidence of EBV or BK polyomavirus infection is not lower in EVR- compared to MMF-treated patients [125].

Side Effects

Clinically relevant adverse effects of SRL and EVR that require a specific therapeutic response or can potentially influence short- and long-term patient morbidity and mortality as well as graft survival include hypercholesterolemia, hypertriglyceridemia, infectious and non-infectious pneumonia, anemia, lymphocele formation and impaired wound healing (Table 67.2). These drug-related adverse effects are important determinants in the choice of a tailor-made immunosuppressive drug regimen that matches the individual patient risk profile. Equally important in the latter decision is the lack of severe intrinsic nephrotoxicity associated with SRL and EVR and its advantageous effects on hypertension, post-transplantation diabetes mellitus and esthetic changes induced by CNIs. Mild and transient thrombocytopenia, leukopenia, gastrointestinal adverse effects and mucosal ulcerations are all minor complications of SRL and EVR therapy that have less impact on the decision for choosing this drug as the basis for tailor-made immunosuppressive therapy.

An additional side effect in the setting of CNI withdrawal and mTOR inhibitor introduction is aggravation of proteinuria in patients with pre-existing proteinuria by a still incompletely defined mechanism. Available data are consistent with the hypothesis that the increase of proteinuria is causally related to CNI withdrawal and not because of initiation of an mTOR inhibitor [129]. On the other hand, it cannot be excluded that SRL and EVR might also affect glomerular permeability in some patients. The potential complication of increased proteinuria, which is an independent risk factor for decreased long-term kidney allograft function, should therefore be considered when converting from a CNI-based to an mTOR-containing maintenance therapy. Preliminary results show that mTOR inhibitor treatment may impair gonadal function after kidney transplantation, but the clinical significance of these effects is unknown [129, 130].

Generic Immunosuppressive Drugs

The number of immunosuppressive drugs prescribed to prevent rejection is relatively small. Not more than 10 different compounds have been used over a period of 50 years. For most of these drugs, the patents have expired (AZA, CSA, TAC, MMF, SRL), or will expire within the next few years (EVR, mycophenolate sodium) [131]. Policy makers consider generic drugs an attractive option to enable savings on medication cost, allowing the savings to be used for funding high-cost medicines. Generic immunosuppressive drugs are available in Europe, Canada, and the US. Between countries, there are large differences in the market penetration of generic drugs in general, and for immunosuppressive drugs in particular. To allow for safe substitution, a number of criteria need to be fulfilled. Generic substitution should not be taken out of the hands of the treating physicians. Generic substitution can only be done safely if initiated by the prescriber, and in well-informed and prepared patients. Payers should refrain from forcing pharmacists to dispense generic drugs in patients on maintenance treatment with brand drugs. Instead, together with transplant societies, they should design guidelines on how to implement generic immunosuppressive drugs into clinical practice. Substitutions must be followed by control visits to check if the patient is taking the medication correctly and if drug exposure remains stable. Inadvertent, uncontrolled

substitutions from one generic to another, initiated outside the scope of the prescriber, must be avoided as they are unsafe. Repetitive subsequent generic substitutions result in minimal additional cost savings and have an inherent risk of medication errors [131].

Adherence to Immunosuppressive Medication

Adherence to medications is defined as the process by which patients take their medication as prescribed. Adherence is of critical importance and it is often lacking, especially in teenagers. Adolescent age at transplant is an independent and significant risk factor for worse long-term renal allograft survival in all major pediatric solid organ transplant types [132]. Adolescents have also been identified to have a multitude of psychiatric and socioeconomic risk factors that deserve greater attention and customized care delivery programs [133]. Immunosuppression obviously only works if it is taken reliably, and there is ample evidence in the literature that this reliability is lacking far too often, leading to adverse outcomes. Moreover, immunosuppressive regimens may need to be adjusted to support adherence, e.g. by combination products to reduce the pill burden and/or by once daily dosing. The latter is realized by a prolonged-release TAC formulation available for older children and adolescents (Advagraf™ in Europe, Astagraf™ in the US). Comparative pharmacokinetic studies have shown that stable pediatric transplant recipients can be converted from immediate-release to prolonged-release TAC at the same total daily dose, using the same therapeutic drug monitoring method [134, 135].

Conclusions

Transplantation in children carries unique challenges. While issues such as controlling rejection and minimizing side effects are similar between adults and children, maintenance immunosuppressant regimens that affect developmental processes have a disproportionate impact on children. This is particularly true for steroids, which have many side effects, including some that can be quite devastating in pediatric patients. Steroid avoidance has been very successful when MMF is combined with TAC and either basiliximab or anti-thymocyte globulin for induction therapy is added; long-term follow-up on these patients with regards to improvements in kidney allograft survival and transplant function will be very informative, as steroid-free patients also have reduction of co-morbidities that drive chronic allograft dysfunction. With the goal of eliminating steroids, the combination of MMF and TAC may strike the correct balance between adequate and excessive immunosuppression. Recently, the use of EVR has been advocated for minimizing CNI exposure after kidney transplantation, but its use is limited by mTOR-related side effects. There is at present no consensus for immunosuppressive therapy following renal transplantation in children.

Newer drugs such as belatacept have not been systematically studied in the pediatric transplant patient population. Since the approval of belatacept in 2011 for use in *de novo* adult renal transplantation, this CD80/86-CD28 co-stimulation blocker has been shown to be a valuable treatment option for maintenance immunosuppression [136]. Belatacept in adults has been associated with a superior GFR as compared to CNI-based treatments because of the absence of nephrotoxicity. Additionally, belatacept avoids cardiovascular side effects (e.g. hypertension and dyslipidemia) caused by a CNI-based-regimen [136]. However, its use is limited to EBV-seropositive patients, since EBV-naïve patients on belatacept have an increased PTLD risk. Nevertheless, there is great interest in belatacept as possible “depot” immunosuppression in adolescent pediatric recipients. The latter population has the highest rates of kidney allograft loss due to immunosuppression non-adherence and will most likely have prior exposure to EBV. Belatacept has been shown to be safe in adolescents, but not yet approved for children or EBV-naïve patients given the additional risk for developing PTLD [137]. Studies are underway to explore safe pro-

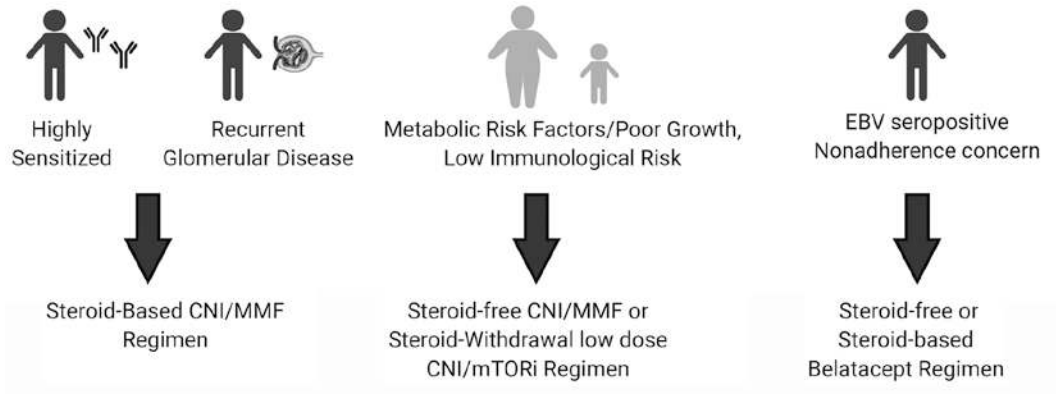

Fig. 67.13 Modern era immunosuppressive strategies in children. The role of a low-dose CNI regimen is illustrated within the context of other maintenance therapies. *CNI* calcineurin inhibitor, *EBV* Epstein-Barr virus, *MMF* mycophenolate mofetil, *mTORi* mammalian target of rapamycin inhibitors. (Reproduced from [138], with permission)

tocols for the use of belatacept in adolescent children.

Currently, there is a paucity of novel maintenance immunosuppressive drugs in the pipeline. Iscalimab, a non-B cell depleting anti-CD40 monoclonal antibody, has been investigated in a phase 2 trial in *de novo* adult kidney allograft recipients with a CNI-free regimen, and other agents targeting co-stimulation blockade are in pre-clinical development [85].

Clearly, much additional work is needed to define optimal immunosuppressive regimens in pediatric renal transplant patients, particularly with respect to newer and evolving regimens. The safety and efficacy of these protocols, with special emphasis on long-term renal allograft survival, PTLD and other malignancies, but also co-morbidities need to be established. The "one size fits all" strategy needs to evolve into tailored strategies based upon a child's medical history and circumstance (Fig. 67.13) [138].

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68 Non-Infectious Post-Transplant Complications: Disease Recurrence and Rejection

Lyndsay A. Harshman, Sharon M. Bartosh, and Stephen D. Marks

Introduction

With improved screening for immunological sensitization and newer immunosuppressants, the early renal allograft survival rates have increased considerably over the past three decades. However, the risk of renal allograft loss due to non-infectious complications—such as rejection or recurrence of primary disease—remains a worrisome and stark reality for some pediatric kidney transplant recipients (pKTR). This chapter will discuss the causes of allograft dysfunction, including recurrence of primary kidney disease, rejection, and acute post-operative complications. The investigations and management of these conditions will be considered with particular emphasis on the diagnosis and treatment of graft dysfunction.

Recurrence of Primary Kidney Disease in the Allograft

Overall, recurrence of disease accounts for 7–8% of graft losses in pKTR and is the fourth most common cause of allograft loss after chronic rejection, acute rejection, and death with a functioning graft [1]. Recurrent diseases leading to allograft loss are most commonly glomerulonephritis (70–80%) and inherited metabolic diseases (Table 68.1) [1].

Disease recurrence may render the graft unsalvageable and lead to years of patient and graft life lost [2]. General features of disease recurrence may include renal allograft dysfunction with elevated serum creatinine, hypertension, oligoanuria, hematuria, and/or proteinuria. Recurrence may occur at variable time points post-transplant, with some diseases, such as C3 glomerulopathy (C3G) and focal and segmental glomerulosclerosis (FSGS), having the potential to recur within hours to days after transplant.

In a competing risk analysis of 1955 European children transplanted before age 20 years, the highest rates of allograft failure were seen in

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Table 68.1 Estimated rates of disease recurrence following transplantation. (Reproduced with permission from Cochat et al., [1])

Primary disease	Recurrence rate	Graft loss to recurrence
FSGS	14–50%	40–60%
Atypical HUS	20–80%	10–83%
Typical HUS	0–1%	0–1%
MPGN type 1	30–77%	17–50%
MPGN type 2	66–100%	25–61%
SLE nephritis	0–30%	0–5%
IgA nephritis (Berger disease)	35–60%	7–10%
Henoch–Schönlein nephritis	31–100%	8–22%
Primary hyperoxaluria type 1	90–100%	80–100%

those children with immune-mediated kidney disease compared to children with end-stage kidney disease (ESKD) secondary to congenital anomalies of the kidney and urinary tract (CAKUT) [3]. Statistically significant differences in 5-year allograft losses were seen for children with FSGS (25.7%) and membranoproliferative glomerulonephritis (MPGN) (32.4%) compared to transplant recipients with CAKUT (14.4%) as the cause of their ESKD [3].

Focal and Segmental Glomerulosclerosis

Nature and Frequency of Primary Disease

FSGS is the third most common primary diagnosis in children and adolescents, accounting for 10–13.2% of cases in children older than 12 years of age, with nearly 50% of affected patients progressing to ESKD [4]. The percentage of children listed as having either FSGS or other glomerular disease as the cause of their ESKD has been decreasing over the past decade, perhaps related to improved therapeutics and success in treating glomerulonephritis in children. Despite improvements in treatment, glomerulonephritis (non FSGS) is the identified cause of ESKD in 6.5% of children undergoing kidney transplantation in the USA [5].

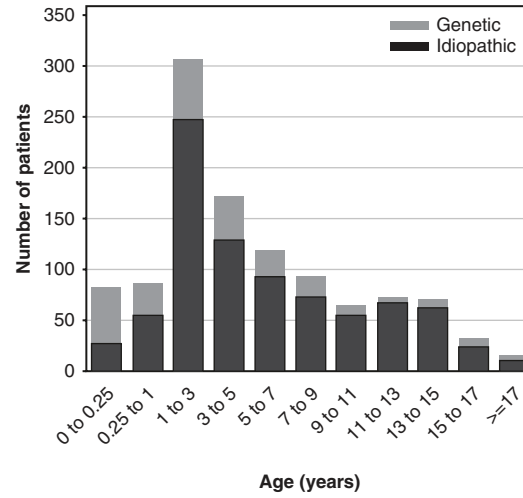


Fig. 68.1 Age at first disease manifestation in children with and without an identified genetic cause of steroid-resistant nephrotic syndrome [13]

Although most cases of FSGS are sporadic (idiopathic), familial forms have been linked to mutations in genes encoding slit diaphragm in addition to other proteins, including nephrin, podocin, WT1, alph-actin-4, CD2AP and TRPC6 [6–12]. The percentage of children having an identified gene mutation is variable depending on the age of the child [13]. In children presenting with NS in the first 3 months of life, 85% have pathogenic mutations. The percentage of children with identifiable mutations decreases with age (for example, 50% in those 4–12 months and approximately 10% in teenagers; Fig. 68.1).

Investigations into podocyte biology and circulating permeability factors have shed light on the pathogenesis of FSGS. A candidate circulating factor, soluble urokinase-type plasminogen activator receptor (suPAR) has been identified to potentially explain the pathophysiology of FSGS at least in some cases of primary, non-familial FSGS [14–16]. The prognostic value of suPAR in the prediction of disease recurrence post-transplant, however, remains unclear [2, 17–20].

Considerations for Transplant Planning: Predicting Risk for Recurrence

In children with ESKD secondary to FSGS, considerations for transplant planning and donor selection are related to the risk of disease recurrence [13]. Much of the early literature relating to recurrence risk did not differentiate between idiopathic cases of FSGS and genetic causes of FSGS. Since genetic FSGS is presumably due to structural alterations in podocyte proteins of the native kidneys, one would not predict recurrence of disease for these patients. Screening for genetic mutations has become a routine part of pre-transplantation evaluation in patients who develop ESKD due to FSGS to allow for appropriate counseling of families and to anticipate the risk of recurrence [6, 21, 22]. Unfortunately, the genotype-phenotype relationship for recurrence risk is not always clear, especially when identified variants are of unknown pathogenic significance.

The clinical course of FSGS prior to ESKD can provide important insight to risk for recurrence. For example, initial steroid responsiveness that evolves to secondary steroid resistance appears to have a higher risk of recurrent disease. In contrast, patients with initial steroid resistance were more likely to have a genetic cause of FSGS and a significantly lower likelihood of developing recurrence post-transplant [23]. Additional clinical risk factors for recurrence include: an aggressive clinical course of primary FSGS with rapid progression to ESKD within 3 years of diagnosis and younger age at onset of nephrotic syndrome [24–26]. The role of race in predicting recurrence of FSGS has been evaluated by several groups. Although African American children tend to have a more aggressive course of initial disease, they do not appear to be at higher risk of recurrence and may be at a lower risk compared to white children [27–29].

Donor type, induction immunosuppression regimen, and timing of corticosteroid withdrawal have all been evaluated using large international registry studies to describe risk for FSGS recurrence after kidney transplant. The TANGO (post-transplant glomerular disease) study evaluated a cohort of 176 patients who presented with nephrotic syndrome and had biopsy proven idiopathic FSGS [30, 31]. FSGS recurrence rate was 32%, with a median time to recurrence of 1.5 months. Among those who had FSGS recurrence, 39% lost their allograft over a median of 5 years. There was a higher risk of recurrence with older age at primary FSGS onset, white race, lower BMI at transplant, and native nephrectomies. No differences were seen for donor type (living or deceased), human leucocyte antigen (HLA) mismatch, induction immunosuppression regimen, pre-transplantation plasmapheresis (PE), or time to ESKD from disease occurrence.

The impact of FSGS on renal allograft survival in children is greatest in transplants after living donation, resulting in loss of the expected living donor allograft survival advantage [28, 32–34]. This effect is particularly evident in adolescents [32]. For this reason, the rationale for use of living donors in children with ESKD secondary to FSGS should be based on factors other than better outcomes typically associated with living donor transplantation [21]. Since the risk of recurrence of FSGS in a second allograft has, in some series, been reported to be as high as 100% [35], further attempts to transplant after allograft loss due to recurrence should be carefully considered [1].

While practices regarding pre-transplant native nephrectomies vary and some experts have supported bilateral native nephrectomy prior to transplantation [36, 37], others have not confirmed this recommendation [38, 39]. While there may be reasons to perform native nephrectomy

prior to kidney transplantation, such as persistence of nephrosis and abnormal coagulation increasing the risk of thrombosis, there is not convincing evidence that native nephrectomies affect FSGS recurrence risk in the pediatric population.

There are no clear data to support a single induction immunosuppression regimen to minimize risk for post-transplant FSGS recurrence. Raafat et al., [40] evaluated retrospective data for 35 pKTR between 1968–1997 at a single center. They found a potentially higher risk of FSGS recurrence associated with use of anti-lymphocyte globulin, with seven of eight patients receiving anti-thymocyte globulin (ATG) experiencing a recurrence. Hubsch et al. [41] compared the incidence of recurrence following induction with ATG and the IL-2 receptor blocker daclizumab. In contrast to data from Rafaat et al., Hubsch found a significantly higher risk of recurrence with daclizumab as induction therapy. More recent multicenter, retrospective data from the North American Pediatric Renal Trials Collaborative Study (NAPRTCS) evaluated patients transplanted between 2002 and 2016, comparing patients with FSGS to other glomerular diseases [34]. Among the cohort of 2010 patients, there was no association between induction agent(s) and allograft survival. Lastly, there are no data supporting use of rituximab as a primary transplant induction agent for prevention of recurrence of FSGS.

As with the data for induction immunosuppression, published literature is mixed as to the impact of corticosteroid avoidance and early corticosteroid withdrawal on potential for FSGS recurrence. Clinical practice regarding use of corticosteroids following transplant is variable. A descriptive survey was performed by the European Society for Pediatric Nephrology to evaluate current practices for recurrence of FSGS after pediatric kidney transplantation [42]. Within this professional cohort, corticosteroids were prescribed for different durations following transplant: (a) life-long (37%); (b) for 3–12 months (17%); (c) 1–2 years (15%); and (4) other or unknown (31%).

Kukla et al., [43] studied the effect of rapid corticosteroid discontinuation on disease recurrence among adult transplant recipients with a history of glomerulonephritis, including recipients with FSGS. They found that corticosteroid avoidance was associated with a higher rate of recurrent glomerulonephritis, but no apparent increase in risk of allograft loss. The 1-, 5-, and 7-year recurrence rate in the glomerulonephritis group completing a rapid corticosteroid discontinuation protocol was 6.7%, 13.7%, and 19.2% and in historic glomerulonephritis recipients maintained on corticosteroids it was 2.4%, 3.8%, and 5.3%, respectively ($P < 0.0001$). Within this sample, rapid corticosteroid discontinuation was also associated with a higher adjusted risk of recurrent disease for all glomerulonephritis types (hazard ratio 4.86; 95% confidence interval 2.34–10.07; $P < 0.0001$). The results of early corticosteroid withdrawal in adult FSGS recipients compared to a historic control group of FSGS patients who received a kidney transplant in parallel with corticosteroid-based immunosuppression was reported by Boardman et al., [44]. There was no significant difference in recurrent FSGS, time to recurrence, or allograft loss.

Given the mixed and limited results across available literature, the use of long-term corticosteroids should be weighed in the context of duration and risk-benefit ratio considering the increased risk for development of new onset diabetes mellitus after transplantation, hyperlipidemia and poor growth.

Treatment of FSGS Recurrence

When FSGS is the cause of ESKD, recurrence is one of the major risk factors for premature allograft loss and has been reported to occur in 14–60% of first transplants and up to 80% of subsequent transplants [1, 25, 27–29, 35, 45–47]. In the NAPRTCS report, 6.6% of index graft failures were due to recurrent disease, with 48% secondary to recurrence of FSGS [48]. There are many approaches for the treatment of recurrent FSGS, and conclusions are limited by small sample sizes and heterogeneity in treatment. The CERTAIN study group recommended initial treatment with early PE or immunoadsorption—

possibly combined with intravenous rituximab—and an angiotensin-converting enzyme inhibitor or angiotensin receptor blocker [21]. If there is inadequate response or patients are dependent on apheresis modalities, then the use of high dose cyclosporin, rituximab, or LDL apheresis should be considered.

Rarely, recurrence may occur intraoperatively at the time of transplant, and approximately 30% of patients with FSGS recurrence have histological evidence of recurrence within the first few days after transplant, with allograft loss in half of these cases [49, 50]. Recurrent disease should be suspected if there is significant proteinuria, which is often accompanied by hypoalbuminemia and other features of nephrotic syndrome. The diagnosis is confirmed histologically with the demonstration of podocyte foot process effacement and without the classical segmental sclerosis of glomeruli [51].

Optimal management of recurrent FSGS remains controversial due to the paucity of well-designed randomized trials and lack of control groups and is often approached in a multimodal manner using various combinations of high dose cyclosporin, cyclophosphamide, intravenous rituximab, PE, immunoadsorption (IA) and LDL-apheresis. To date, no controlled clinical trials comparing different treatment strategies have been performed, and current management recommendations have been adapted from anecdotal reports and small case series.

High dose cyclosporin, with doses of 13–35 mg/kg/day or intravenously at 3 mg/kg/day for up to 3 weeks, has been used for treatment of post-transplant recurrence of FSGS and has been found in retrospective series to achieve remission in approximately 75% of pKTR [52–54]. The reported studies are confounded by the inclusion of patients who had also received plasma exchange in addition to high dose cyclosporin, although the best outcomes were observed when combination therapy was utilized [55]. The CERTAIN study group recommended initial treatment with early PE or immunoadsorption—possibly combined with intravenous rituximab—and an angiotensin-converting enzyme inhibitor or angiotensin receptor blocker [21]. If there is inadequate response or patients are dependent on apheresis modalities, then the use of high dose cyclosporin, rituximab, or LDL apheresis should be considered.

Pre-emptive rituximab monotherapy has not been shown to prevent recurrence of nephrotic syndrome post-transplantation [56]. The addition of rituximab to PE may be considered, with 44–50% of patients treated with rituximab achieving complete remission and 20–25% achieving partial remission, and the reported number of doses given varying from 1 to 6 [57–61]. The optimal timing of rituximab administration and the required number of doses remain unclear. Likewise, a correlation between CD19/CD20 B-cell depletion and clinical efficacy has not been demonstrated. Side effects of rituximab include hypogammaglobulinemia and increased risk for infection.

Due to the successful treatment of children with steroid-dependent or frequently relapsing nephrotic syndrome with oral cyclophosphamide, this treatment has also been used to treat FSGS recurrence. Experience with oral cyclophosphamide in this setting is quite limited but has been reported to be well tolerated and to lead to long-term remission in some patients [35, 45, 62]. In one series of pediatric subjects, a cumulative dosage of 115–121 mg/kg of cyclophosphamide was used over a 3-month period to induce remission following disease recurrence [63].

Clinical and experimental evidence supports the existence of a circulating factor responsible for FSGS, with several candidate factors proposed including galactose, cardiotrophin-like cytokine-1 and soluble urokinase receptor (suPAR) [64]. Due to the possibility of a circulating permeability factor causing progressive podocyte injury leading to FSGS (primary or recurrent), the use of extracorporeal systems aimed at removal of this factor such as PE, IA, and low-density lipoprotein-apheresis (LDL-A) have been introduced. Several groups have reported the favorable effects of PE on remission of recurrent FSGS and on overall allograft survival although these retrospective case series differed in numbers of patients treated, underlying immunosuppression regimens, definition of

recurrence, and ethnicity of the patients [1, 25, 50, 54, 65–68]. Different PE protocols have been reported, with colloid replacement including 4.5–5% albumin, intravenous gamma globulin and fresh frozen plasma. In most reports, PE was started early, within 7 days of developing proteinuria. The outcome of PE in recurrent FSGS may be linked to the number of sessions performed, typically ranging from 5 to 13 treatments. After initiation of PE, the time to remission is also highly variable, ranging from 5 to 27 days in the reported literature. Immediately post-transplant, the exclusive use of 5% albumin should be avoided due to the increased risk of post-operative bleeding. Some centers recommend prophylactic use of PE even before nephrotic syndrome develops [67], although others have not found that preemptive therapy is effective [65, 69]. It is important to note that in reported cases, PE is almost universally used in conjunction with immunosuppressive therapy, including corticosteroids, cyclophosphamide and calcineurin inhibitors (CNIs).

Another therapy for recurrent FSGS which could potentially remove inciting circulating factors is IA [66, 70, 71]. A comprehensive review of the extracorporeal therapies, particularly with respect to the technical aspects, has recently been published [72]. Case series data suggests that IA seems to have comparable efficacy to PE although there are no head-to-head comparisons; thus, the exact mechanism of IA remains unclear. In a multicenter French study of recurrent FSGS in children, 10 of 12 treated with IA responded (eight with complete remission and two with partial remission) [68]. A decrease in proteinuria occurred within the first 10 sessions. Within 3 months of IA, eight pKTR were IA dependent, with two patients able to maintain remission without IA.

Apoproteins play a role in lipid transport and possibly in maintaining the integrity of the glomerular filtration barrier. Loss of these factors may play a role in the pathogenesis of recurrent FSGS [73]. There are many proposed mechanisms through which hyperlipidemia, hypercholesterolemia, and abnormal lipoproteins ultimately lead to progressive renal disease involving oxidative stress, vasoactive substances, and inflammatory factors [74]. LDL-A is a primary selective extracorporeal system that has been studied in FSGS [72]. In addition to inducing remission, LDL-A is purported to improve response rate to corticosteroid and immunosuppressive therapy. Currently, LDL-A is commonly performed using the Liposorber LA-15 system, which was approved by the FDA in the USA for a Humanitarian Device Exemption in 2013 for use in the treatment of pediatric patients with drug-refractory FSGS as well as post-transplant FSGS.

Lastly, renin-angiotensin-aldosterone blockade may be considered in post-transplant recurrence of FSGS, particularly to help diminish protein excretion and to address the suggested role of angiotensin in the role in the pathogenesis of recurrent FSGS [24, 75].

Atypical Hemolytic Uremic Syndrome

Nature and Frequency of Primary Disease

The prevalence of atypical hemolytic uremic syndrome (aHUS) in patients less than 20 years of age is estimated at 2.21–9.4 per million people [76] with the highest disease prevalence occurring in children between 0–4 years of age [77]. Uncontrolled overactivation of the alternative complement pathway (ACP) at the level of the endothelium is a primary immunological feature of aHUS [78]. There are known genetic abnormalities within the ACP which predispose to aHUS, including complement factor H (CFH), complement factor I (CFI), complement component C (C3), complement factor B (CFB), and membrane cofactor protein (MCP). In addition, some patients have aHUS secondary to autoantibodies to CFH. The clinical hallmark of aHUS is a thrombotic microangiopathy (TMA) with a microangiopathic hemolytic anemia, thrombocytopenia and renal dysfunction. Direct kidney injury is largely driven by damage to the glomerular endothelium from the complement-

associated membrane attack complex (MAC) composed of complement components (C) C5b-9 [79]. Kidney damage results in acute kidney injury, difficult to control hypertension, microscopic hematuria, and proteinuria.

Considerations for Transplant Planning

Kidney transplantation should be delayed at least 6 months after starting rescue therapy with eculizumab (a recombinant, humanized monoclonal antibody against the complement protein C5 [80]) as there may be limited recovery of kidney function that occurs within the first several months of eculizumab initiation [81–83]. Furthermore, aHUS-associated hematological features and extra-renal manifestations should be resolved prior to transplantation [81].

The risk for aHUS recurrence post-transplant is strongly linked with several pathogenic complement-based mutations. CFH, CFI, and C3 mutations have the highest risk for aHUS recurrence (68–90%, 70–80%, and 40–50%, respectively) [84]. It is important to note that approximately 30–50% of patients with aHUS have no identifiable genetic mutation or CFH autoantibody using currently available testing platforms [85, 86]. Pediatric patients and families should meet with a genetic counselor having expertise in complement-mediated genetic abnormalities. When counseling families of children and young people affected with aHUS without identification of a genetic mutation or autoantibody, it should be emphasized that there still may be an underlying genetic contribution to aHUS that could confer recurrence risk post-kidney transplant.

The diagnosis of aHUS has implications for evaluation of potential living donors. For example, kidney donation from a living-related donor has historically not been advised given the potential for the related donor to have the same genetic susceptibility factor(s) as the recipient [87]. As noted, if there is no identifiable complement genetic mutation in the patient, then living-related donation is contraindicated given the risk for an unidentified, underlying genetic mutation which could adversely impact the donor [88]. Conversely, if a pathogenic gene variant is identified in the recipient and not present in a potential living-related donor (and the donor has no other evidence of abnormal complement activation), then living-related donation may be feasible [87]. Living donation may, in fact, confer a decreased risk for complement activation secondary to ischemia-reperfusion injury typically encountered with deceased donation [89].

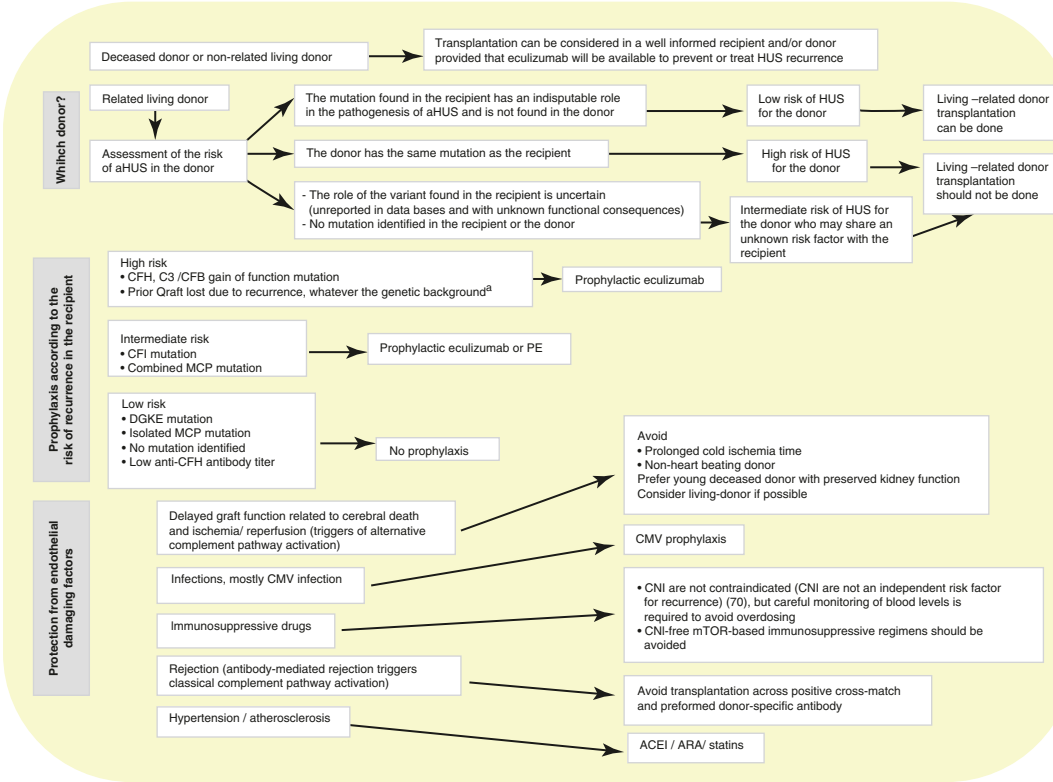
Risk Factors for Recurrence and Treatment of Recurrence

Most aHUS recurrences occur within the first year following kidney transplant. The strongest risk factor for aHUS recurrence is the presence of genetic complement abnormalities [86, 87, 90]. Feitz et al. [84] provide an excellent review of the estimated risk for aHUS recurrence based on complement gene mutation. The development and availability of eculizumab has drastically changed how transplant nephrologists approach prevention of aHUS recurrence. For patients with a known genetic mutation conferring risk for recurrence, intravenous eculizumab should be initiated within 24 h prior to transplantation, with an additional dose on the first post-operative day [87].

Current guidelines from the Kidney Disease Improving Global Outcomes (KDIGO) consensus report provide expert opinion regarding prophylaxis strategies against aHUS recurrence post-transplant based on a risk-assessment strategy (Table 68.2). For example, patients with persistently negative factor H autoantibody and/or isolated MCP mutations can potentially be transplanted without prophylactic eculizumab [81, 91]. In this situation, the child should be followed closely post-transplant for disease recurrence, with a low threshold to initiate intravenous eculizumab therapy. Markers of disease recurrence might include dropping C3, anemia, thrombocytopenia, low haptoglobin, elevated lactate dehydrogenase, new onset hypertension, microscopic hematuria, and/or proteinuria.

There are no data to support that nephrectomy prior to or coincident with transplant decreases

Table 68.2 Expert opinion regarding prophylaxis strategies against aHUS recurrence post-transplant based on a risk-assessment strategy. (Reproduced with permission from Loirat et al., [91])



risk for recurrence. Available, limited data suggest that targeted transplant protocols attempting to minimize endothelial damage may decrease risk for aHUS recurrence in patients not receiving prophylactic eculizumab. For example, induction therapy with basiliximab (interleukin-2 receptor blocker) may be preferable to use of lymphocyte depleting agents [92] in addition to decreasing the target trough CNI levels [93]. A case series [94] demonstrated excellent patient and allograft outcomes using an induction regimen with intravenous basiliximab, reduced-dose tacrolimus, and high-dose mycophenolate mofetil (MMF) in conjunction with early, strict blood pressure control, statin therapy, and angiotensin-converting enzyme inhibition to diminish the risk for endothelial injury that might up-regulate complement activation within the allograft. Acute rejection is an additional risk factor for aHUS recurrence; thus, intensified monitoring for aHUS recurrence may be required during rejection episodes [95].

Risk of Disease Recurrence in the Era of Eculizumab

Prior to the widespread use of eculizumab, kidney transplantation was not a viable option for many aHUS patients given the substantial morbidity and mortality associated with disease recurrence. Without eculizumab, the risk of recurrent disease after kidney transplantation was estimated to be 50–80%, with an overall 5-year graft survival of 36 ± 7% in patients with a recurrence compared with 70 ± 8% in patients without a recurrence [96, 97]. In the absence of effective anti-complement treatment, nearly 30% of the pediatric patients and half of adult patients with aHUS who survived in the acute phase of disease recurrence required long-term dialysis [85, 96]. The 2016 KDIGO consensus report on aHUS suggests that withdrawal of eculizumab should not be considered in patients treated for post-transplant recurrence until there are data demon-

strating the safety of this approach [91]. Limited case-series data suggest that cessation of eculizumab after the first year post-transplant may be a viable, safe option [98]; however, there is currently limited consensus to support this approach.

Additional Post-Transplant Considerations

Due to the risk for sepsis from encapsulated organisms, recipients receiving eculizumab should receive meningococcal and pneumococcal vaccinations prior to transplantation with additional booster vaccinations as necessary following transplantation [99]. The recipient additionally requires prophylactic antimicrobial coverage with ciprofloxacin or penicillin-V for the duration of eculizumab use [99]. The use of eculizumab, MMF, and CNI constitutes triple immunosuppression; thus, terminal CNI levels can potentially be targeted at the lowest end of the clinician's goal range to avoid overimmunosuppression and infectious complications. For example, this may correlate with targeting tacrolimus levels to 3–5 ng/mL after the first 12 months post transplantation in the absence of rejection (expert opinion).

C3 Glomerulopathy

Nature and Frequency of C3G

C3 glomerulopathy (C3G) is an umbrella term that encompasses both C3 glomerulonephritis (C3GN) and dense deposit disease (DDD). C3G is caused by overactivation of the alternative complement pathway. Abnormal complement activation typically may result from loss of function of one of the complement regulatory proteins (factor H or factor I) or from gain-of-function mutations in C3 that lead to resistance to regulation by factor H [100] (see Fig. 68.2). Overactivation of the complement pathway can

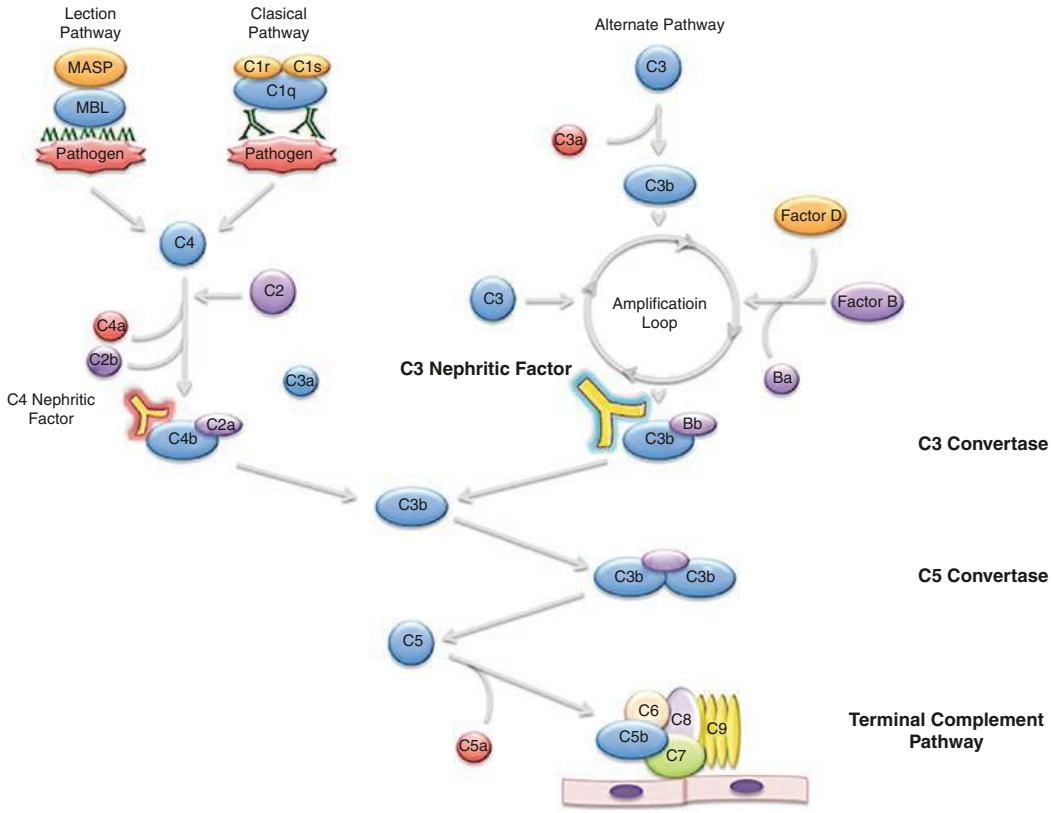


Fig. 68.2 Complement Inhibition in C3G. (Figure used with permission from Nester et al., [100])

also be secondary to generation of a C3 convertase-stabilizing autoantibody, C3 nephritic factor (C3NeF), or production of an autoantibody to factor H. Ultimately, these abnormalities result in overactivity of the C3 convertase and consumption of complement. Thus, low C3 level is a feature of C3G in approximately 75% of cases [78, 101].

C3GN and DDD have an overlapping spectrum of pathological and clinical features. Clinical features are consistent with active glomerulonephritis: nephrotic range proteinuria, microscopic hematuria, hypertension, and renal dysfunction with elevated serum creatinine. The diagnosis of C3G requires a percutaneous renal biopsy demonstrating significant C3 deposition within the kidney, specifically the glomerulus. C3 deposition occurs in the *absence* of immunoglobulin deposition on pathological examination (e.g., negative/near absent immunoglobulin G, immunoglobulin A, and immunoglobulin M) [102, 103]. Electron microscopy findings of electron-dense, "sausage-shaped" deposits within the glomerular basement membrane are pathognomonic of DDD whereas in C3G the deposits are less dense and primarily located within the mesangium.

C3G is an ultra-rare disease with an incidence of around 0.2–1 per 1,000,000 [104, 105]. C3G i has a 10-year kidney survival of 50%, leading to eventual need for kidney replacement therapy [78].

Considerations for Transplant Planning

As with aHUS, planning for transplant in the setting of C3G should include an evaluation with a team experienced in the genetics of complement-mediated kidney disease. This evaluation should include genetic testing of complement genes, measurement/assessment of complement function, and screening for complement autoantibodies. Mutation screening of complement regulatory genes (e.g., CFH, CFI), activation protein genes (C3, CFB), autoantibodies (C3NeF and antibodies to CFH), and assessment of copy number variation across the CFH-CFH-related (CFHR) locus should be done on a case-by-case basis given the need for expert interpretation and clinical validation [81, 102]. Genetic and functional studies may provide insight regarding utility and efficacy of targeted anti-complement therapy (e.g., eculizumab or novel anti-complement therapies in development) should C3G recur following transplant.

Living-related donor kidney transplantation should be approached with caution for both the presumed healthy donor and recipient with C3G. Current international recommendations are that all potential *recipients* of a living-related kidney be screened for genetic abnormalities within the complement system [81]. If a genetic abnormality is found, the donor should subsequently be tested. The presence of an identical genetic abnormality may not constitute an absolute contraindication to donation; however, the individual case be evaluated in conjunction with expert teams in complement genetics and C3G. Furthermore, for donors with identified complement genetic abnormalities, the donor team must disclose the theoretical risks that donation may trigger new disease onset.

There are no published data supporting a specific induction agent; thus, selection is based on center preference. There are no data to support pre- or peri-transplant nephrectomy to prevent disease recurrence. The presence of active disease, specifically heavy proteinuria, is a relative contraindication to transplantation; transplantation should be delayed until there is resolution of nephrotic-range proteinuria, which may require nephrectomies in some patients [81].

Risk Factors for Recurrence and Treatment of C3G Recurrence

C3G recurs at a high rate, with allograft loss due to C3G in approximately 50% of those patients [81]. Patients and families should be counseled on the high risk for disease recurrence. The reported recurrence rate of C3GN is estimated as greater than 50% [106, 107]. The recurrence rate of DDD is much higher and approaches 80–100% [108, 109].

Pediatric patients have lower long-term graft survival. It has been hypothesized that this may be due to more significant complement disruption and aggressive disease in children compared to adults. In one case series, 10-year graft survival was 11% and 21% in pediatric and adult allograft recipients, respectively [110].

Pre-transplantation C3 levels may also predict allograft outcomes. In one small case series, low C3 levels were present pre-transplantation in more than 50% of patients with recurrence; C3 levels were normal pre-transplantation in all patients without recurrence [111]. Other factors associated with an increased risk of C3G recurrence include high levels of circulating autoantibodies (C3NeF and FH autoantibody), rapid progression to ESKD in the native kidneys (crescentic disease) and living-related kidney transplantation [112].

Diagnosis of C3G recurrence requires pathological features of the disease and should be supported by clinical history. Following transplant, patients with history of C3G should be closely followed for signs of recurrence, including proteinuria, hematuria, reducing complement C3 levels and elevated serum creatinine. Up to 90% of C3G allograft recipients will show histological C3 deposition [109, 113]. Data support that protocol biopsies from C3G transplant recipients can show deposition of C3 as early as the first month post-transplant in the absence of clinical disease [113, 114]. Furthermore, glomerular C3 deposition in the absence of other clinicopathological findings is independently associated with a higher risk of allograft failure [114].

Unfortunately, even when recurrence is diagnosed early therapeutic options in the setting of recurrence are very limited. The decision to utilize any therapy for C3G recurrence should be done in parallel with clinical and pathological data as well as comprehensive complement biomarker assessment. There are insufficient data to recommend routine use of plasma exchange for C3G recurrence unless there is an identified complement factor deficiency, such as CFH [81, 102]. Insufficient data exist to support routine use of eculizumab for C3G recurrence [81, 102]. One study [115, 116] suggests that the lowest incidence of allograft loss (33%) among patients with recurrent C3G are those treated with eculizumab. Among those who received no treatment for C3G due to stable allograft function, there is a high incidence of allograft loss of 32% in C3GN and 53% in DDD [115]. Consideration to plasma exchange and/or eculizumab should incorporate assessment of patient complement biomarkers [115]. For example, soluble membrane attack complex levels may help to select good responders to eculizumab.

Due to the mechanistic complexity of C3G, there may not be a single therapeutic option, such as eculizumab for aHUS, that provides comprehensive treatment of C3G recurrence. More promising, perhaps, is the development of complement inhibitors (e.g., inhibition of C3 or complement factor B) which could provide targeted therapy for recurrence of C3G following transplant. Early clinical trial (phase II) data for the novel complement factor B agent, LNP023, demonstrate resolution of proteinuria and stability of kidney function in native kidneys [117]. Data from allograft recipients with C3G recurrence have not been published. With the current lack of treatment options for C3G recurrence post-transplant, loss of a prior allograft due to recurrent C3G indicates a high risk of recurrence upon subsequent transplantation and this should be a major consideration in determining candidacy for re-transplant [112].

IgA-Mediated Kidney Disease

IgA vasculitis (IgAV; previously Henoch-Schönlein purpura) and IgA nephropathy (IgAN) are thought to be related diseases with nearly identical pathology. IgA dominant mesangial deposits are characteristic of both IgAV and IgAN. IgAV is classically described in children, whereas, IgAN occurs more often in early adulthood. IgAV, unlike IgAN, has extra-renal manifestations, including a purpuric lower extremity rash. IgA vasculitis and IgAN are comparatively rare causes of ESKD in pediatric patients.

IgA Vasculitis

Based on limited data, the recurrence rate of IgAV) after transplantation is similar to that of IgAN. A matched retrospective cohort study patients with IgAV in the Organ Procurement and Transplantation Network/United Network for Organ Sharing (OPTN/UNOS) database reported allograft failure from recurrent disease in 13.6%, but no difference in 10-year allograft survival compared to the matched cohort [118].

In a study from six European transplant centers [119], overall allograft survival rates were 84%, 66%, and 56% at 5, 10 and 15 years, respectively. Histologic recurrence occurred in 33% on for-cause biopsies. Clinical recurrence occurred in five patients at a median time of 96 months post-transplant. Allograft loss occurred in three patients resulting in an actuarial risk of allograft loss from recurrence in a first graft of 7.5% at 10 years post-transplant. Severity of disease at presentation and type of immunosuppression post-transplant did not affect recurrence. Although not reaching significance, 60% of those with clinically significant recurrence had living donors compared to 16% of living donors in the cohort who did not experience.

IgA Nephropathy

Nature and Frequency of IgA Nephropathy

IgAN is characterized by a highly variable course ranging from a benign condition to rapidly progressive renal failure and affecting 10–20% of all persons undergoing kidney biopsy, rendering IgAN the most prevalent primary chronic glomerular disease worldwide [120]. Prevalence of IgAN differs among populations of different ancestries, being most frequent among persons of Asian descent, rare in those of African descent, and with an intermediate prevalence among those with European descent.

IgAN is thought to occur due to a primary, inherited defect leading to preferential production of IgA with galactose-deficient O-glycans in the hinge-region. IgA deficient in galactose elicits the production of anti-glycan autoantibodies that lead to the formation and subsequent glomerular deposition of immune complexes. IgA-based activation of alternative complement pathway plays a critical role in the pathogenesis of IgAN. For example, C3 is frequently involved in the formation of circulating immune deposits inducing mesangial stress, podocyte damage and progressive deterioration of kidney function. On this basis, IgAN can be classified as an autoimmune glomerular disease. While the pathogenesis of the disease resulting in IgA1 subclass deficient in galactose is not completely clarified, genome-wide association studies have identified multiple susceptibility loci for IgAN implicating independent defects in adaptive and innate immunity, and alternative complement pathways that potentially influence the different pathogenetic steps towards development of disease [121].

IgAN generally has an indolent course, with a 10-year native kidney survival rate of 90% in adults and children with normal renal function at diagnosis; however, 71% of patients will demonstrate worsened hematuria and/or proteinuria in upwards of 20 years follow-up [122]. Clinical risk factors for progression to ESKD include heavy proteinuria, decreased estimated glomerular filtration rate (eGFR) at diagnosis and uncontrolled hypertension, although the ability to accurately predict individual patient-level risk remains limited [123].

Risk Factors for Recurrence

The reported frequency of histologic or clinically significant recurrence of IgAN post-transplantation varies in the literature. An excellent review of recurrence rates cis available [124]. The recurrence rate in a study from the Australia and New Zealand Dialysis and Transplantation Registry (ANZDATA) was 5.4% and 10.8% at 5 and 10 years, respectively, with a median time to recurrence of 4.6 years [125]. Analysis of data from the ANZDATA showed no increased risk of recurrence in a second allograft after loss of a first allograft to recurrence despite prior reports of increased risk.

Recurrence of IgAN can be "histologic only" when diagnosed on protocol biopsies in asymptomatic patients or "clinical" when associated with urinary abnormalities and/or graft dysfunction. Histologic recurrence in protocol biopsies in adults, with or without evidence of clinical disease, is common, with IgA mesangial deposition being found in up to 32–58% of grafts [126, 127]. In children with IgAN, the recurrence of IgA deposits in the allograft following transplantation is very common, but clinically relevant recurrent disease has been reported to be infrequent [128]. Hematuria, the hallmark of IgAN in the native kidney, is not a reliable manifestation of recurrence, being absent in 52% of cases diagnosed by protocol biopsy [126]. Given the lack of a prospective study involving allograft protocol biopsies in pKTR, the true risk of significant allograft dysfunction and allograft loss from recurrent disease in the pediatric population remains unclear.

No single parameter, including age, gender, race, donor source, HLA typing, pre-transplantation course or biochemical characteristics of serum IgA, has been shown to reliably predict recurrence. Risk factors for recurrence in IgAN have been suggested to be younger age at transplant, male gender, and rapidly progressive course of original disease, but there is not consensus. Furthermore, one study of native kidney biopsies in adults with IgAN showed that younger age at onset of IgAN and greater burden of crescents predicted recurrence after transplant [129]. Longer time following transplantation may also be a risk factor for disease recurrence and supports the suggestion that recurrence may be a time-dependent event [127]. The relationship between donor type and recurrence of disease is discussed below.

Considerations for Transplant Planning

The relationship between the risk of recurrence and the donor type remains controversial, with conflicting reports in the literature [130–135]. There have been no large, prospective studies defining the risk of recurrence in patients with IgAN who receive either living donor or deceased donor renal allograft although there are large registry reports. An analysis of ANZDATA found recurrence was significantly more frequent in the zero HLA-mismatched living donors at 17% vs 7% in the cohort overall. In this report, allograft survival was the same for recipients of zero HLA-mismatched donors and those with one or more HLA mismatches (and no recurrence), suggesting loss of the survival advantage expected with zero HLA-mismatched transplants [136]. The authors concluded that despite increased recurrence risk, since allograft survivals were similar, there is no reason to avoid living donor-recipient pairs with zero HLA-mismatches in IgAN. In the same study, there were no differences seen in recurrence rates in those with HLA B12, B35 or DR4. A more recent report from the same registry, showed a 10-year recurrence rate of 16.7% in living donors, 7.1% in living-unrelated donors and 9.2% with deceased donors, with a significant HR of 1.7 for living-related vs deceased donors [137].

Genome wide association studies have identified abnormalities in the cCFH and CFHR genes in patients with IgAN [121, 138, 139]. Although it is unclear whether these variants increase the risk of recurrence following transplant, familial IgAN should be rigorously excluded in potential living-related donors since familial IgAN is associated with a high risk of ESKD [140].

The effect of immunosuppression regimen on IgA recurrence risk is unclear. Despite initial enthusiasm, newer immunosuppressants seem ineffective in preventing recurrence [141]. Retrospective data suggest that induction with ATG or anti-lymphocyte globulin is associated with a lower risk of recurrence compared to interleukin receptor-2 blockade [142–144].

Corticosteroid avoidance has become a major goal in pediatric kidney transplantation and has been successful in select transplant recipients [145, 146]. There are conflicting reports on the effect of rapid corticosteroid withdrawal or corticosteroid avoidance on IgAN recurrence risk and allograft survival. In a retrospective analysis of adults in the OPTN/UNOS database with IgAN receiving a first kidney transplant, early corticosteroid withdrawal was associated with increased risk of recurrence

compared to patients in the corticosteroid continuation group. Patient survival and death-censored allograft survival were not different [147]. Similarly, in an analysis of the ANZDATA of adult recipients of a primary transplant for IgAN, corticosteroid use was strongly associated with a reduced risk of recurrence, after adjusting for age, sex, HLA mismatch, dialysis duration and transplant era [148]. In this report, 12.6% of graft loss was attributed to recurrence. Conversely, a study of pediatric patients within the OPTN database reported that receiving a corticosteroid avoidance regimen in patients with a pre-transplantation diagnosis of glomerular kidney disease was not associated with an increased risk of allograft failure, although this study was unable to examine recurrence rates [149].

Treatment of IgAN Recurrence

Just as with native kidney IgAN, no therapy for recurrent IgAN has been shown to be effective. In the setting of disease recurrence, KDIGO guidelines recommend treatment strategies to reduce proteinuria, optimize blood pressure, and reduce inflammation [150].

Use of corticosteroids as well as intravenous rituximab to treat recurrence in small numbers of patients has been described [151–153]. Data from Japan have reported favorable outcomes after tonsillectomy in patients with recurrent IgAN, but these results have not been confirmed in other populations [154–156]. The effect of fish oil on recurrent IgAN has not been systematically examined for risk reduction or treatment of IgAN. One study reported a reduction in proteinuria and stabilization of kidney function after budesonide administration in native kidney IgAN patients, possibly by targeting the intestinal mucosa directly, suggesting a possible role in patients with recurrence post-transplant [157].

Impact of IgAN on Graft Function and Survival

Recurrent disease was thought to have little impact on allograft outcomes; however, recent studies with longer duration of follow-up suggest that recurrent disease may contribute substantially to allograft injury. The rate of allograft loss due to recurrence of IgAN varies based on time from transplant, with less early allograft loss attributed to IgAN and significantly more at 10 years post-transplant [52, 54]. True estimates of allograft loss purely attributable to disease recurrence are difficult given the interplay with acute or chronic rejection and calcineurin toxicity, particularly if histology close to the time of graft loss is not available [127, 158, 159].

Lupus Nephritis

Nature and Frequency of Disease

Systemic lupus erythematosus (SLE) is an autoimmune inflammatory disease that is characterized by antibodies directed against self-antigens, resulting in multiorgan damage. SLE in children is more severe than in adults, and there is a higher incidence of lupus nephritis (LN) [160, 161]. Risk factors for development of ESKD due to LN include International Society of Nephrology and the Renal Pathology Society class IV LN, male gender, black race, hypertension, nephrotic syndrome, anti-phospholipid antibodies, high glomerular staining for monocyte chemoattractant protein-1, chronicity on biopsy, poor response to induction therapy, and occurrence of nephritic kidney flare [162, 163]. Lupus nephritis is responsible for approximately 3% of ESKD leading to transplantation in North America [48].

Risk for Disease Recurrence

The reported risk for recurrent lupus nephritis (RLN) after renal transplantation has been quite variable, ranging from <5% [112] to between 30–50% [164] in studies implementing protocol biopsies to evaluate serially for recurrence. The variability in reported recurrence rates has been attributed to varying indications for renal allograft biopsy across transplant centers; single-center versus registry-based study design; follow-up duration; and varying races and ethnicities in study samples [165].

Large-scale data derived from UNOS between 1987 and 2006 estimated period prevalence as well as predictors of RLN and assessed the effects of RLN on both allograft failure and recipient survival [166]. The period prevalence of RLN within the cohort was 2.44%. Non-Hispanic black race, female gender, and age < 33 years were independent risk factors for RLN. In another study, pre-transplantation antiphospholipid autoantibodies confer a higher risk for RLN [167].

Considerations for Transplant Planning

Data are mixed regarding the impact of donor type on RLN. Data from over 30 years ago suggest that grafts from deceased donors are a better option for patients with lupus nephritis than grafts from living-related donors based on lower 1-year graft survival, presumably due to possible familial inheritance through the HLA system [168, 169]. In contrast, more recent data show no difference in allograft loss between the two types of donors [170].

Pre-transplantation clinical condition and immunosuppressive history are important considerations in transplant planning. KDIGO guidelines recommend that lupus should be clinically quiescent and that the patient is receiving minimal (no) immunosuppression prior to transplantation [112]. Delay of transplantation may be necessary for those patients who have received high levels of pre-transplantation immunosuppression or long-term glucocorticoid therapy to minimize the cumulative risk of prior therapy on top of the need for potent induction immunosuppression at the time of transplant [171]. As noted previously, the presence of antiphospholipid autoantibodies should be carefully considered in transplant planning due to the risk of vascular thrombosis and early allograft failure [172, 173]. Anticoagulation in the peri- and post-transplant period should be considered in patients with antiphospholipid autoantibodies to reduce the risk of vascular thrombosis [112, 174]; however, this risk should be balanced by the potential for bleeding in the immediate post-transplant period [173].

The post-transplant immunosuppression for LN patients does not differ from that normally used. The OPTN/UNOS database was utilized to compare rates of allograft loss due to disease recurrence between transplant patients receiving cyclosporin plus azathioprine (CSA + AZA) compared to those receiving cyclosporin plus MMF (CSA + MMF) [175]. There was no difference in the rates of allograft loss due to RLN among recipients receiving either CSA + AZA or CSA + MMF maintenance immunosuppressive therapy at 10-year follow-up. In patients with LN recurrence, an intensification of immunosuppression should be reserved for the exceptional cases showing a severe (life threatening) lupus flare due to the potential risks of serious or lethal infection post-transplant [165].

Impact of RLN on Graft Function and Survival

Retrospective multicenter data from the North American Pediatric Renal Transplant Cooperative Study (NAPRTCS) database demonstrated that kidney transplant outcomes in young patients with LN were comparable to those seen in an age-, ancestry-, and gender-matched control group, in spite of an unexplained increase in recurrent rejections in the living donor LN patients [176].

In an analysis of UNOS data, allograft failure occurred in 93% of those with RLN, 86% of those with rejection, and 19% of control subjects without rejection [166]. Although recipients with RLN had a fourfold greater risk for allograft failure compared with control subjects without rejection, only 7% of allograft failure episodes were attributable to RLN compared with 43% due to rejection. Mortality was similar (11–18%) between those with RLN, rejection, and controls.

Acute Graft Dysfunction in the Early Post-Operative Period

Graft Thrombosis

One cause of acute graft dysfunction in the early post-operative period is graft thrombosis. Around

2–3% of all renal grafts in the pediatric population thrombose and not surprisingly this is seen more frequently in the early post-transplant period [177, 178]. Thrombosis is encountered more commonly in younger children, particularly those less than 2 years of age at the time of transplant and in those receiving a deceased donor graft [177–180]. The size of the native vessels is undoubtedly a contributing factor, and the frequent discrepancy between donor and recipient vessels increases the surgical difficulty of the anastomosis. Previously, high rates of thrombosis were seen when very young donors were used and this is one of the reasons why children under the age of 5–6 years are now usually excluded from being donors [35, 181]. Thrombosis is now the third most common cause of graft loss, accounting for 9.8% of losses overall and for 7% of index graft losses since January 2000 [182].

Thrombosis is reported to occur more commonly in grafts from deceased donors, in those with prolonged cold ischemic time beyond 24 h and in retransplants [177, 180, 181]. Furthermore, there are data to suggest that pr—transplantation peritoneal dialysis increases the risk of thrombosis [181, 183]. Interestingly, the most recent NAPRTCS data suggest that the previously reported risk factors may not be significant and that the use of IL-2 receptor blockers more than halves the risk of graft thrombosis [178]. Further studies are required to establish the true risk factors for this often devastating complication.

There is an apparent higher incidence of thrombophilic risk factors in the ESKD population. In one study of pediatric pre-transplantation patients, 27% were found to have an increased risk of thrombophilia, whereas another group found that almost 90% of children screened pre-transplantation had at least one thrombotic risk factor [182]. There was a higher risk in the "non-anatomical" etiological group of patients where lupus anticoagulant was commonly detected [48]. The routine use of prophylactic anticoagulation remains controversial and the evidence for its use is limited. Currently, there are many different protocols, ranging from no anticoagulation, tailored anticoagulation according to risk and the use of one or more anticoagulants routinely. One study has compared the use of routine unfractionated heparin with historical controls given no anticoagulation and found no reduction in the rate of allograft thrombosis [177]. Others have reported reduced thrombosis rates with the use of low-molecular-weight heparin [184, 185]. Several units where patients are routinely screened for thrombophilic risk factors have shown good outcomes using anticoagulation protocols stratified for risk, but the numbers of patients included have been small and it is difficult to extrapolate the data to the general pediatric ESKD population [150, 185]. Until there is a large, randomized, controlled trial, the benefit of anticoagulation remains undetermined.

Obstruction

Obstruction to the flow of urine from the allograft can occur at any time but is most common in the early post-operative period. Complete obstruction will result in no urine output, but partial obstruction may only be detected on ultrasound. The scan may show dilatation of the renal pelvis with or without urine in the bladder depending on the level of the obstruction. The major concerns with obstruction are the increased pressure on the ureteric anastomosis with the risk of rupture and the long-term effects of increased pressure on the renal parenchyma. There are many reasons for the early increased risk of obstruction, including intravesical clots, stenosis of the ureter, particularly at the anastomosis site, and external compression or kinking of the ureter due to hematomas and other fluid collections. There is inevitable bleeding at the time of transplant, and it is not unusual for clots to form. The majority of patients post-transplant will have either a urethral or suprapubic catheter to drain the bladder, or rarely a urinary diversion, and this should facilitate the passage of clots, though it is possible for clots to block the catheter and flushing of the catheter is often required in the first few days after the transplant. Inadequate drainage of urine can also be the result of dislodgement of the catheter. Many centers also routinely place a stent into the ureter of the allograft at the time of transplantation and this

generally remains in situ for 2–6 weeks to minimize the risk of stenosis or effects of external compression. External compression of the ureter may be caused by a hematoma, a urinoma, a lymphocele, stool, or any other mass in the vicinity of the graft. Ultrasound will usually be able to follow the length of the ureter and will detect any mass compressing it. Transplant ureters are also at risk of kinking, especially if a large adult donor has been used without appropriate trimming of the ureter. Rarely, further surgical intervention is necessary.

Beyond the early post-operative period, obstruction is more frequently encountered in patients with known bladder problems and in those with incomplete bladder emptying. Double voiding regimes, clean intermittent catheterization or indwelling catheter drainage may be necessary to relieve the obstruction.

Urine Leak

This potentially devastating complication is fortunately rare, and the incidence appears to be decreasing. Leakage is usually due to ureteral necrosis around the anastomosis site, and urine then collects in the abdomen. If an abdominal drain has been placed at the time of surgery, the drain fluid can easily be tested for the creatinine concentration, which would be comparable to that of the urine rather than the blood. If there is no drain, then fine needle aspiration of the fluid and measurement of the creatinine concentration will aid in the diagnosis. Occasionally, nuclear medicine imaging studies may also be considered. The adult literature has demonstrated poorer wound healing in patients treated with sirolimus and subsequent increased urine leak [186].

Investigation of Delayed Graft Function and Peri-Operative Allograft Dysfunction

If no urine is obtained post-operatively, then a systematic approach should identify the cause. Assessment of the patient, ensuring that there is adequate volume replacement to maintain a good blood pressure and central venous pressure is critical. The position of the urinary catheter should be assessed, and it should be checked for any blockage. A Doppler ultrasound will demonstrate the degree and pattern of renal perfusion. If the kidney looks well-perfused and the patient is well-hydrated, then the most likely cause of the delayed graft function (DGF) is acute tubular necrosis, although most would advocate an early biopsy to rule out hyperacute rejection.

If good urine volumes were present but then decrease, factors such as inadequate fluid replacement or a blocked catheter should be considered initially. These problems are common and are easily resolved. If the patient is assessed to be normo- or hypervolemic, then a trial of a loop diuretic may be appropriate. If there is still no response, then more serious complications such as urinary leak, graft thrombosis and hyperacute rejection need to be investigated. A Doppler ultrasound, with or without a mercaptoacetyltriglycine or diethylenetriamine pentaacetic acid scan, will assess the perfusion of the kidney. Ultrasound will also detect obstruction to the kidney, fluid collections around the kidney suggestive of a urine leak or lymphocele, and clots in the bladder. A renal biopsy is required to rule out rejection. In exceptional cases, surgical re-exploration is required to assess the viability of the kidney.

Treatment of Delayed Graft Function

By the most used definition of DGF, dialysis will be required. Data from the OPTN show that 50% recover adequate function by the tenth post-transplant day. The use of CNIs is often minimized or delayed until good graft function is obtained. These patients are often treated with ATG, until graft function improves, or for a maximum of 10 days, as an alternative to CNIs, as there is evidence to suggest less DGF with ATG [187].

Acute Rejection and Chronic Allograft Dysfunction

Acute rejection and chronic allograft dysfunction require clinical, immunological, and histopathological input to understand and appropriately

treat. With the advent of new techniques for detecting anti-HLA antibodies and a better understanding of histological changes, it is becoming easier to define the causes of acute rejection and chronic allograft dysfunction, although it is important to remember that more than one factor may be operating in any individual patient. Interstitial fibrosis and tubular atrophy (IFTA) are the final common pathway of chronic allograft dysfunction; however, early investigation may pinpoint the underlying mechanism involved and allow intervention prior to irreversible allograft fibrosis.

International data from single center reports and registries have shown improved long-term patient and renal allograft survival rates for children and young people after kidney transplantation, including the NAPRTCS [48]. Most studies have shown improvement inpatient and renal allograft survival rates over the last three decades, which may be due to changes in immunosuppressive drug regimens, improvements in HLA matching, and a reduction in cold ischemia times. Pre-emptive and living donor renal transplantation are preferred given improved outcomes for living donation as compared to deceased donation with donation after brain death (DBD) and donation after circulatory death (DCD). A national study from the United Kingdom showed 1-year DBD renal allograft survival for those transplanted from 2012 to 2016 was 98%, compared with 72% for those transplanted from 1987 to 1991 [188]. Within this cohort, renal allograft survival for first kidney only transplants at 1, 5, 10, 20 and 25 years were 89%, 79%, 65%, 42% and 33%, respectively. Superior survival with living donation was maintained throughout the study period with 25-year renal allograft survival at 33% compared with 31% from deceased donation ($p < 0.0001$). These are similar to long-term data published from the Dutch LERIC study; 20-year allograft survival was 49% and 29% for LD and DD, respectively [189]. The 20-year allograft survival in Norway of allografts children since 1970 was 45%; 52% of these allografts were pre-emptive and 84% were from LD [190].

Predictors of Pediatric Renal Allograft Survival

Renal allograft function is calculated using eGFR, which requires the plasma creatinine and height according to modified Schwartz or other formulae, although other serum markers such as cystatin C have been proposed as providing increased accuracy [191].

Living donation confers a distinct advantage over deceased donation as the process of brain death and longer cold ischemia may predispose to acute tubular injury and DGF. DGF, prior transplantation, primary renal disease, and degree of HLA matching appear to influence long-term allograft outcome [192]. Recipient age is a predictor for graft survival, with older children having better early allograft survival but worse 5-year allograft survival [193]. Pre-transplantation dialysis and early acute rejection adversely affect allograft survival [194]. Additionally, some studies have found a deceased donor age over 40 years and prolonged cold ischemia time to be predictive of reduced allograft survival [195].

Proteinuria is common after pediatric renal transplantation, occurring in up to 80% of patients and can be glomerular or tubular in origin. Proteinuria can be both a contributor to and sign of chronic allograft damage [196]. A reduction in nephron mass results in increased intra-glomerular pressure in the remaining nephrons, causing proteinuria, which is injurious to the kidney via glomerular and interstitial inflammation, resulting in interstitial fibrosis and glomerulosclerosis [192]. Proteins entering the interstitium are processed by dendritic cells [197]. It has even been postulated that activated dendritic cells may then predispose to the development of rejection [194]. Transplant glomerulopathy is associated with proteinuria, presumably linked to damage to the glomerular basement membrane following antibody mediated rejection [198]. The use of mammalian target of rapamycin inhibitors has also been associated with the development of proteinuria [199].

Chronic Allograft Dysfunction

Allograft lifespan can be influenced by a variety of factors including peri-implantation injuries, history of allograft rejection, cardiovascular disease, and (recurrent) infection—all of which may influence the rate of decline of renal allograft function. However, the contribution of each of these factors will vary in individual patients. Post-transplant factors can be divided into early or implantation stresses and later injuries to the allograft. It is likely that multiple factors operate in individual patients. Over the last decade, there has been an appreciation that repeated episodes of stress damage the allograft resulting in nephron loss. This cumulative burden of injury eventually results in allograft failure [200].

Pre-Implantation Injury

Long-term outcome data clearly demonstrate that LD transplantation confers a survival advantage compared with deceased donation. This is observed in living-related and unrelated donors, suggesting that reduction in cold ischemia time and avoidance of brain death are important factors. The process of brain death, through hemodynamic and neuro-hormonal changes, reduces the viability of DD kidneys, although optimal management of brain-dead donors and their kidneys can improve outcome [201–203].

Advanced donor age for living and deceased donors is associated with a worse long-term outcome in adult recipients, which may represent reduced functional renal mass, although other factors such as donor hypertension or arteriosclerosis may be important [204]. However, older donor age is associated with a greater risk of renal allograft failure for pediatric recipients. Donor organ quality is also important; more functioning nephrons lead to better outcomes. The presence of interstitial fibrosis on implantation biopsies (reflecting nephron loss) is predictive of late outcome as is glomerular size, which is an inversely related surrogate for renal mass [205–207]. Extended criteria donors are rarely used in pediatric practice, and include donors older than 60 years, or 50–59 years with two out of three of the following: hypertension, cerebrovascular cause of death or plasma creatinine >130 μmol/l (1.5 mg/dL), as they have a 70% increased risk of long-term allograft failure compared with standard criteria donors [208].

Implantation Injury

Cold ischemia, preservation injury and ischemia/reperfusion injury, with or without the effects of brain death, cause allograft injury and DGF, which may result in nephron loss if there is incomplete recovery. There is increased expression of cytokines, adhesion molecules and HLA molecules with ischemia, and DGF can be associated with acute rejection episodes and worse outcomes.

Post Implantation Injury

There are further stresses on the transplanted kidney, including acute or chronic rejection, recurrent disease, hypertension, hyperlipidemia, nephrotoxic medications, and bacterial and viral infections. These insults may cause further loss of nephrons with increasing IFTA. Rejection, as the major cause of chronic allograft dysfunction, is discussed in detail below.

The significant contribution of the immune system to chronic allograft dysfunction is supported by reduced allograft survival in the setting of previous acute rejection episodes, in highly sensitized patients, where there is de novo production of anti-donor HLA antibodies, and where there is evidence of under-immunosuppression.

Acute Allograft Dysfunction and Rejection

Acute allograft dysfunction or transplant acute kidney injury may result from pre-renal, renal, and post-renal conditions. The commonest pre-renal conditions include hypovolemia caused by dehydration (including viral gastroenteritis), hypotension and renal arterial or venous thrombosis, although thrombosis is rare after the immediate post-operative period. Intrinsic renal disease is more common with acute rejection, nephrotoxicity (including CNI nephrotoxicity) and infection (including bacterial infections, such as

transplant pyelonephritis, and viral infections) and recurrent and de novo glomerulonephritis (see above). Post-renal causes are detailed below and include transplant ureteric stent obstruction and dislodgement, ureteropelvic and ureterovesical junction obstructions, bladder dysfunction and rarely perirenal hematoma or fluid. Percutaneous renal transplant biopsies may be required to make the correct diagnosis; these are called indication biopsies as opposed to protocol or surveillance biopsies, which are routinely performed at a specific time point post-transplant.

Allograft Rejection

The Banff Classification was originally devised in 1991 and was introduced to standardize reporting of acute and chronic rejection (Table 68.3) [209]. The Banff criteria have subsequently been updated on a regular basis to reflect increased understanding of antibody-mediated rejection and place more emphasis on the type rather than the degree of rejection [210–212]. Four main types of acute rejection are defined: T cell mediated rejection (TCMR) with or without vasculitis and AMR with or without vasculitis [213]. Antibody mediated vascular rejection (AMR with vasculitis) is associated with a poor outcome. A better understanding of the underlying mechanisms of rejection will allow more appropriate treatment and hopefully better outcomes. There is improved long-term survival with aggressive treatment of early TCMR, as early TCMR can rapidly lead to renal allograft loss if left untreated. However, it may result in a degree of nephron loss and IFTA, which will still be evident in later percutaneous renal biopsies but will not have progressed.

Chronic Allograft (Late) Rejection

Chronic allograft dysfunction has been intensively studied over the last decade, resulting in increased understanding of late acute and chronic rejection. Rejection episodes occurring 3–6 months after transplantation are labeled acute or chronic depending on the speed of development, severity of allograft dysfunction and the

Table 68.3 Acute allograft rejection according to the Banff 2017 classification. (Table used with permission from Haas et al., 2018 [256])

Acute T cell–mediated rejection (TCMR)
• Ia >25% interstitial inflammation with moderate tubulitis (t2)
• Ib >25% interstitial inflammation with severe tubulitis (t3)
• IIa mild-to-moderate intimal arteritis (v1)
• IIb severe intimal arteritis (v2)
• III transmural arteritis and/or fibrinoid necrosis (v3)
Acute antibody-mediated rejection (ABMR): Should have all three criteria below
Histologic evidence of tissue injury:
• Acute tubular injury
• Microvascular inflammation (g > 0 and/or ptc > 0)
• Arteritis (v > 0)
• Thrombotic microangiopathy without apparent cause
Evidence of current/recent antibody interaction with endothelium:
• Positive C4d staining of peritubular capillaries
• Moderate microvascular inflammation (g + ptc ≥ 2)
• Increased expression of gene transcripts in biopsy tissue strongly associated with ABMR
Serologic evidence of donor-specific antibodies (DSA):
• Positive C4d staining/presence of ABMR-associated gene transcripts may substitute for DSA

Key: i0—No inflammation or in <10% of unscarred cortical parenchyma. i1—Inflammation in 10–25% of unscarred cortical parenchyma. i2—Inflammation in 26–50% of unscarred cortical parenchyma. i3—Inflammation in more than 50% of unscarred cortical parenchyma

t0—No mononuclear cells in tubules or single focus of tubulitis only, t1—Foci with 1–4 mononuclear cells/tubular cross section (or 10 tubular cells), t2— 5–10 mononuclear cells and t3—Foci with >10 mononuclear cells/tubular cross section or the presence of ≥2 areas of tubular basement membrane destruction accompanied by i2/i3 inflammation and t2 elsewhere. G: glomerulitis, none (g0), segmental or global glomerulitis in 75% of glomeruli (g3), PTC: peritubular capillaries- at least 1 leukocyte in ≥10% of cortical PTC but 10 leukocytes in most severely involved PTC (ptc3)

biopsy findings. Late acute rejection is associated with a worse outcome [214]. The timing of a biopsy for allograft dysfunction is predictive; indication biopsies (performed for allograft dysfunction) in adult recipients after 12 months are associated with worse outcome than a biopsy performed during the first post-transplant year [215]. Dorje et al. report similar findings with AMR; allograft survival at 4 years was 75% in those with early AMR in the first 3 months post-transplant, compared with 40% in those with AMR diagnosed after 3 months [216]. Non-adherence or suboptimal immunosuppression were more common in the group with later AMR.

There is increasing research on the link between late allograft dysfunction and subsequent allograft loss. Previously, used causes of allograft loss have often been imprecise diagnoses such as chronic allograft nephropathy (CAN) or IFTA [209, 217]. These terms describe the final common pathway of several different processes and have been shown to be unhelpful in determining the underlying cause of allograft failure. Recruitment to a large multicenter study of factors that affect long-term kidney transplant function was commenced in 2005 [218]. Biopsies performed for allograft dysfunction were frequently reported by local pathologists as showing CAN or CNI toxicity. However, further follow-up showed no difference in outcome for patients with or without a diagnosis of CAN. This study demonstrated that late allograft dysfunction in a previously stable allograft was associated with a high risk of renal allograft loss. Further reports showed that a diagnosis of CNI toxicity was associated with a reduced rather than increased risk of allograft failure, and, unexpectedly, a high incidence of antibody-mediated rejection was found on biopsies for late allograft dysfunction [219, 220].

A precise diagnosis of allograft failure was made in a detailed prospective study of adult kidney transplant recipients of whom 19% had allograft loss, which was correlated with findings on earlier indication biopsies, clinical and serological data [221]. The causes of allograft failure were rejection in 64% of cases, glomerulonephritis in 18%, polyoma virus in 7% and intercurrent events in 11%. The most common cause of allograft failure was AMR, with all allografts that failed from rejection having some degree of AMR, whereas no losses were attributable to TCMR alone. A substantial proportion of AMR losses were associated with non-adherence. Neither a diagnosis of IFTA alone without evidence of inflammation nor CNI toxicity were common causes of allograft failure in this study. Antibody mediated rejection is the most common cause of late allograft loss, and it is important to differentiate late acute rejection into TCMR or AMR to allow appropriate treatment.

Antibody Mediated Rejection

Endothelial inflammation and injury in the microvasculature of the kidney allograft is the underlying pathology in AMR [222]. This can be due to donor specific anti-HLA antibodies (DSA) causing direct toxicity to antigen expressing endothelial cells through activation of the classical complement cascade or through cell-mediated toxicity after binding to the Fc receptors of mononuclear cells [223, 224]. The spectrum of histological lesions range from mild changes with acute tubular necrosis to severe changes with vascular fibrinoid necrosis [225]. There is swelling of the injured endothelia in the glomeruli and peritubular capillaries, which are infiltrated with mononuclear cells. The Banff criteria for AMR include signs of microcirculatory inflammation and the presence of complement split product C4d staining, which reflects activation of the classical complement pathway [226]. The Banff criteria require both of these markers to be present for the diagnosis of AMR, with or without the presence of DSA, although it is now appreciated that C4d staining is not always present in AMR [227].

Chronic injury in AMR is characterized by remodeling of the microvasculature and larger arteries. There is multilayering of the basement membrane in the peritubular capillaries, often with associated secondary IFTA, with or without C4d staining. The changes on electron microscopy may be present before there are obvious changes on light microscopy. The glomeruli are enlarged, and the capillary walls are thickened

with duplication of the glomerular basement membrane causing double contours with interposition of mesangial matrix, usually with an increase in mesangial matrix. There may be subendothelial accumulation of deposits on electron microscopy. These chronic changes are referred to as transplant glomerulopathy (TG), which is associated with a poor outcome despite treatment [228]. There is now better understanding of the role of the endothelium and microcirculatory inflammation and repair in AMR [229].

The presence of C4d staining of peritubular capillaries has been associated with a worse outcome than C4d negative AMR, but the category of C4d negative AMR is now established and is well-recognized as a cause of allograft loss [230]. Deposition of C4d has been shown to have a high specificity but a poor sensitivity for AMR [231]. Fifty percent of AMR is missed if reliance is placed on the presence of C4d to make a diagnosis. Signs of microcirculatory injury, including glomerulitis, are a better predictor of late allograft loss than the presence of C4d.

Deposition of C4d on biopsy is associated with basement membrane multilayering, TG and the accumulation of mononuclear cells within the peritubular capillaries. Deposition of C4d in biopsies from low immunological risk patients which show normal glomerular morphology is associated with the development of TG on later biopsies. C4d deposits in the absence of other signs of AMR can be seen in ABO incompatible transplantation and may be a sign of accommodation [212, 232]. Molecular studies have shown that the presence of inflammatory cells in areas of fibrosis contribute to rejection and are predictive of outcome. It is therefore recommended that fibrotic areas should be included when calculating the degree of inflammatory infiltrate on a percutaneous renal biopsy [233]. Gene expression analysis has also proved useful in discriminating biopsies classified histologically as showing borderline histological changes of rejection into those patients with and without rejection [234]. However, there is the hope for point of care testing of biomarkers in pediatric renal transplantation to predict rejection in the future. Studies linking findings on indication biopsies with later allograft loss have used a broader diagnosis of AMR than traditional Banff criteria with the requirement for microcirculatory inflammation, DSA or C4d.

Donor Specific Antibody

The consequences of developing de novo DSA on allograft outcome has been known for some time [235]. In both adults and children, the natural history of de novo DSA in pKTR is well-documented, with evidence of complement activation leading to renal allograft injury [134, 236]. The development of sensitive solid-phase assays has helped to detect the presence of DSA and establish its role as a major cause of late allograft dysfunction and failure. The sensitivity of the assay and the cut-off median fluorescent intensity (MFI) used will affect the ability to distinguish de novo DSA from antibody present prior to transplantation. Using rigorous methods to exclude antibody already present in the recipient at the time of transplantation, no de novo DSA was detected prior to 6 months, 2% had developed DSA by 1 year and the median time to develop DSA was 4.6 years in adult kidney transplant recipients, although other adult studies have found that de novo antibodies can develop earlier [237, 238]. The prevalence of de novo DSA in pediatric renal transplant populations has been reported to be between 14 and 34% and can be associated with allograft loss [239].

HLA molecules are expressed on peritubular capillaries and can be upregulated during infectious or inflammatory processes by interferon gamma. This may result in cellular rejection or allow the expansion of B cells producing DSA. Early cellular rejection has been shown to be a risk factor for DSA development [240]. More intense peritubular capillaritis during cellular rejection is associated with the development of DSA. The degree of immunosuppression is important; reduction in immunosuppression or the use of less potent immunosuppressants is associated with the development of DSA. DSA occur more frequently with the use of cyclosporin than with tacrolimus, with everolimus compared to cyclosporin and with azathioprine compared to MMF [241]. HLA mismatching is a

predictor of the development of DSA, especially HLA class II mismatch. In addition younger age predicts development of DSA, which may reflect a more robust immune system in the young or non-adherence [242]. De novo DSA precedes the onset of allograft dysfunction by months or years. Most DSA is directed against class II HLA, including DQ and DP, which are not included in most matching algorithms. There are other non-DSA anti-HLA and non-HLA antibodies. Anti-HLA antibodies that are not donor specific have been reported to be pathogenic in some studies, whereas in other studies there has been no association with outcome [243]. The development of de novo DSA post-transplant has been associated with 10-year allograft survival that is almost 40% lower than those without DSA [244].

Ginevri has published a detailed study in pediatric renal transplant recipients [245]. Thirty-seven of 82 consecutive recipients of first allografts developed de novo anti-HLA antibodies; 19 had DSA (6 also had non-DSA) and 18 had non-DSA only. Two patients who had only HLA Class I mismatches developed only HLA Class I DSA. The other 17 had mismatches at both HLA Class I and class II; 11 developed HLA Class II antibody and 6 developed both HLA Class I and II. HLA Class II DSA were almost exclusively directed against DQ. In the non-DSA group, 15 developed mostly HLA Class I with or without HLA Class II antibodies that recognized cross-reactive epitope group specificities related to the donor mismatch. The MFI of the non-DSA were significantly lower than the MFI of the DSA. DSA appeared at a median time of 2 years post-transplant with a range of 3–60 months. DSA were more likely to remain detectable compared with non-DSA and were more common in patients receiving cyclosporin compared with tacrolimus. Renal allograft function decreased significantly in the DSA group during 4.3 years of follow-up. Five allografts were lost in the DSA groups; four were due to AMR. Seventeen (89%) DSA patients needed an indication biopsy compared to 44% in the non-DSA group and 11% in those with no antibody ($p < 0.0001$). Eleven of the biopsies showed AMR, and 8 were C4d positive. One recipient in the non-DSA group had evidence of microcirculatory inflammation in keeping with probable AMR. These data suggest that it is prudent to monitor DSA post transplantation.

Prevention and Treatment of Rejection

Prevention of chronic allograft dysfunction confers significant health and economic benefits and is likely to be a more successful strategy than treatment. Many preventative strategies have been touched on already, including the use of living donors, which carries an allograft survival benefit, as does the appropriate selection of deceased donors. Optimal management of the deceased donor can attenuate the effects of ischemia reperfusion injury [246]. Prevention and treatment of rejection is an important part of post-transplant management. In our opinion, the most effective way to prevent allograft rejection is through regular adherence counseling, increasing parent-patient engagement in post-transplant care, and close laboratory and clinical follow-up after transplant.

Early acute rejection is most likely to be TCMR and this is usually responsive to corticosteroid treatment and indeed recent improvements in early allograft survival have been attributed in part to newer, more potent immunosuppressants which have significantly reduced early acute rejection rates. Accurate diagnosis of early rejection episodes into either T or B cell-mediated with or without a vascular component is important in determining outcome.

The treatment of late AMR remains a challenge. The outcome after late AMR is worse than for early AMR, where good results have been obtained using PE or intravenous immunoglobulin (IVIG) and rituximab [247–250]. There are no RCT data available regarding the treatment of late AMR but a number of different agents have been tried. High-dose corticosteroids, IVIG, PE, IA, rituximab and bortezomib have been used [251]. Baseline immunosuppression is commonly augmented to include MMF and tacrolimus [252]. Varying results have been reported,

which are likely to be influenced by the baseline function of the allograft and the degree of injury that has already occurred by the time of AMR diagnosis [253]. Rituximab, used in combination with other therapies, and bortezomib both appear less effective for late AMR than early AMR [254]. Many reports describe reduced immunosuppression or non-adherence prior to the development of AMR; prevention of late AMR is likely to be a more effective strategy than treatment. Late TCMR seems more amenable to treatment and in studies of late allograft dysfunction does not appear to be a cause of allograft loss.

Adequate Immunosuppression

Late allograft dysfunction is often due to AMR with non-adherence or under-immunosuppression as the underlying mechanisms. Non-adherence and under-immunosuppression with lower doses of tacrolimus, cyclosporin and MMF may in part be related to a concern that calcineurin nephrotoxicity contributes to chronic allograft dysfunction. Calcineurin nephrotoxicity is a concern clinically, but not a frequent cause of allograft loss. The Mycophenolic Renal Transplant Registry of the de novo use of MMF showed that reductions in dose during the first-year post-transplant were associated with increased rejection, and if tacrolimus levels were also low, with increased allograft failure [255]. An observational analysis of over 25,000 low-risk, adult recipients showed that withdrawing maintenance cyclosporin, tacrolimus or MMF, or reducing the dose below certain thresholds after the first post-transplant year, was associated with a significant risk of allograft failure. These data indicate the importance of adequate immunosuppression for allograft survival.

Conclusion

Transplant planning and care require a thorough understanding of the underlying cause of ESKD and close monitoring for non-infectious complications in the post-operative period. In most instances, transplantation confers significantly reduced morbidity and mortality and improvements in quality of life. Rejection remains a lifelong concern for kidney transplants, as does the risk for disease recurrence in certain populations. For most, the risk of disease recurrence is low; however, exceptions are notable in the case of complement-mediated kidney disease where risk for disease recurrence may be greater than 50%. Most patients with recurrent disease due to glomerulonephritis still have generally equivalent patient and allograft survival compared to those with non-recurrent etiologies, such as CAKUT.

Despite scientific advances, the management of disease recurrence and allograft rejection in pediatric kidney transplant recipients remains challenging; however, emerging therapies for high-risk diseases, such as aHUS and C3G, provide hope for improved allograft survival amongst these pediatric kidney diseases at highest risk for recurrence. Our understanding of optimal treatment for both allograft rejection and recurrent disease is limited by the lack of systematic, randomized studies, particularly in pediatric patients. Systematic use of international registries and prospective multicenter collaborative studies is necessary to allow improvement in pre-transplantation risk evaluation and facilitate data-driven yet individualized post-transplantation management.

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Prevention and Treatment of Infectious Complications in Pediatric Renal Transplant Recipients

Jodi M. Smith, Sarah J. Kizilbash, and Vikas R. Dharnidharka

Introduction

Over the last few decades, significant advances have been made in the outcomes of pediatric kidney transplant recipients, with marked improvement in patient survival and early allograft survival. However, the more potent immunosuppressive therapy that successfully reduced the incidence of acute rejection has resulted in a higher incidence of infectious complications [1]. This increase has manifested as (1) an increase in the total frequency of infection [2]; (2) infection becoming the primary reason for post-transplant hospitalization [3]; and (3) the successive emergence of new viral infections in the past several decades. Specifically, cytomegalovirus (CMV) infections have been common in kidney transplant recipients since the 1980s, followed by Epstein Barr virus (EBV) related post-transplant lymphoproliferative disorder (PTLD) since the 1990s, and BK virus associated allograft nephropathy (BKVAN) in the last 15 years. Infections are not only a significant source of morbidity and hospitalization, but they also can lead to graft loss and patient death. Even when adjusting for death, infections represent an additional risk factor for worse graft survival [4–6], thus in part accounting for the less significant improvement in longer-term allograft survival [7]. Excessive PTLD resulted in the early termi-

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nation of a large multi-center immunosuppression trial in pediatric kidney transplantation in the US [8]. Hospitalizations due to infection occurred in 47% of children within the first 3 years after kidney transplant, higher than in adults with a kidney transplant or in children on dialysis [2]. Unlike adults, the total incidence of infections did not drop in children in more recent years. From 2019 onwards, the global SARS-COV-2 (COVID19) pandemic greatly affected organ transplant recipients.

Special Considerations in Pediatric Transplantation

Organ transplant recipients are at greater risk for infection than immunocompetent individuals. The immunosuppressive medications currently in use are non-selective in nature, suppressing immune responses to alloantigens, as well as to infectious organisms. An organ transplant is a major surgical procedure with the infection risks of any major surgery. Chronic kidney failure itself suppresses the immune system to some extent. Cytopenias are common post-transplant due to medication side-effects, and can raise the infection risk.

Further, children are exposed to some unique infection risks. Many of the main viral infections that occur post–transplant are associated with higher morbidity when they are primary infections. A primary viral infection is defined as infection in a recipient who is seronegative at the time of transplant, with no prior immunity. Reactivation infection occurs in the setting of a patient who is seropositive and has some prior immunity. Pediatric patients are at higher risk for primary infection due to higher rates of recipient seronegativity at the time of transplant. Recent US data demonstrated that approximately 50% of pediatric kidney transplant recipients were EBV seronegative and 65% were CMV seronegative at the time of transplant compared to 10% EBV and 40% CMV seronegativity among adults [9]. The grafts to children come most often from adult (therefore most likely seropositive) donors, thereby introducing the virus at transplant.

Viral Infections

Cytomegalovirus

CMV, now called human herpesvirus 5 (HHV5), a double-stranded DNA virus of the herpes virus family, is perhaps the single most important pathogen in solid organ transplantation [10]. CMV not only causes significant morbidity by direct infection, but its immunomodulatory effects also predispose to other infectious complications [10]. CMV infection and CMV disease are different from each other. CMV infection is defined as evidence of CMV replication regardless of symptoms (differs from latent CMV). CMV disease is defined as evidence of CMV infection with attributable symptoms. Three patterns of CMV infection may be seen post-transplantation: Primary infection, reactivation infection, and superinfection. Primary infection occurs in transplant patients who were CMV seronegative prior to transplant, most commonly via transmission from a graft from a seropositive donor [10–12]. Without preventative therapy, the incidence of CMV disease in such recipients is 50–65% [10]. Reactivation infection is due to activation of latent virus in seropositive recipients, while superinfection is activation of virus from a seropositive donor in a seropositive recipient. Infection with CMV usually presents in the first few months post-transplant and can manifest as CMV syndrome, characterized by fever, myalgias, malaise, leukopenia and thrombocytopenia, or CMV disease, in which there is clinical evidence of organ involvement by the infection [10, 13]. The transplanted kidney is at higher risk for CMV infection than are the native organs, but pulmonary, liver and gastrointestinal tract infection are common, regardless of the organ transplanted [14–16]. As stated above, in addition to causing direct infection, CMV has significant indirect effects,

including an increase in the overall state of immunosuppression leading to increased risk for opportunistic infection [10]. CMV infection has been demonstrated to increase the risk of EBV-associated PTLD [10, 16]. In addition, CMV and acute rejection are interrelated. CMV infection is a risk factor for acute rejection, while rejection leads to release of tumor necrosis factor, triggering the process that ultimately leads to CMV replication [17].

Prevention of CMV infection can be accomplished with either (1) universal prophylaxis: the administration of anti-CMV therapy to all patients except seronegative recipients of a seronegative organ; or (2) preemptive therapy: viral monitoring and initiation of the treatment dose of anti-viral medication when a certain positive threshold is reached. There is some controversy as to the optimal strategy, as both methods have advantages and disadvantages. Consensus guidelines from the American Society of Transplantation (AST), Kidney Disease: Improving Global Outcomes (KDIGO), and The Transplantation Society International CMV Consensus Group recommend universal prophylaxis for high risk patients (seronegative recipients of seropositive organs or seropositive recipients of seropositive organs in the setting of anti-T-cell antibody immunosuppression), based on the available data suggesting better graft survival and clinical outcomes [11, 12]. Preemptive therapy has not been well studied in pediatrics. Although several agents are available for prophylaxis, valganciclovir has revolutionized both CMV prevention and treatment [18]. It is a prodrug of ganciclovir and is approximately 60% bioavailable, which is tenfold more than ganciclovir [19]. While the dosing of valganciclovir is well established in adults, the dosing in pediatric patients is somewhat more complex due to the dependence on metabolic activation, renal clearance and variable absorption. Since 2009, the manufacturer recommends normalization of the adult dose for BSA and creatinine clearance. Other centers have employed a weight based approach. Due to the challenges, particularly in infants and young children, ganciclovir levels may be helpful to guide therapy. Leukopenia is a common side effect of valganciclovir therapy. The duration of prophylaxis is an area of debate. Consensus recommendations guide the duration of therapy based on the serostatus of the donor and recipient [11, 12] (Table 69.1). For CMV Donor (D)+/Recipient (R)- patients, 3–6 months of prophylaxis with oral ganciclovir or valganciclovir is recommended. For CMV R+ patients, 3 months is recommended but 6 months should be considered if anti-lymphocyte induction is used. No prophylaxis is recommended in the CMV D−/R- patient. In addition, treatment of rejection with antilymphocyte antibodies in at risk recipients (D+/R-) should prompt re-initiation of prophylaxis or preemptive therapy for 1–3 months [11, 12, 22]. For treatment of CMV disease in pediatric patients, IV ganciclovir is recommended [12]. Therapy should continue until the CMV is no longer detectable. Reduction of immunosuppression in life-threatening CMV disease is indicated in cases of persistent disease despite treatment.

Late onset CMV disease is defined as disease occurring after prophylaxis has been discontinued and has been reported in 25–40% of patients on universal prophylaxis [20, 23]. Late onset CMV is associated with significant morbidity and high mortality, underscoring the ability of anti-viral prophylaxis to delay but not prevent

Table 69.1 Recommendations for duration of CMV prophylaxis [20, 21]

CMVD+/R-	• 3–6 months of prophylaxis with oral ganciclovir or valganciclovir is recommended. • In addition, treatment of rejection with antilymphocyte antibodies in at risk recipients (D+/R-) should prompt re-initiation of prophylaxis or preemptive therapy for 1–3 months.
CMV D+/R+ CMV D−/R+	• 3 months is recommended but 6 months should be considered if anti-lymphocyte induction is used.
CMVD−/R-	• No prophylaxis is recommended.

Table 69.2 Expert recommendations regarding BK virus screening

	2003 Polyoma-virus associated nephropathy interdisciplinary group [24]	2009 AST infectious diseases group [25]	2009 KDIGO transplant work group [12]
Screening	Urine screening, various techniques, every 3 months till month 24 (grade A-II) and annually thereafter till fifth year post-transplant (grade B-III) or with allograft dysfunction Biopsy if urine BK DNA > 1×10^7, VP1 mRNA >6.5×10^5 or plasma DNA > 1×10^4	Urine screening every 3 months in first 2 years then annually until fifth year post-transplant (grade II-B). If plasma screening performed, then at monthly intervals Biopsy if urine BK DNA > 1×10^7, VP1 mRNA > 6.5×10^5 or plasma DNA > 1×10^4	Plasma BK nucleic acid testing monthly for first 3–6 months, then every 3 months till month 12, or if elevated serum creatinine or after treatment for acute rejection
Intervention	Various approaches discussed, none specifically endorsed	Reduce immunosuppression for presumptive BKVN (plasma BKV loads >1×10^4 for >3 weeks)	Reduce immunosuppression if plasma nucleic acid load persistently >1×10^4

Adapted from [139]

CMV. Thus, careful clinical follow-up and virologic monitoring is recommended after completion of prophylaxis.

Antiviral drug resistance should be suspected and tested for in the setting of a patient who has had cumulative ganciclovir exposure of more than 6 weeks and there are rising viral loads or progressive disease after 2 weeks at full dose [11]. Risk factors include prolonged antiviral drug exposure (median 5–6 months), ongoing active viral replication, lack of prior CMV immunity (D+/R-), and inadequate drug delivery. Currently, genotype testing includes the UL97 kinase and UL54 DNA polymerase, with the UL97 mutation appearing in 90% of cases.

The timing and frequency of screening for CMV is largely center-specific and influenced by donor and recipient CMV serostatus, as well as whether universal or preemptive therapy is employed. Published guidelines recommend regular monitoring using a quantitative viral load assay for the first year post-transplant; however, the duration and frequency may vary depending on the type of CMV prevention strategy [11, 12]. Table 69.2 summarizes the characteristics of many commonly used assays for the different viral infections. The recent development of an international standard for CMV is promising as it will permit determination of appropriate standardized trigger points for intervention and allow comparison among sites.

Epstein-Barr Virus

EBV is another herpes virus that causes significant morbidity post-transplantation. Distinctions are made between EBV infection and disease. Active, asymptomatic EBV infection is defined by the presence of a detectable EBV viral load as measured by a nucleic acid amplification assay. Uncommonly, asymptomatic infection may also be identified in lymphoid rich histopathologic specimens. EBV disease is defined by the presence of active EBV infection with symptoms or signs attributable to the virus. The spectrum of clinical manifestations of EBV in transplant recipients includes nonspecific viral syndrome, mononucleosis, lymphoproliferative disorders and malignant lymphomas.

Like CMV, EBV commonly infects immunocompetent people sometime in childhood and establishes a prolonged latency in reticuloendothelial cells. Thus, the patterns of infection are identical to CMV: primary infection (often from the graft of a seropositive donor), reactivation or superinfection. Again, like CMV, the primary infection in an immunosuppressed transplant recipient is more virulent. Unlike CMV, EBV infection does not seem to have many indirect effects except for the development of PTLD. PTLD is a major complication and is covered in detail in the next chapter. This section deals with EBV infection only.

Prospective viral surveillance studies revealed that subclinical EBV infection occurs in 35–40% of pediatric renal transplant recipients [6, 26]. In a recent cohort study of adult kidney transplant recipients, 40% had subclinical viremia [27]. EBV viremia often precedes the development of EBV disease and PTLD by 4–16 weeks [28, 29]. Thus, early identification of EBV viremia may allow for intervention that could prevent progression to EBV disease and PTLD. KDIGO recommends the following post-transplant EBV screening schedule for high risk D+/R- patients: once in the first week after transplant; at least monthly for the first 3–6 months; then at least every 3 months until the end of the first year with re-initiation of monitoring after treatment for acute rejection. While D−/R– patients might be at decreased risk of developing EBV disease compared to D+/R–, they are still at increased risk relative to R+ patients and therefore warrant close monitoring. Some centers may choose to measure EBV loads more frequently. Beyond the first year, selective monitoring, such as in those with persistently high viral loads or in those with higher than normal immunosuppression, may be performed based on center preferences. Some centers recommend continued monitoring for an indefinite period for all patients. For seropositive individuals, selective monitoring may be considered in the setting of increased immunosuppression or clinical concern.

The reader should note that PCR techniques to detect EBV DNA amplification vary greatly based on the type of sample and laboratory standards. Thus, PCR values from peripheral blood leukocytes and whole blood generally correlate with each other but not with PCR values from plasma [30, 31]. To date, there is no defined standard sample site for EBV. In practice, the most important strategy is to follow the viral load in the same lab using the same type of sample consistently over time and to be careful to not compare viral loads from one lab to another.

There is no universally accepted treatment for subclinical EBV infection post-transplant. Options include reduction of immunosuppression, antiviral therapy, intravenous immunoglobulin (IVIG), and monoclonal antibody therapy directed toward infected B lymphocytes [21, 29, 32–34]. Currently, the only consensus recommendation is for a reduction of immunosuppression in EBV seronegative patients with an increasing EBV viral load. The utility of antiviral therapy to prevent PTLD is controversial, with little evidence to support the role of acyclovir or ganciclovir in response to an elevated or rising EBV viral load without a concomitant reduction of immunosuppression. These agents seem to delay the onset of infection rather than reducing its incidence. Two studies suggest that anti-viral prophylaxis has an additional benefit of preventing the progression from EBV disease to PTLD [19, 35]. IVIG does not appear to be of added benefit [36]. Preemptive use of rituximab in response to subclinical EBV infection began in the hematopoietic stem cell population and has recently been reported in the adult kidney transplant population [37, 38]. It is important to remember, however, that children, in particular, can develop a chronic high load carrier state without ever progressing to PTLD [39–44]. Nevertheless, the majority of reports indicate that higher EBV PCR values are associated with a greater risk for subsequent PTLD [45–47]. An EBV vaccine, directed against an EBV-glycoprotein, was tested in the United Kingdom but failed [48]. Unlike with CMV, we are not aware of any cost-benefit analysis of EBV monitoring or preventative treatment strategies.

BK Virus and BKVAN

BK virus (BKV) was first isolated from the urine of a kidney transplant recipient in the 1970s [49], but it was not until the late 1990s that this virus emerged as a significant problem in kidney transplantation [50, 51]. BKV is a part of the polyoma group of viruses. Though this virus is not from the herpesvirus group, it shares the characteristics of herpesviruses of infecting most immunocompetent people during childhood and establishing a prolonged latency. Unlike the herpesviruses, the virus does not establish latency in reticuloendothelial cells but in the uroepithelium. This propensity for the uroepithelium is responsible for the clinical manifestations: hemorrhagic

cystitis in bone marrow transplant recipients and allograft nephropathy in kidney transplant recipients. The incidence of BKVAN in pediatric kidney transplantation appears to be the same as in adult kidney transplants at 3–8% [52–56]. Risk factors include the intensity of immunosuppression, recent treatment for acute rejection, and placement of a ureteral stent, though the data implicating specific immunosuppressive agents is conflicting [54, 57–59].

BKVAN and BKV infection are two separate entities. BKVAN is defined as the presence of virus in the renal parenchyma, with accompanying evidence of either tubulointerstitial nephritis or elevated serum creatinine, as defined by a working group of the AST [2]. BKVAN is more prevalent in the medulla of the kidney, so at least one core should be deep enough to include medulla. A negative biopsy result does not rule out BKVAN due to the possibility of sampling error and the focal nature of the infection, so sensitivity is not 100%. In cases where the biopsy is negative, but there is high clinical suspicion for BKVAN, a repeat biopsy may be indicated. The histologic patterns of BKVAN have been divided into three types, as reviewed by Liptak et al. and the AST Transplant Infectious Diseases Group [25, 60]: Type A has intranuclear viral inclusions only, Type B has additional acute inflammation but very little chronic fibrosis, and Type C has significant chronic fibrosis and atrophy. The value of this classification lies in its prognostic value of clinical outcomes: the incidence of progression to end-stage kidney disease (ESKD) was only 13% with Type A, 55% with Type B and 100% with Type C [61]. BKVAN represents a diagnostic challenge because the condition may resemble acute rejection. Symptoms are often minimal or absent. Serum creatinine elevations are found on clinical lab monitoring. Since the treatment of acute rejection (intensifying immunosuppression) is the opposite of the treatment of BKVAN (reduction in immunosuppression), making the correct diagnosis is critical.

Early identification of BKV infection (detectable viral load in blood or urine) may permit intervention that may prevent BKVAN. Data suggest that the BK viremia precedes BKVAN by a median of 8 weeks [24]. BKV viral load >10,000 copies/L has a high positive predictive value for BKVAN [59]. Indications for biopsy vary among centers but many include viral load >10,000 copies/L with or without an elevated creatinine.

Routine screening is the most important tool used to identify patients at risk for BKVAN. Various schedules of surveillance are shown in Table 69.3. Intervention options include reduction of immunosuppression and use of other agents such as cidofovir, leflunomide, or IVIG. Stepwise immunosuppression reduction is recommended for kidney transplant recipients with plasma BKV-DNAemia of >1000 copies/ml sustained for 3 weeks or increasing to >10,000 copies/ml, reflecting probable and presumptive BKVAN, respectively [64]. The approach to immunosuppression reduction varies among centers, with varying levels of supporting evidence, and includes the following: (1) switching from tacrolimus to cyclosporine (CSA) or sirolimus; (2) mycophenolate mofetil (MMF) to azathioprine or sirolimus or leflunomide; (3) decreasing tacrolimus (trough levels <6 ng/ml), MMF (dosing ≤1 g/day), and CSA (trough levels 100–150 ng/ml); or (4) decreasing tacrolimus or MMF (maintain or switch to dual therapy with calcineurin inhibitor (CNI) and prednisone, sirolimus/prednisone, MMF/prednisone) [12]. While the reduction of immunosuppression raises concerns about the unintended consequence of rejection, several studies have reported successful preemptive intervention with no increase in rejection [65, 66].

There are virtually no randomized controlled trials to test any of these strategies head to head for any of the viral infections. Anti-viral therapy against BKV is more complicated than for CMV or EBV, since acyclovir, ganciclovir or their analogues are not active against BKV. Cidofovir is one anti-viral drug that has been tried with some success [67, 68]. Higher doses of cidofovir can be very nephrotoxic. Probenecid in combination with the higher dose cidofovir or intermediate dose cidofovir prevents the nephrotoxicity [69]. Fluoroquinolones are not recommended for either prophylaxis or treatment [64].

Table 69.3 Recommended vaccinations for pediatric transplant candidates and recipients [62, 63]

Vaccine	Inactivated/live attenuated (I/LA)	Recommended before transplant[a]/ strength of recommendation	Recommended after transplant/ strength of recommendation	Monitor vaccine titers?	Quality of evidence
Influenza, injected	I	Yes/A	Yes/A	No	II
Hepatitis B	I	Yes/A	Yes[b]/B	Yes[b]	II
Hepatitis A	I	Yes/A	Yes/A	Yes	II
Pertussis	I	Yes/A	Yes/A	No	III
Diphtheria	I	Yes/A	Yes/A	No	II
Tetanus	I	Yes/A	Yes/A	No	II
Polio, inactivated	I	Yes/A	Yes/A	No	III
Hemophilus influenzae	I	Yes/A	Yes/A	Yes[c]	II
Streptococcus pneumoniae[d] (conjugated/ polysaccharide)	I/I	Yes/A	Yes/A	Yes[c]	III
Neisseria meningitidis[e]	I	Yes/A	Yes/A	No	III
Rabies[f]	I	Yes/A	Yes/B	No	III
Varicella	LA	Yes/A	No/D	Yes	II
Measles	LA	Yes/A	No/D	Yes	II
Mumps	LA	Yes/A	No/D	Yes	III
Rubella	LA	Yes/A	No/D	Yes	II
Bacille Calmette-Guérin [g]	LA	Yes/B	No/D	No	III
Smallpox[h]	LA	No/C	No/D	No	III
Anthrax	I	No/C	No/C	No	III

Adapted from (a) Advisory Committee for Immunization Practices 2013; (b) The American Society of Transplantation (AST) Handbook of Transplant Infections, 2011

[a]Whenever possible, the complete complement of vaccines should be administered before transplantation. Vaccines noted to be safe for administration after transplantation may not be sufficiently immunogenic after transplantation. Some vaccines, such as Pneumovax, should be repeated regularly (every 3–5 years) after transplantation

[b]Routine vaccine schedule recommended prior to transplant and as early in the course of disease as possible; vaccine poorly immunogenic after transplantation, and accelerated schedules may be less immunogenic. Serial hepatitis B surface antibody titers should be assessed both before and after transplantation to assess ongoing immunity

[c]Serologic assessment recommended if available

[d]Children older than 5 years should receive 23-valent pneumococcal polysaccharide vaccine. Children less than 2 years should receive conjugated pneumococcal vaccine. Those 2 years–5 years of age should receive vaccination based on age and number of previous immunizations with conjugated pneumococcal vaccine

[e]Vaccination with conjugated meningococcal vaccine recommended in United States for all children aged 11–12 years of age and adolescents at high school entry or 15 years of age, whichever comes first

[f]Not routinely administered. Recommended for exposures, or potential exposures due to vocation or avocation

[g]The indications for Bacille Calmette-Guérin administration in the United States are limited to instances in which exposure to tuberculosis is unavoidable and where the measures to prevent its spread have failed or are not possible

[h]Transplant recipients who are face-to-face contacts of a patient with smallpox should be vaccinated; vaccinia immune globulin may be administered concurrently if available. Those who are less intimate contacts should not be vaccinated

Varicella

Varicella-zoster virus (VZV) is the most infectious of the human herpesviruses. Primary infection with VZV results in chickenpox. Following primary infection, the virus remains in the body in a latent state from which it may be reactivated, resulting in cutaneous herpes zoster, or shingles. Most adult kidney transplant recipients have experienced primary infection in childhood and, therefore, are at risk for reactivation and the development of herpes zoster with the introduction of immunosuppressive medication post-transplant [70]. Historically many children were VZV naive at the time of transplantation and primary infection was a significant cause of morbidity and mortality [71, 72]. With the development of a safe and effective VZV vaccine, routine immunization of pediatric kidney transplant candidates has been documented to reduce the incidence of primary VZV infection post-transplantation [73]. Given these findings, it is recommended that all transplant candidates over 9–12 months of age receive immunization with the VZV vaccine [74]. Studies in children with chronic kidney disease and on dialysis suggest that two doses, rather than one, may be necessary to elicit protective antibody levels, so it is recommended that antibody levels be obtained at least 4 weeks following immunization, and a second dose given if necessary [74–76]. Although some studies have evaluated the use of this vaccine in post-transplant patients, both the American Academy of Pediatrics Committee on Infectious Diseases and the Centers for Disease Control and Prevention's Advisory Committee on Immunization Practices (ACIP) advise against the use of this live viral vaccine in immunocompromised patients [77, 78]. Thus, it is imperative that immunization be provided and protective antibody levels documented prior to transplant whenever possible.

Patients who are varicella-naive at the time of transplant, i.e. no history of chicken pox or VZV immunization, or fail to develop protective antibody after immunization, and who are exposed to varicella should receive prophylactic therapy. Previous recommendations included the delivery of varicella zoster immune globulin (VZIG); however, this product is no longer being manufactured [79]. In North America, an investigational VZIG product, VariZIG (Cangene Corporation, Winnipeg, Canada) has become available under an investigational new drug application and the ACIP recommends that use of this product be requested if an immunocompromised patient is exposed to varicella infection [79]. If this product is not available, IVIG, which contains some anti-varicella antibody, may be given. Any prophylactic therapy should be given as soon as possible, up to 96 hours after exposure. Patients who develop infection, either primary or secondary, should receive treatment with intravenous or oral acyclovir, with consideration of reduction of immunosuppression [12, 80, 81].

COVID-19

The coronavirus disease 2019 (COVID-19), characterized by significant respiratory and multiorgan disease, is caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS–COV–2). This virus first emerged in December 2019 in Wuhan, China [82]. Droplets expelled during talking, coughing, sneezing, or eating are the most common mode of transmission. Transmission may also occur through aerosol; however, it is unclear if this is a significant mode of transmission outside of laboratory settings. Common symptoms of COVID-19 infection include fever, dry cough, shortness of breath, fatigue, myalgias, nausea and vomiting, diarrhea, headaches, weakness and rhinorrhea. Common complications include pneumonia, acute respiratory distress syndrome, liver injury characterized by elevation of liver enzymes, cardiac injury marked by troponin elevation, acute heart failure, myocarditis, prothrombotic coagulopathy, acute kidney injury, and acute cerebral vascular disease. Rare complications include cytokine storm and macrophage activating syndrome. Patients become contagious about 2–3 days prior to the onset of symptoms until about 8 days after symptom onset [82]. Nearly 80% of patients with COVID-19 have mild manifestations, 15% develop severe illness and 5% become critical.

Data are emerging on the impact of COVID-19 on kidney transplant recipients. The incidence of COVID-19 in solid organ transplant (SOT) recipients is 10- to 15-fold higher than in the general immunocompetent population and adult SOT patients with COVID-19 appear to be at higher risk of poor outcomes on the basis of their chronically immunosuppressed state and underlying medical comorbidities [67, 68]. Initial reports in pediatric kidney transplant recipients demonstrate a decreased risk for infection and less severe disease when compared to adult kidney transplant recipients [83, 84].

Transplant Candidate Considerations

Vaccination is recommended to occur prior to transplantation, ideally completing the vaccine series a minimum of 2 weeks prior to transplant. Living donor candidates should self-quarantine or follow strict social distancing for a 14 day period prior to the transplant. All candidates should also have a negative nucleic acid amplification test (NAT) documented prior to surgery.

Post-Transplant Vaccination

The AST recommends vaccination against SARS CoV-2 using the locally approved vaccines for pediatric kidney transplant recipients [85]. Information about COVID-19 vaccine responses in transplantation is rapidly evolving. However, antibody responses to COVID-19 vaccines in transplant recipients are diminished compared with the general population [86–97]. Data suggest that providing a third dose of mRNA vaccine to SOT recipients that have previously received two doses of mRNA vaccine can increase antibody titers to SARS-CoV-2 [98–100]. In a recent, double-blind, randomized placebo-controlled trial, a third dose mRNA vaccine provided 2 months after the second dose significantly increased antibody titers, neutralizing antibody, and cellular immune response to SARS-CoV-2 compared to a third dose placebo [94]. Based on this, a third dose of mRNA vaccine is recommended for SOT recipients who have previously completed a 2-dose mRNA vaccine series. The use of a third dose should, until further evidence is available, be based on individual patient's unique situation and must depend on local availability of vaccines and local regulations. In addition, vaccination is recommended for all eligible household and close contacts. Routine antibody testing following vaccination is not recommended by the FDA.

COVID-19 and Donor Considerations

The AST published guidelines for organ donor screening for COVID-19. All deceased donors should be tested for SARS-CoV-2 infection using RT-PCR from the upper respiratory tract within 72 hours, but ideally as close to organ recovery as possible. For donors previously known to have had COVID-19, organ acceptance can be considered if the following circumstances are met: negative SARS-CoV-2 RT-PCR testing from the respiratory tract, symptoms of COVID-19 have resolved, AND at least 21 days have transpired since the date of disease onset. Data regarding the safety of organ donation from donors with previous COVID-19 are limited at this time and consultation with transplant infectious disease experts is recommended. Living donors should be advised to follow universal masking precautions and strict social distancing for 14 days prior to donation. All living donors should undergo respiratory tract SARS-CoV-2 RT- PCR testing within 3 days of donation. Donors should be encouraged to self-quarantine after the preoperative COVID-19 test [101].

Bacterial Infections

Urinary Tract Infection

Urinary tract infection (UTI) is the most common bacterial infection in kidney transplant recipients, in both adults [102, 103] and children [104].

UTIs develop in 20–60% in the first year post-transplant and 40–80% by 3 years post-transplant [2, 102–105]. UTI is not only a cause of morbidity but is also associated with higher rates of graft loss and patient death [106, 107]. Early UTI (within 6 months of transplant) elevated the risk for graft loss in children, while late UTI did not [108]. The urogenital tract is the most common entry point for systemic sepsis [109]. Numerous risk factors have been identified for UTIs post-transplant. Urologic anomalies such as neurogenic bladder, urinary tract obstruction, vesicoureteral reflux, bladder augmentation, ureteral stents and intermittent catheterization have all been associated with an increased risk of UTI post-transplant [105, 110–112]. UTI risk is highest in the first 3–6 months post-transplant but some risk remains at later time points. While the organisms implicated are usually the same as in immunocompetent individuals, such as Enterobacter species (e.g., *Escherichia coli* and *Klebsiella)*, a higher percentage of UTIs in transplant patients are due to unusual organisms such as *Pseudomonas* species [113]. Clinical symptoms may include fever, dysuria, graft tenderness and foul-smelling or cloudy urine. In some patients, symptoms may be masked due to immunosuppression. A rise in serum creatinine may occur and can mimic acute rejection. UTIs can also precipitate acute rejection.

The diagnosis of UTI is usually made by urine culture, though patients on trimethoprim-sulfamethoxazole prophylaxis for *Pneumocystis jiroveci* may not demonstrate positive cultures. Treatment is with antimicrobial therapy. Initially, the antimicrobial prescribed should cover the common gram negative organisms, such as the beta-lactams or the quinolones [114]. Once the organism is known, the most specific and cost-effective antimicrobial can be prescribed. Treatment route and total duration are determined by the severity of infection, recipient age, and other risk factors. Kidney allograft pyelonephritis can be associated with bacteremia and significant morbidity. If allograft pyelonephritis is suspected, hospitalization and treatment with intravenous antibiotics for up to 14 days is recommended [12]. Shorter 5–7 day oral courses, as are used in immunocompetent individuals, can be used for milder cystitis episodes in older children [92]. KDIGO suggests that all kidney transplant recipients receive UTI prophylaxis with daily trimethoprim-sulfamethoxazole for at least 6 months post-transplant based on data showing a decrease in the frequency of UTIs [115]. For patients who are allergic to trimethoprim-sulfamethoxazole, the recommended alternative is nitrofurantoin. Currently, the available evidence does not support routine treatment of asymptomatic bacteriuria [114].

Other Bacterial Infections

Other bacterial infections, such as wound infections, line sepsis and pneumonia are seen with significant frequency in kidney transplant recipients. Wound infections and line sepsis are commonly due to gram-positive *staphylococcus* and *streptococcus*.

Pneumonia can be due to multiple etiologies (bacterial, viral or fungal), but bacterial pathogens are responsible for approximately 44% of cases [116]. In adult transplant recipients, cellulitis and bacterial abscesses are frequent problems, largely due to co-morbid diabetes mellitus. In general, these complications are less common in the pediatric population. The treatment of these infections is generally no different than standard treatment in immunocompetent hosts, though duration of therapy may be longer.

Bartonella henselae infection (also known as cat-scratch disease) has been reported in pediatric organ transplant recipients, including kidney transplants [117].This infection typically presents as fever and lymphadenopathy, and thus must be included in the differential diagnosis for PTLD. However, unlike PTLD, this infection is treated with antimicrobial therapy.

The incidence of *Mycobacterium tuberculosis* infection in kidney transplant recipients varies geographically, occurring in less than 2% of kidney transplant recipients in North America and Europe, but 5–15% in Asia and Africa [118–120]. This infection may present at any time post-transplant, but is most common in the first post-

transplant year [119]. *M. tuberculosis* infection presents with a myriad of symptoms, including weight loss, cough, fever and lymphadenopathy, again mimicking PTLD. The diagnosis of tuberculosis in the transplant recipient is similar to that in other populations, although the tuberculin skin test may be positive in only a third of kidney transplant recipients with tuberculosis [120]. Early diagnosis is best achieved by staining for acid-fast bacilli or using PCR from sputum, bronchoalveolar lavage or gastric aspirates. Interferon-gamma release assays such as QuantiFERON and T-SPOT.TB are alternative methods used to detect latent infection. Management of tuberculosis is complex and evolving and has long been directed by recommendations developed, updated and disseminated by expert panels [12, 120–122]. A four-drug regimen, similar to the regimen recommended in the general population, should be used in case of active tuberculosis after transplantation [123]. Rifampin is associated with numerous drug interactions through its activation of the CYP3A4 pathway which can impact levels of CNIs and mTOR inhibitors, sometimes necessitating higher CNI doses. Alternatively, rifabutin could be used instead of rifampicin given its milder interactions [123].

Other Infections

Pneumocystis Jiroveci Pneumonia

Pneumocystis jiroveci was previously known as *Pneumocystis carinii* and classified as a protozoal disease. The classification has evolved based on DNA sequence analysis such that *P. jirovecii* is now classified as a fungus. Human pneumocystis is now called jirovecii as *Pneumocystis carinii* only infects rats. *P. jirovecii* pneumonia (PJP) is an important opportunistic infection that has fortunately decreased in frequency due to the widespread use of sulfamethoxazole/trimethoprim prophylaxis in the immediate post-transplant period. Patients typically present with fever, dyspnea and nonproductive cough, interstitial infiltrate on chest x-ray and hypoxemia. Elevated lactate dehydrogenase and hypercalcemia are characteristic biochemical findings supporting the diagnosis. The diagnosis is established by demonstration of pneumocystis in lung secretions obtained from bronchoalveolar lavage or in tissue from lung [124]. Gomori stain or toluidine blue staining will demonstrate the cysts and Giemsa staining will identify the sporozoites. CMV infection is the major differential diagnosis. Many children may have dual infection, in which CMV infection predisposed to superinfection with *P. jirovecii*. Treatment recommendations include high dose intravenous trimethoprim-sulfamethoxazole, corticosteroids and a reduction in immunosuppression. Chemoprophylaxis with three times a week oral sulfamethoxazole/trimethoprim (5 mg/kg trimethoprim component/dose) has reduced the incidence of PJP from 3.7% to 0% [125]. Daily dosing may be easier for patient adherence and is recommended by KDIGO [12]. Prophylaxis is recommended in all transplant recipients for 3–6 months post-transplant. Some also advocate its use after anti-rejection therapy, particularly with anti-T cell antibodies.

Parasitic Infections

Although several parasitic infections have been reported in pediatric recipients of solid organ or bone marrow transplantation, there are few reports of such infections in pediatric kidney recipients. Several parasitic infections deserve mention, however, as they have been reported as transmitted by the transplanted graft in adult kidney transplant recipients. *Strongyloides stercoralis* is an intestinal nematode that infects tens of millions of people worldwide. It is endemic in tropical and sub-tropical regions. The highest rate of infection in the US is in the Southeast [126]. *S. stercoralis* may remain in the human intestinal tract without symptoms for decades, and then cause disseminated infection with the introduction of immunosuppressive medication post-transplant [126]. In addition, there are case reports of transmission of strongyloidiasis by kidney transplantation in an adult recipient [127]. Interestingly, CSA but not tacrolimus has effects

against *S. stercoralis* and may reduce the risk for disseminated strongyloidiasis [128, 129]. Active infection typically presents with cutaneous, gastrointestinal and pulmonary symptoms as well as eosinophilia [130]. With disseminated disease, fever, hypotension, and central nervous system symptoms may be present [130]. In uncomplicated infections, diagnosis is made by detection of larvae in stool, although 25% of infected patients may have negative stool examinations [131]. In disseminated disease, larvae may be found in stool, sputum, bronchoalveolar lavage fluid, and peritoneal and pleural fluid [126, 132]. Serologic testing using ELISA may also be of value, but may be falsely negative in immunocompromised hosts [132, 133]. Thiabendazole, previously the treatment of choice for *S. stercoralis,* has been replaced by ivermectin, with albendazole as an alternative [134].

Other parasitic infections reported in kidney transplant recipients as transmitted by the transplanted graft include Chagas' disease and malaria [126, 135, 136]. Chagas' disease is caused by *Trypanosoma cruzi* and is found only in the southern US, Mexico and Central and South America. The manifestations of Chagas' disease classically include megaesophagus, megacolon, and cardiac disease, although CNS involvement has been reported in kidney transplant recipients [135]. The diagnosis is routinely made serologically and treatment typically consists of benznidazole or nifurtimox. Post-transplant malaria, transmitted from donors living in high-risk areas, is a frequent occurrence [136]. The discussion of these infections is meant to illustrate the potential problem of parasitic infections post-transplant. Policies to screen potential recipients and donors for these and other parasitic infections should be based on the presence of risk factors, including residence in or travel to an endemic area.

Fungal Infections

In general, serious invasive fungal infections such as aspergillosis are less common in pediatric kidney transplant recipients than thoracic organ recipients. Candida is the most common organism affecting kidney transplant recipients, either as oral and esophageal thrush, vaginitis, nail infection or UTI. The diagnosis of thrush is by clinical examination or demonstration of hyphae on a smear. Candidal UTI is diagnosed by urine culture. Treatment for topical candida is by topical nystatin or clotrimazole. Prophylactic measures include oral clotrimazole lozenges, nystatin, or fluconazole for 1–3 months post-transplant and for 1 month after treatment with an anti-lymphocyte antibody [12]. Treatment of invasive disease typically requires amphotericin. Fluconazole may be used for treatment of less severe disease, or for infections that have stabilized after initial therapy with amphotericin. Dose adjustment and close monitoring of the levels of CNIs are necessary when fluconazole is used due to the drug-drug interactions. In addition, there are potential drug-drug interactions between CNIs and clotrimazole [137].

Immunizations

One of the cornerstones of preventative care in pediatrics is the delivery of routine childhood immunizations. Unfortunately, the complicated medical care required by many children with chronic kidney disease may result in only sporadic delivery of routine well-child care, including immunizations. Complete immunization is especially important in children with ESKD as they approach transplantation given the increased risk for vaccine preventable disease post-transplant. In general, children with chronic kidney disease should receive immunizations according to the recommendations for healthy children in the region. Because they may also be more susceptible to or at risk for more serious infection from pathogens that are not typically problematic in healthy children, candidates for or recipients of kidney transplantation may also benefit from supplemental or additional vaccinations [76]. Table 69.3 provides a list of vaccinations recommended specifically for pediatric transplant candidates and recipients. Because children with chronic kidney disease and on dial-

ysis may have sub-optimal response to many immunizations, or lose immunity prior to transplantation, it is important not only to ensure timely delivery of routine childhood immunizations, but also to monitor antibody titers or levels and revaccinate when indicated [138]. This is especially true of the live viral vaccines, including measles, mumps, rubella and varicella zoster vaccine, which are contraindicated in the immunosuppressed patient post-transplant.

In the post-transplant period, immunizations may be given after immunosuppressive medications have reached a baseline level, typically 6 to 12 months post-transplant. Again, live viral vaccines are generally contraindicated in the post-transplant period. Because the presence of immunosuppressive medications may impair response to vaccines, maximal protection requires universal immunization of health care workers, family members and household contacts [74]. In particular, annual immunization with injectable influenza vaccine is required [74].

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Long-Term Outcome of Kidney Failure in Children

70

Jaap W. Groothoff

Introduction

> …The patient remained unconscious during dialysis, showing cramps from time to time ...suddenly he vomited, showing massive gingival bleeding...13 h the patient started shaking after a piece of wood had been placed in the device against the spatter of dialysate...an example of a smooth dialysis session….

These thrilling notes are from Willem Kolffs' thesis in which he describes the first dialysis sessions ever performed in humans. The experiments took place during World War II in Kampen, a small town in the Netherlands [1]. Only one of the 12 patients, notably a sympathizing Nazi, survived the treatment. After the war Kolff emigrated to the United States where he developed the first production artificial kidney, the Kolff Brigham Artificial Kidney, which laid the foundation for modern hemodialysis treatment.

Yet, it would take until the end of the 1960s before chronic kidney replacement therapy (KRT) became available on a routine basis for children with end-stage kidney disease (ESKD). Initially, many physicians were reluctant to start such invasive therapy in children. Among those who refused to start dialysis in children were some very distinguished pediatric nephrologists. As both the dialysis technique and supportive therapy improved during the 70s and 80s chronic renal replacement therapy in children became gradually more accepted. In particular, better nutrition, the introduction of bicarbonate-buffer replacing acetate in hemodialysis, the introduction of continuous cycling peritoneal dialysis, the use of recombinant human erythropoietin and growth hormone therapy, and the introduction of cyclosporine after transplantation contributed to a marked decrease in morbidity and mortality and increase of children taken into therapy.

Despite all these improvements in therapy, RRT in children remained controversial at least until the early nineties of the last century. Even today, many physicians feel uncomfortable in offering chronic RRT to young children and question whether it should be offered to all children, especially in case of multiple comorbidities [2]. The fear for acute casualties has been replaced by concerns about the long-term prospects for children on RRT. One of the first questions parents ask when confronted with the necessity of chronic dialysis or transplantation for their child is what his or her future will be like. Will he or she ever be able to participate in society as an independent individual and have an acceptable life?

Unfortunately, even to date few extensive data exist on long-term outcome. This chapter reviews the very few late outcome studies performed to

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date. By definition, these data are based on outcomes of patients who started RRT in the early experimental years of dialysis and transplantation in children. Extrapolation of outcomes to the current generation of children on RRT is therefore hazardous as both the treatment quality and policies have importantly changed over time. Most western registry data show, for instance, a significant and continuous rise in pre-emptive transplantation rates in children over time [3–5]. Yet, despite all improvements, the most important problems of dialysis and transplantation have not been solved to date. Therefore, the situation of the first generation of now middle-aged patients with pediatric chronic renal replacement therapy may indeed give at least an impression of the potential threats for the current generation of children with ESKD.

Chronic Pediatric Renal Replacement Therapy in Developing Countries

A shortcoming of this review is that the reported data apply to patients from highly developed countries. Few data exist on the situation in resource limiting settings, but from the few existing reports it is obvious that the situation there is incomparable with that in developed countries. In some countries, chronic renal replacement therapy in children does not even exist at all. In an IPNA survey in 2017 among all countries with more than 300,000 inhabitants and a response rate of 80%, chronic RRT for children was reported to be absent in 10 out of 94 countries [6]. However, this is most likely an underreport, as nearly all not-responding countries were low-income countries. Also, the existence of a program for pediatric renal replacement therapy does not automatically imply access to RRT for all children. In China and India, for instance, the world's largest countries with 32% of the global childhood population, the estimated prevalence of pediatric RRT was in 2017 less than 10% of that observed in Western countries [6]. A recent report from Nigeria described outcomes of peritoneal dialysis in children aged 3–11 years for acute kidney injury after 18 months of follow up and reported 27.6% mortality [7]. A report from India mentioned 72% one-year survival, falling to 30% after 106 months of 66 children median aged 12.3 years on PD [8]. Another recent paper on country disparity within Europe showed that children on RRT from low-income countries have less access to transplantation but equal graft survival of those who received a kidney graft. Access to transplantation should also therefore be also the main aim for countries with limited financial means [9].

Course of Treatment Over Time

"A child with end-stage renal disease should receive a kidney transplant as soon as possible; a child that cannot be transplanted should not be started on chronic renal replacement therapy at all."

This paradigm has been a guideline for most pediatric nephrologists. Unfortunately, there are several circumstances in which dialysis is the only—temporary—option for children with ESRD. Moreover, as renal grafts may fail over time, the longer a patient is on RRT the higher is the chance that a patient will have a sequence of periods of living with a functioning graft alternating with periods on dialysis.

Most outcome studies have followed patients either on dialysis or after transplantation, ignoring the fact that most patients living with RRT for decades spend variable periods on both treatment modalities. The interpretation of such studies is troublesome, as it is the cumulative time spent on dialysis vs. the time with a functioning renal graft that predominantly affects the overall outcome.

In a long-term study of all 249 Dutch patients born before 1979 who started RRT before age 15 years between 1972 and 1992 with follow-up to 2010 (the LERIC study), patients spent on average 25.5 years on RRT. Of this cumulative time, 19.7 (0.03–39.6) years (77%) were spent with a functioning graft and 5.8 (0–36.5) years on dialysis. Among the 231 (93%) transplant recipients, 71 (31%) lived with a single transplant -not necessarily their first- for more than 20 consecu-

tive years, up to 37.2 years. Transplantation was performed twice in 84 patients (36.4%), three times in 43 (18.6%), four times in 8 (3.5%), five times in 1 (0.4%) and six times in 3 patients (1.3%). Patients changed treatment modality between 1 and 11 times during the study period. Only 2 of the 249 patients (0.8%) lived with a functioning graft during the entire follow-up (median survival 25.3 years) while 18 patients (7.2%) only received dialysis (median survival 3.7 years) [10].

In an equally long follow-up study using data of the Australian and New Zealand database (ANZDATA) concerning 1634 patients with RRT onset <19 years between 1963 and 2002, patients received RRT for on average for 11.3 years, of which 3.5 years were spent on dialysis and 7.8 years (69%) with a functioning graft [11]. The difference towards a longer time on dialysis in the latter study is probably explained by the 10 years earlier observation period [11].

Possibly, the average lifetime on dialysis may further decrease in the future as the last decade has shown a trend towards more pre-emptive transplantations in children, at least in the Western countries. All registry data show rises in rates of pre-emptive transplantation, varying from 26% in 1997–2001 to 32.9% in 2007–2011 in the United Kingdom ($p < 0.05$), from 10% to 25% between 1992 and 2007 in Canada and from 9.6% in 1985–1989 to 18.4% in 2000–2004 in the countries connected to the ERA-EDTA database [3–5]. According to the ANZDATA base, the proportion of children who underwent transplantation as the initial RRT modality had remained stable over time, being about 19–20% of all children starting RRT. On the other hand, the incidence of pre-emptive transplantation in children increased from 0.58 transplantations per million age related population (pmarp) in 1967–1971 to 1.65 transplants pmarp in 2002–2006 [12].

A recent report from the ESPN/ERA-EDTA database showed a 2% increase from 26.4 per million age-related population (pmarp) in 2007 to 32.1 pmarp between 2007 and 2016 of the prevalence of pediatric transplant recipients aged <15 years, with an unchanged incidence (5.5–6.6 pmarp), reflecting a better graft survival and promising a prolonged time on transplantation over time [13]. These shifts will certainly impact on the course of RRT as well as the impact on adult life in the current population of children with ESRD.

Impact of RRT Modality Over Time: Transplantation vs. Dialysis

All late outcome studies favor early transplantation in children with ESKD, which appears to have a beneficial effect on overall mortality, morbidity and psychosocial development. In the LERIC study, the cumulative duration of dialysis in relation to years with a renal graft was the strongest factor associated with nearly all adverse outcomes, especially with cardiovascular death, but also with impaired cognitive performance, loco motor disorders and social independency [10, 14–18]. The impact of dialysis on physical condition is also reflected by the sharp difference in physical health perception of dialysis and transplanted patients. On the other hand, the ANZDATA data showed that a short period of dialysis (up to 2 years) did not affect overall mortality, a finding that was confirmed by ERA-EDTA data on mortality in young adults with pediatric-onset ESKD [11]. None of the studies showed significant differences in outcome of peritoneal dialysis and hemodialysis.

Although dialysis is the most unfavorable mode of RRT, transplantation is associated with considerable late morbidity. Disabling co-morbidity was reported by 40% of all transplant recipients in the LERIC study of whom most patients were transplanted at time of investigation [19]. Apart from clinical bone disease, the most frequently reported disabling problems were severe daily headaches, tremors and severe itching, most of them appearing in transplanted patients. Malignancies, infection, hypertension related LVH and arterial wall stiffening are the most life-threatening problems after transplantation. Although current insight shows a much lower mortality in transplant recipients with childhood ESKD than in patients who remain on dialysis, with the passage of time the

'dark side of Camelot' may become more lucid. The longer the period of transplantation, the more patients become at risk for life-threatening infections and malignancies at relatively young age [20]. The current trend towards the use of more potent, and hence potentially more carcinogenic, immunosuppressive therapy may bear significant future consequences.

Mortality

According to long-term outcome studies, the overall mortality of patients with pediatric onset of chronic renal replacement therapy is about 30 times as high as among people of the same age without the need for RRT [10, 20–23]. Mortality rates per 100 patients decreased in most long-term outcome studies from 4.4 (ANZDATA) and 3.6 (LERIC) in 1972–1983 to 1.2–1.8 years after 1983 (ANZDATA, LERIC, Canadian Registry) [4, 10, 11, 20]. After on average 30 years of cRRT, the Dutch cohort study showed a U-shaped course of mortality risk over time: the added mortality risk, expressed as Mortality Rate Ratio, was extremely high during the first years of RRT in 72–'89, then decreased from 53 to 19.7 in the following period '90–'99, but increased again to 26.8 in the period '00–'10 [20]. After on average 30 years of RRT, mortality risk in the Dutch cohort study showed a U-shaped course over time: the added mortality risk, expressed as Mortality Rate Ratio, was extremely high in the 1970s and 1980s, decreased from 53 to 19.7 in the 1990s, but increased again to 26.8 in the first decade of this century [20]. This suggests that the added mortality risk may increase with ageing.

To some extent, these figures may overestimate the mortality risk for children currently on RRT as the dialysis technique certainly has improved over time and more children are transplanted pre-emptively. All studies on patients with ESKD indeed show a substantial decrease in mortality over the last 40 years, especially in the very young age groups. Yet, at the same time, this trend toward improvement of survival of ESKD has also slowed dramatically during the last 25 years [10, 11]. In fact, the survival trends are quite disappointing, considered the experimental nature of renal replacement therapy among children during the early years. Both McDonald's study on the ANZDATA and the Dutch cohort showed no increase in survival after 1983 [10, 11]. These outcomes are in line with other registry data. A Canadian registry study on survival rates of pediatric dialysis and transplantation since 1992 showed no improvement over time [4]. The USRDS data show a slightly different picture; here the mortality hazard ratio among transplanted patients decreased from to 0.83 in 1988–1994, dropped further to 0.77 in 1995–1997, stabilized between 1998–2001 and further dropped to 0.69 in 2002–2006. Yet, the latest figure might be biased by the maximum time to follow up as the authors also showed an increase of mortality with increasing time, becoming significant after 10 years of transplantation. Ageing and, more importantly, return to dialysis seem to explain this effect. Taken this into account, one can conclude that the actual mortality risk has not changed since the mid 90ies.

The lack of improvement might have been due at least partly to a shift towards acceptance of more severely disabled and younger children for chronic renal replacement therapy over the last two decades. Unfortunately, data on the referral and selection of children for chronic RRT do not exist, yet from oral communication this tendency over time towards accepting sicker children for chronic RRT accounts for most Western countries. Nevertheless, it is beyond discussion that the stable mortality rate over the last 20 years is unacceptably high.

Low-income countries that do offer chronic RRT to children show fewer favorite outcomes with respect to survival compared to high income countries. A recent report from the ESPN/ERA-EDTA database showed important disparities between European low- and high-income countries. A high-income country like France had a mortality rate (9.2) of more than 3 SDs better, in contrast to relatively low-income countries like Russia (35.2), Poland (39.9), Romania (47.4), and Bulgaria (68.6) had mortality rates more than 3 SDs worse than the European average. At the same time, the number of children on RRT was significantly lower in low income compared to high

income countries. Public health expenditure was inversely associated with mortality risk (per SD increase, aHR 0.69, 95% CI 0.52–0.91) and explained 67% of the variation in renal replacement therapy mortality rates between countries. Child mortality rates showed a significant association with renal replacement therapy mortality, albeit mediated by macroeconomics (e.g., neonatal mortality reduced from 1.31 [95% CI 1.13–1.53], p = 0.0005, to 1.21 [0.97–1.51], p = 0.10). After accounting for country distributions of patient age, the variation in renal replacement therapy mortality rates between countries increased by 21% [24].

Factors Associated with Mortality

Age of onset of RRT before 6 years, a long-time burden of hypertension, onset of RRT before 1982 and most significantly, the cumulative duration of dialysis during RRT were associated with premature death in the LERIC study. The risk ratios with respect to overall mortality of a relatively long period of dialysis and relatively long-standing hypertension were 7.2 and 3.1, respectively. Patients who started RRT between 1972 and 1982 had a 1.7-fold chance of premature death compared to those who started between 1982 and 1992, and patients aged below 6 years a 2.2-fold risk compared to older patients [10].

These outcomes are in line with those of the larger ANZDATA study and the Canadian Registry. In the ANZDATA study, the hazard ratio for death among those who started RRT before 12 months of age was 3.7 as compared to those who started at 15–19 years, and of those who started RRT between 1963 and 1972 the risk ratio was 4.2 compared to 1993–2002. In the Canadian study, the hazard ratio of patients aged younger than 1 year at onset of RRT and of those aged 2–10 were 7.8 and 1.5, respectively, compared to patients aged 10–18 years [4] (Fig. 70.1).

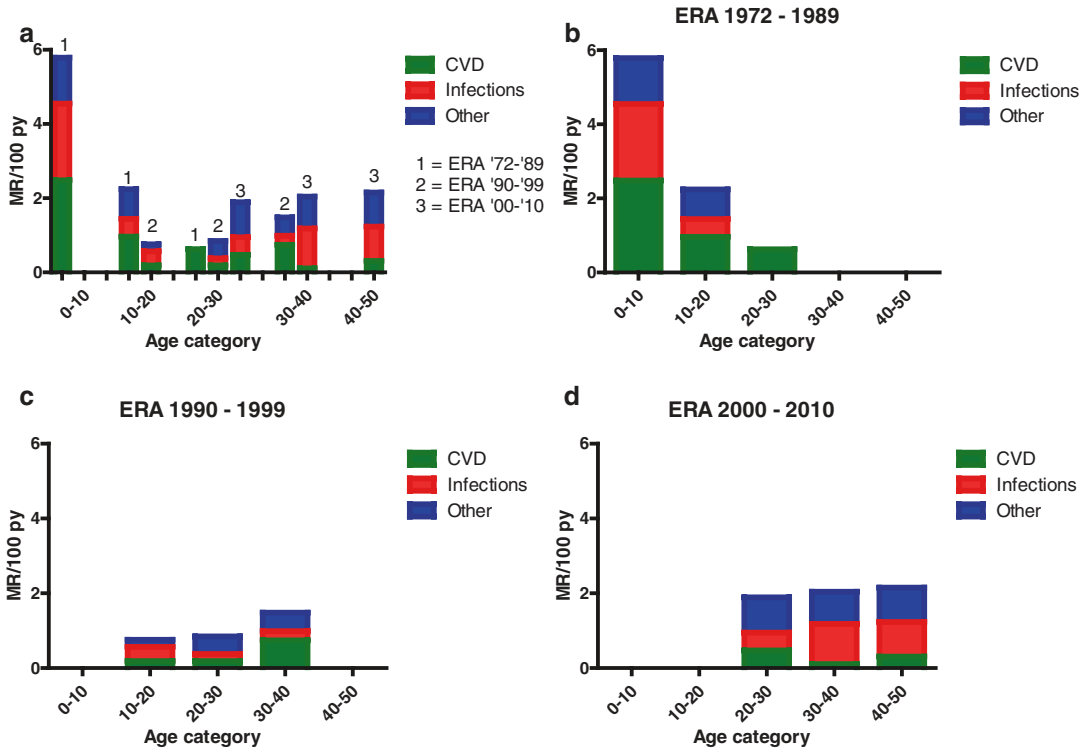

Fig. 70.1 CVD, infections and other causes related mortality rates per 100 persons in the LERIC Cohort (all Dutch patients with ESKD at age <15 years in 1972–1992 with follow-up to 2010) per age category per ERA (**a**), in the ERA 1972–1989 per age category (**b**) in the ERA 1990–1999 per age category (**c**) and in the ERA 2000–2010 per age category (**d**). (From Ref. [20]: Vogelzang JL et al, Nephrol Dial Transplant. 2013;28:2082–2089)

Rates of survival also varied with the type of renal replacement therapy. Overall mortality rates were 4.8 per 100 patient-years among patients receiving hemodialysis, 5.9 among those receiving peritoneal dialysis, and 1.1 among those with a functioning renal transplant. The Canadian Registry study showed that graft failure with return to dialysis was strongly associated with greater mortality risk in an adjusted model compared with a functioning graft (HR 7.2) [4]. According to both the ANZDATA and the ERA-EDTA registry study a short period of dialysis did not influence mortality; patients with pre-emptive transplantation had equal mortality to those with a maximum period of 2 years dialysis vintage [11, 13] (Fig. 70.2).

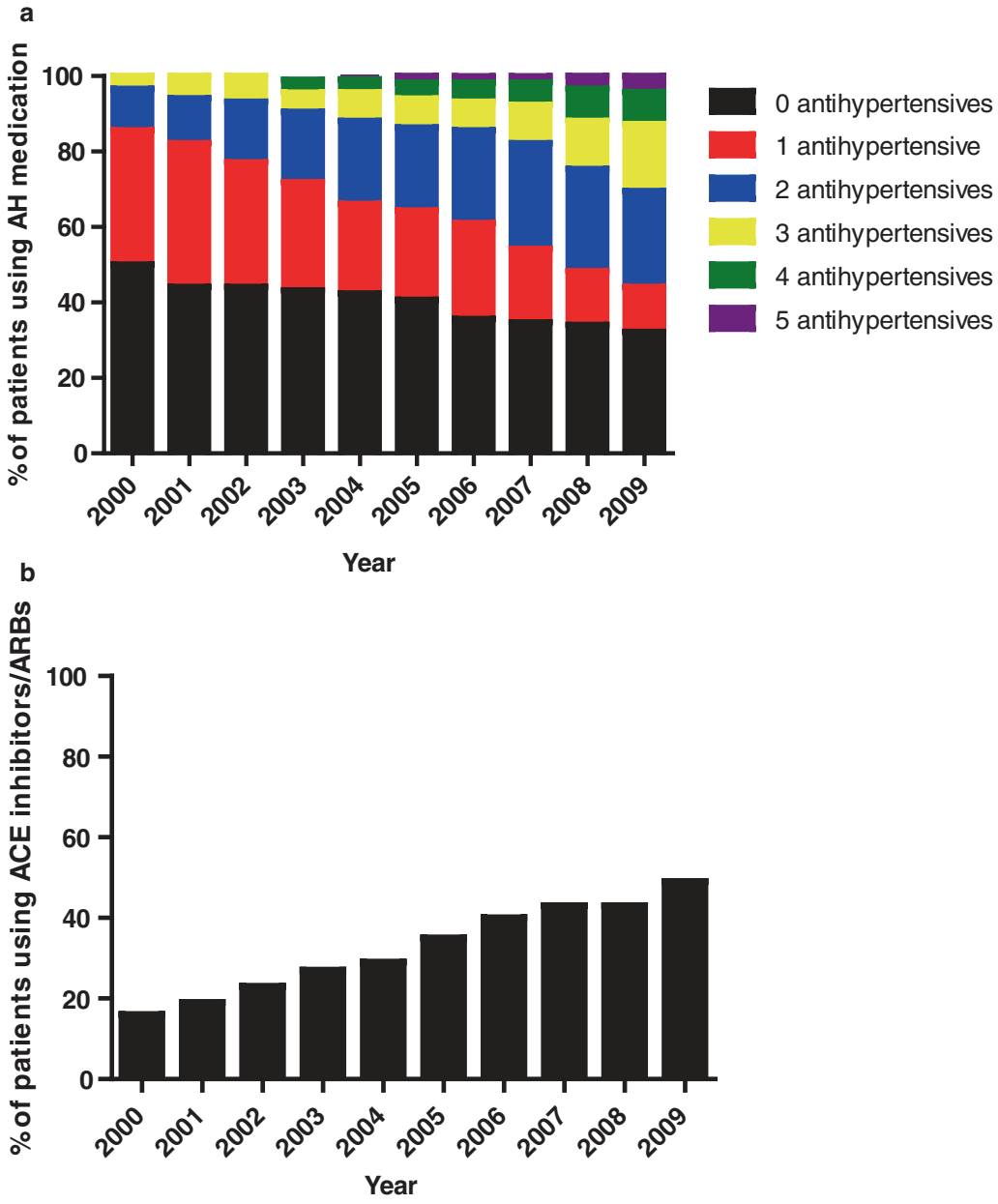

Fig. 70.2 Number of antihypertensive used per patient in each calendar years (**a**) and ACE-inhibitor (**b**) prescriptions in the LERIC Cohort (all Dutch patients with ESKD at age <15 years in 1972–1992 with follow-up to 2010) over time. (From Ref. [42]: Vogelzang JL et al, Nephrol Dial Transplant. 2013;28:2545–2552)

Causes of Death

Cardiovascular disease accounts for most casualties in pediatric RRT, followed by infection and malignancies, at least according to most outcome studies. Between 35 and 50% of all deaths are attributed to cardiovascular disease [10, 11, 20, 22, 25–27] Although one has to be cautious in interpreting "cardiac death" as a genuine cause of death, these figures reflect the excess risk of cardiovascular disease in children with ESRD. Prolonged hypertension has been shown to be independently associated with increased morality [10]. Other recent studies show that left ventricular hypertrophy (LVH) occurs early in children with ESRD and that it is strongly associated with hypertension [28–30]. Mitsnefes et al. found that 69% of all children already had LVH at the onset of dialysis therapy which persisted for 2 years after transplantation in 56% and that regression of the LVH could be induced by controlling systolic blood pressure [28–30]. These data emphasize the pivotal role of blood pressure in both dialyzed and transplanted children. Both the ESPN registry and NAPTRCS have shown that uncontrolled hypertension occurs in 40–65% of patients [28, 31].

Despite the high prevalence of hypertension in transplanted patients, the overall cardiovascular profile of conventional dialysis is by far more adverse than that of kidney transplantation. In line with this notion, late outcome studies have identified extended time spent on dialysis as the strongest predictor for premature death from cardiovascular disease. Patients on dialysis who do not receive a transplant have a four times higher risk of death than transplant recipients; in patients who have spent more time on dialysis than with a renal graft, mortality rates are seven times higher [10, 11]. A long-term outcome report on pediatric transplant data of the USRDS showed that after the first post-transplant year, each additional year with a functioning graft was associated with a 16% decrease of cardiovascular mortality [32]. The same study showed a significant 6% increase in mortality risk for each year on dialysis prior to transplantation, whereas the ANZDATA and the ERA-EDTA data only found an increase in mortality risk of patients with a pre-transplant dialysis period of more than 2 years [11, 31, 32]. Nevertheless, the beneficial effect of transplantation on survival is beyond discussion.

In adults, increasing the intensity of hemodialysis, especially nocturnal hemodialysis, has shown to be effective in reducing cardiovascular disease, improve overall condition and quality of life and prolong survival [33]. Frequent hemodialysis ideally preformed as nocturnal home hemodialysis should be considered in children who cannot be transplanted and are long-time bound to dialysis treatment.

A younger age at onset of RRT was consistently identified as a considerable mortality risk factor across all studies [10, 11, 26, 32]. However, it is encouraging to notice that the survival of very young patients has improved over time. Considering the fact that more infants have been accepted for chronic RRT during the last 20 years, it seems that we indeed have overcome a great deal of the specific technical problems concerning delivering RRT to very young children, at least in terms of survival.

Trends Over Time in Causes of Death: From Cardiovascular Disease to Infections?

Up to the late 1990s, few physicians, internists as well as pediatric nephrologists were fully aware of the extreme cardiovascular burden that threatened their patients. Growing awareness and adjustment of therapy accordingly might be the basis of a remarkable trend in change of outcome according to some very recent data. In an extension of the Dutch LERIC study, a significant shift from cardiovascular disease to infections as main cause of death was found [20]. In this study following up to 2010 all Dutch patients with RRT onset before 15 years of age between 1972 and 1992, the overall mortality rate as well as the added risk to the overall population, the Mortality Rate Ratio (MRR), stabilized over time. At the same time, the MRR for cardiovascular death decreased from 660 in 1972–1989 to 70 in 1990–1999 and further to 20 in 2000–2010. Conversely,

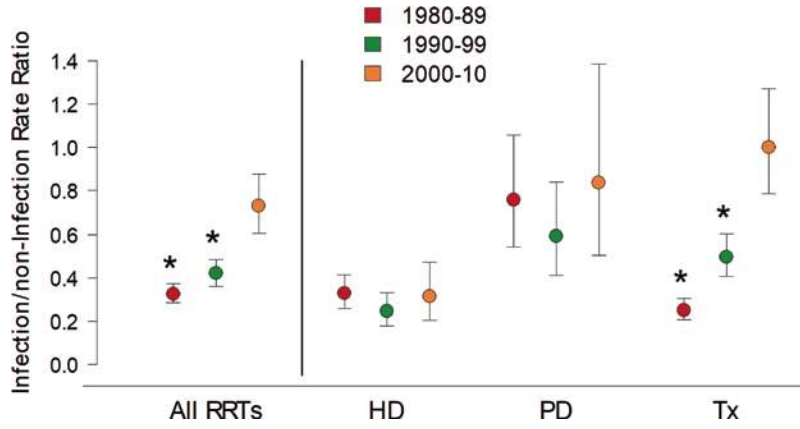

Fig. 70.3 Infection-non infection for Hospital Admission Rate Ratio according to decade and Renal Replacement Therapy modality in the LERIC Cohort. *p < 0.001 vs. decade 2000–2010. Whiskers represent 95% CI> *HD* hemodialysis, *PD* peritoneal dialysis, *Tx* transplantation (From Ref. [46]: Lofaro D et al, Pediatr Neph. 2016)

the MRR for infectious death showed a U-shaped curve; it decreased from 503 in 1972–1989 to 102 in 1990–1999 and increased again to 350 in 2000–2010. In the period 2000–2010, infections became the most prevalent cause of death (44%). In 2000–2010 cardiovascular mortality had decreased by 91% since 1972–1989 (p = 0.003) whereas infectious mortality had doubled over time, although not significantly (adjusted HR 2.12, p = 0.09) [20] (Fig. 70.3).

More recent data confirm this trend of infections gradually replacing cardiovascular disease as the most important cause of death. Recent USRDS data show a declining burden of cardiovascular mortality among the dialysis patients of all ages over the last years (MR 120 in 2001 to MR 83 in 2008 per 1000 patient years without changes in other causes of death over time (MR 100 per 1000 patient years in 1998, 2001 and 2008) [34]. This trend was similar in patients aged 20–44 years, with the cardiovascular MR declining from 40.5 per 1000 patient years in 2001 to 31.3 in 2008 [34]. Also ANZDATA showed decreasing cardiovascular mortality rates for all dialysis patients (MR 9.0 in 1992 to 6.4 in 2005 per 1000 patient years), but not among the younger patients aged 35–54 years [35].

Co-morbidity

Many studies have shown that end-stage renal disease affects many organs, especially the cardiovascular system and the loco motor system, and that it has an important impact on the overall physical condition of patients. Yet, very little data exist on the exact physical burden of pediatric ESKD later in life. In the LERIC study, 40% of survivors aged between 20 and 40 years. 75% being carriers of a functioning renal graft at time of investigation, indicated an important co-morbidity that significantly affected their activities of daily life, ranging from chronic fatigue to symptoms of cardiovascular disease or disabilities due to loco motor disorders [19].

Cardiovascular Disease

Asymptomatic cardiovascular disease, to an extent that it might induce sudden death, has been reported to be highly prevalent in transplanted as well as in dialysis patients with pediatric ESKD [10, 14, 18, 22, 30, 36, 37]. Yet, for transplanted patients, previous dialysis vintage is the strongest factor for developing cardiac and vascular abnormalities [10]. In a German single center late outcome study of 283 patients that had been transplanted between 1970 and 1997 and of whom 42 patients had died, in 50% of cardiovascular disease, CT scans were performed in 39 survivors, aged between 19 and 39 years [22]. Coronary artery calcifications were present in 92% of patients; calcium scores exceeded the 95th age- and sex-specific percentiles tenfold on average. Carotid IMT was significantly increased compared with matched control subjects. Both coronary calcium scores and IMT were associ-

ated with cumulative dialysis and ESKD time and the cumulative serum calcium-phosphate product [22]. In the Dutch LERIC study, nearly 50% of all living male and 40% of all female patients aged between 20 and 42 years were found to have moderate to severe left ventricular hypertrophy (LVH), 75% being transplanted at the time of investigation in 2000 [18]. Like LVH, cardiac valve calcification and arterial wall stiffening caused by media proliferation and secondary calcification are highly prevalent in young adults with childhood ESRD, both in dialysis and transplant recipients [14, 22]. All these abnormalities are associated with an increased risk of death. Coronary ischemia and cardiac conduction defects due to myocardial calcification are the most probable links between aortic valve calcification and mortality. Aortic valve calcification may occur early in ESKRD patients and reflects a more generalized artery disease with calcification of the coronary arteries and the myocardium [38]. Vascular calcification appears to already start at a young age. Eifinger et al. described coronary calcifications in 16-year-old dialysis patients [38]. Chronic hypertension, a high calcium phosphate product and a chronic state of inflammation are among the most important potential determinants of cardiovascular disease in ESKD [36, 39–41].

Yet, as previously discussed, increased awareness among physicians of the impact of pediatric ESKD on the cardiovascular system might profoundly change outcomes in the near future. To our surprise, we found in the extension of the LERIC study in 2010 that between 2000 and 2010, most people had died of infections and only 1 due to CVD [20]. Among the survivors, the prevalence of cardiovascular risk factors decreased from 41.3% in 2000 to 18.8% in 2010. The odds ratios in 2010 relative to 2000 for left ventricular hypertrophy, hypertension and hypercholesterolemia were 0.26, 0.22 and 0.04, respectively. The rate of non-fatal cardiovascular events dropped, although not significantly, from 1.75 in 1972–2000 to 0.95 in 2000–2010 per 100 patient years. RAS antagonists and cholesterol lowering medication were significantly more often prescribed in the period 2000–2010 (OR 7.4 and 11.5) [42]. Trends were similar among those who survived and those who did not survive the last decade. Although a causal relationship between the two cannot be inferred from this study, our data strongly suggest that strict blood pressure control, with preferential use of RAS antagonists, and reduction of dyslipidemia may be effective in reducing cardiac threat in patients with end-stage renal disease even after a long-lasting burden of cardiovascular disease (Fig. 70.4).

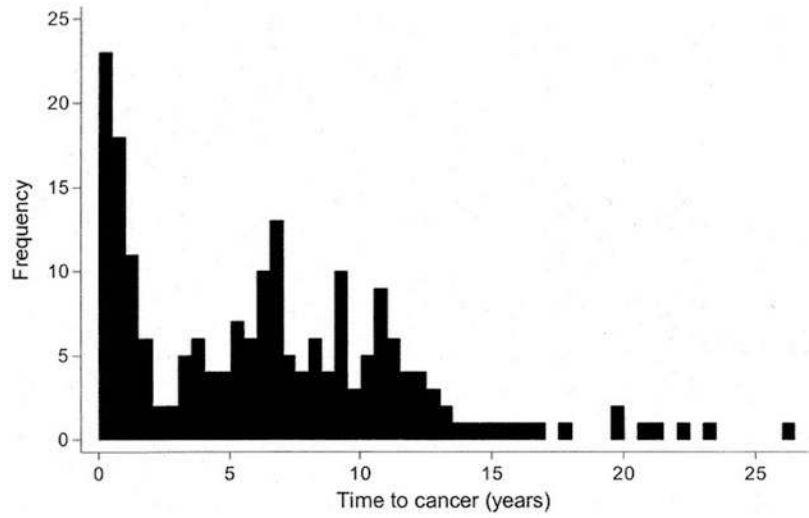

Fig. 70.4 Histogram of occurrence of PTLD against time since transplantation (Data from the Australian and New Zealand Transplant Registry. (Used with permission from Ref. [60]: Faul RJ et al, Transplantation. 2005:80:193–197)

Infections

In adults, infections are the leading cause of death in the first year after kidney transplantation [43]. In a single center study, only 7 out of 129 consecutively transplanted patients did not have an infection for which medical intervention was necessary [44]. Urinary tract infections (69.8%) and CMV infection/reactivation were most prevalent. Yet, USRDS data show that infections, especially urinary tract infections are also highly prevalent much later after transplantation. In a retrospective cohort study on 28,942 Medicare renal transplant recipients the cumulative incidence of urinary tract infections was 60% for women and 47% for men at 3 years after transplantation. Late urinary tract infection was significantly associated with an increased risk of subsequent death (adjusted hazard ratio-AHR 2.93) and graft loss (AHR 1.85). The association of urinary tract infection with death persisted after adjusting for cardiac and other infectious complications, and regardless of whether urinary tract infection was assessed as a composite of outpatient/inpatient claims, primary hospitalized infections, or solely outpatient infections [44]. This contrasts somewhat with findings in children; according to USRDS data the risk for graft loss was increased for early but not late urinary tract infection. This finding might, however, be biased by a relatively short follow-up time [45].

Data on the prevalence of severe infections over a very long time in pediatric ESKD come from the LERIC study. This study shows an increasing importance of infections over time. Between 2000 and 2010, 34 out of 186 patients died; 44% of these died of infections in contrast to only 12% of cardiovascular disease. There was a 60% reduction of the number of hospitalizations over time, from 1202 in the period 1980–1989 to 484 in 2000–2010. This was entirely due to a reduction in non-infection-related hospitalizations by 74%, whereas infection-related hospitalizations in allograft recipients did not change at all. Consequently, the hospital admission incidence rate ratio of infections and other causes increased from 0.25 to 1.0 over time in transplanted patients [46]. Urinary tract infections (UTI) became more prevalent over time in transplanted patients; the UTI/non-UTI infections hazard rate ratio in transplanted patients for which hospitalization was required increased from 0.42 in 1980–1999 to 0.72 in 2000–2010 [46].

Most of the transplanted patients in LERIC have a relatively mild immunosuppression as compared to currently used strategies. In adult studies, there is some evidence suggesting that the incidence of infection is associated with the intensity of the immunosuppressive regime. Most studies show that the dose rather than the type of immunosuppression is responsible for the increased risk of infections. The ELITE (Efficacy Limiting Toxicity Elimination)-Symphony study compared outcome of either low-dose of ciclosporin, tacrolimus or sirolimus with standard dose ciclosporin and with equal MMF dose in both groups. Opportunistic infections were more common in the standard-dose ciclosporin group [47]. Infections were also more common if patients use a standard dose of ciclosporin as compared to low-dose ciclosporin combined with everolimus [48]. The TERRA (Tacrolimus Evaluation in Renal Transplantation with Rapamycin) study compared 0.5 mg with 2.0 mg sirolimus and equal dose tacrolimus in both groups; more infections were found in the 2 mg sirolimus group [49].

In conclusion, ageing in pediatric RRT goes with a high risk for premature death by infections. Urinary tract infections are probably the most prevalent late life-threatening infections. Lowering of the immunosuppressive load, especially in patients with a prolonged rejection-free period after transplantation, might help reducing the number of fatal infections in these patients.

Malignancies

Incidence

In young adults with pediatric ESKD, the cumulative incidence of cancer varies between 0.8 and 17%. Malignancy is believed to occur about ten times more frequently than expected for age [50–54]. The most prevalent forms of malignancies seen are non-Hodgkin lymphomas and above all,

skin cancer at older age. Cancer was found in 24 cases of all 536 transplanted children in Sweden since 1970 with a mean follow-up of 12.5 (0.04–34) years [52]. In a French single center study with 20 years follow-up, 16 malignancies were found in 219 kidney transplant recipients at a median age of 20.8 (4.1–36.5) years [53]. A large German long-term follow-up study of transplanted children with a mean follow-up of 13 years showed a relatively low percentage of malignancies of 2.6% [25]. In a long-term follow-up study on 187 renal transplant recipients who had reached adulthood at time of assessment, Bartosh et al. found 12% malignancies [50].

The NAPRTCS reported recently on the prevalence among "more than 10,000" pediatric transplant recipients since 1987 and found 35 cases of malignancy, including 5 cases of renal cell carcinoma [54]. The estimated prevalence of 72.1 cases per 100,000 person-years reflects a 6.7-fold increased risk compared with the general population [54].

Yet most studies suffer from a relatively short follow-up period and the distribution over time after onset of RRT suggests that most casualties might come at a later age. In the 10 years extension of the Dutch LERIC study, 54 (21%) of the originally 249 patients had developed 105 *de novo* malignancies after an average RRT time of 23 years (age survivors 30–50 years) [55]. In this study, the Cancer-free survival after 10, 20, 30 and 35 years of follow-up was 97%, 87%, 68% and 58% respectively. The mean age at malignancy diagnosis was 36.9 (range 11.1–50.4) years. The mean interval from first transplantation to diagnosis of cancer was 23.7 (range 0.1–36.2) years. Death was attributed to the malignancy in 13 out of the 54 (24%) patients who developed cancer at time of assessment [55].

Type of Cancer

Post-Transplant Lymphoproliferative Disease (PTLD)

The term Post-transplantation lymphoproliferative disorder (PTLD) stands for different types of lymphoid neoplasms that may develop after kidney organ transplantation. The World Health Organization (WHO) distinguishes four subtypes of PTLD: an early, a polymorphic, a monomorphic and a classical Hodgkin-lymphoma-like type, each with its different therapeutic approach. The risk of PTLD is biphasic in time. The highest incidence is within the first year after transplantation. These so-called early onset PTLD types are for 95% associated with Epstein-Barr virus (EBV) and have a relatively good prognosis. A second peak occurs after 5–10 years [56]. Late onset PTLD is related to the impact of relative high burden of immunosuppressive therapy most often independent from EBV and has a worse prognosis than the early onset form [56, 57]. PTLD occurs in about 0.8–2.8% in kidney transplant recipients [58–60].

Pathophysiology of EBV Associated PTLD

EBV-related PTLD occurs because of a de novo EBV infection or reactivation from latent B-cells driven by immunosuppressive therapy. An EBV-seronegative recipient who gets an allograft from an EBV-seropositive donor (D+/R-) may undergo a primary EBV infection, a situation which is the most important risk factor for PTLD. This explains why PTLD occurs 2–8 times more often in pediatric than adult transplant recipients [56, 61]. EBV is a lymphotropic virus which enters the B-lymphocytes during the initial infection and remains in the host nucleus in a state of latency for that individual's lifetime. This latent state is associated with EB nuclear antigen production and Latent Membrane Proteins which protect the B-cell from apoptosis and stimulate ongoing viral application which is controlled by a cytotoxic T cell driven EB specific surveillance. Impairment of this cytotoxic T-cell protection is believed to be essential for developing PTLD [62]. Lack of T-cell protection leads to uncontrolled viral replication and an increase of virus-carrying B-cells in the circulation [62]. Cytokine responses, induced by immunosuppression, may lead to uncontrolled B-cell proliferation and ultimately neoplastic PTLD.

Risk Factors for PTLD

Epstein-Barr Virus (EBV)

EBV seronegativity and the use of potent immunosuppressive therapy are the two most important PTLD risk factors. In children, over 70% of PTLD is EBV-associated. The risk of being EBV naïve has been established in various studies. Sero-negativity of EBV is associated with a 4–7 times overall increased risk and a 20 times increased risk with in the first years after transplantation [63–65].

CMV/Hepatitis C

Reports on the role of CMV and hepatitis C are conflicting. Studies from the 1990s have revealed a four-to-six-fold excess risk of PTLD of CMV sero-mismatch (i.e., a negative recipient and a positive donor) after non-renal transplantation [66]. A *de novo* CMV infection was associated with polyclonal B-cell proliferation. The same role has been suggested for hepatitis C [67], but neither the CMV nor the hepatitis C link could be confirmed by large registry reports from the USRDS and ANZDATA [60, 68].

Immunosuppressive Therapy

All longitudinal evaluations of the incidence of PTLD show a trend over time toward increased incidence and earlier onset of PTLD up to 2000–2005. The exact role of immunosuppressive drugs in this trend remains to be elucidated. The concurrent increase in potency of the immunosuppressive therapy and incidence of PTLD is obvious, but links between the use of specific drugs and PTLD are unclear. It is believed that MMF is of less influence than calcineurin inhibitors but that statement is not supported by hard evidence and even contradicted by some case reports [69]. The previously supposed protective effect of mTOR is also contradicted by recent data [70].

Other

Apart from the increased overall risk of children, UNOS data have shown that young male Caucasians were at greatest risk for PTLD [65] A complete HLA-DR mismatch associates with a twofold increased risk, probably as a result of the greater immunosuppressive requirements [71].

Monitoring and early detection. Elevated EBV levels in mononuclear cells or in plasma assessed by PCR is the key to early detection of PTLD. The most important monitor is serial EBV quantitative PCR assessment as a rising level is more predictive than a single positive EBV quantitative PCR. EBV+ PTLD most often occurs in the first 1–2 years post-transplant, so monitoring should be intensified during this period [72]. Unfortunately, there is no standardized assay for EBV quantitation. Thresholds for elevation of the EBV load therefore differ per individual lab [72]. A negative EBV PCR does not exclude PTLD. Unexplained anemia, leukopenia or thrombocytopenia and high uric acid and LDH levels are non-specific abnormalities that are associated with PTLD or another malignant blood or lymph disease. Patients should be checked for tonsil abnormalities; if they do occur, a biopsy is indicated. Radiological monitoring includes MRI, CT and positive positron emission tomography (PET) scanning to evaluate the spread of PTLD. In case of CNS involvement, a lumbar puncture with cerebral spinal fluid (CSF) analysis (EBV PCR in CSF fluid) is indicated. Histopathologic examination of the tumor can only determine the definitive diagnosis.

A new development in monitoring is the detection of a certain viral load as proxy for decrease of immuno-competence [73]. Data of a study in 96 heart and lung transplant recipients suggest that a high anellovirus load may be indicative of over-immunosuppression. In line with this, metagenomic shotgun sequencing of tissues from PTLD patients showed that more than 50% of the specimens contained anellovirus sequences, and the anellovirus levels, but not EBV levels, were associated with death within 5 years in a univariate analysis [74]. This presumes that virome changes, and even specific viruses such as anellovirus, may provide indirect measures of the immune status which can promote the development of EBV+ PTLD.

Therapy/prevention of PTLD. Reduction of immunosuppressive therapy in case of an increase in EBV load in EBV-seronegative recipients and

incase of EBV disease in all recipients is the first step in preventing PTLD (KDIGO level 2D and 1C) [75]. Rituximab is the cornerstone of 1e first line therapy of PTLD. In a multicenter trial, 152 transplant recipients with CD20 + PTLD that was unresponsive to immunosuppression reduction, were initially treated with 4 doses of weekly rituximab. In case of complete remission, rituximab treatment was continued with another 4 gifts; other patients received 4 R-CHOP courses. The overall response and CR rates were 88% and 70%, respectively; the most significant prognostic factor respect to overall survival and time to progression was the response to the first 4 courses of rituximab with [76].

Skin Tumors

Skin cancer is the most frequent malignancy after transplantation, also after pediatric transplantation, but hardly occurs in childhood. Most tumors only become manifest 10–15 years after transplantation. According to IPTTRS data, 16 out of 101 skin cancers found in pediatric solid-organ transplant recipients developed during childhood [77], 10 of them being squamous cell carcinomas (predominantly of the lower lip) and 6 melanomas. Death occurred in 8 patients, five from squamous cell carcinoma and three from melanoma [77].

The mean age at onset of skin cancer has been reported to be about 27 years [51, 54, 77–79]. However, recent data of the LERIC study shows that most tumors occur at later age. In the Dutch study, the risk for non-melanoma skin cancer (NMSC) was over 200 times higher than in the age-related population. Of all 249 patients, 63 had died in 2000 and 97 in 2010. At that time, 39 patients had developed 82 non-melanoma skin cancers (78% of all tumors). The mean age at developing a NMSC was 39 (range 22–50) years [55].

UV exposure and human papilloma virus (HPV) in combination with immunosuppression are thought to be the most important causative factors for the development of post-transplant skin tumors. UV radiation is probably one of the most important factors [78] This explains the extremely high incidence in Australia, a country with a majority of genetically ill-protected Caucasian people and a very high sun exposure, with a 93% proportion of skin cancer among all post-transplant malignancies [80]. Sun protection is therefore of utmost importance, in combination with Vitamin D supplementation to avoid Vitamin D deficiency.

Skin cancer may appear in the absence of any pre-existing skin lesion, but is often preceded by actinic keratosis, which suggests the involvement of HPV in its pathogenesis. Additional evidence for the role of HPV comes from a study on post-transplant squamous cell carcinomas (SCC) that occurred in a cohort of 500 allograft recipients; HPV DNA could be detected in nearly 50% of all SCC [81, 82].

Calcineurin inhibitors have been regarded as most important immunosuppressive drugs that may increase the risk of skin cancer [83]. CNIs may express their oncogenic effect by interference with the p53 pathway and nucleotide excision repair and by promotion of malignant cell differentiation [84, 85]. In contrast, mTOR inhibitors have anti-tumor properties. Indeed, several studies have showed that diversion to mTOR in transplanted patients with NMSC could prevent the occurrence of new tumors [86, 87]. A recent study showed that the combination of low dose CNI and sirolimus could also be effective as prevention against new tumors [88].

Squamous cell carcinoma and basal cell carcinoma (non-melanoma skin cancers, NMSC). Squamous cell carcinoma (SCC) accounts for more than 50% of all skin cancers after transplantation. The ratio of basal cell carcinoma (BCC) to SCC is reversed in transplant patients compared with the general population in which basal cell carcinoma is the most common one. SCC was 81 times more prevalent in a cohort of Danish renal allograft recipients than in the general population [82]. The predominance of SCC over basal cell carcinoma (BCC) is more pronounced in pediatric than in adult transplant recipients (SCC: BCC 2.8:1 vs. 1.7:1) [77]. SCC is for the most part found in parts of the body exposed to daylight [80]. In pediatric transplant recipients, lip cancers account for 23% of all skin cancers [77].

Skin cancers may develop rapidly, and recurrences are common. In the LERIC study NMSC was most common to recur and/or metastasize of all malignancies. Including metastases, 114 NMSC (78 SCC and 36 BCC) occurred in 39 patients at a mean age of 39 (21.8–50.4) years [55]. The Incidence Rate and Incidence Rate Ratio's for SCC were 1.5 and 744, respectively. The IRR was 992 in the 25–30 and 2610 in the 45–50 year age group. Two patients died of SCC; far more patients had important morbidity because of SCC metastases.

Melanoma

Melanoma seems to be more prevalent in pediatric than in adult transplant recipients (12 vs. 5%) [77]. The Dutch cohort study counted 5 melanomas on 105 malignancies, occurring at a mean age of 29 years [55]. Data of the IPTTRS and the Dutch cohort study suggest an earlier onset of melanoma compared to NMSC. The IPTTRS noted 25% of deaths were due to melanoma [55, 77].

Melanocytic nevi, a risk factor for melanoma, may develop in excess after transplantation [89]. In transplantation, melanoma can be transmitted by the donor. Any person with a history of melanoma should be excluded from donation [90]. It has been speculated that growth hormone use might increase the growth rate of melanocytic nevi, but to date no association between GH therapy and the occurrence of melanomas has been found [91]. Sun protection and removal of suspect nevi are the most important protective measures.

Kaposi's Sarcoma

Kaposi's sarcoma is caused by HHV8 virus infection. The Norwegian study found 3 times more Kaposi Sarcoma's in kidney transplant recipients than in the general population [92]. The IPTTRS reported 2% Kaposi sarcoma's, nearly all of which occurred during childhood [78]. Only one child presented with skin cancer. All had various visceral localizations. The age of occurrence ranged from 5 to 17 years, the onset was typically within a few months after transplantation. Six of the eight reported patients had a fatal outcome.

Other Tumors

There are very few data on other solid tumors after pediatric renal transplant recipients. The NAPRTCS transplant registry found a rate of non-lymphoproliferative solid tumors of 72.1 per 100,000 person-years which implies a 6.7-fold increased risk compared with the general pediatric population (10.7 cases per 100,000 person-years). Non-LPD malignancy was diagnosed in 35 subjects at a median of 726 days post-transplant. The most common type of malignancy was renal cell carcinoma. No specific type of immunosuppression was identified as a risk factor [54]. In the LERIC study, 9 solid non-skin/non lymphoproliferative tumors were found, consistent with 158 per 100,000 patient years, at a mean age of 41.7 years [55]. All 9 tumors were from different origin.

Other Somatic Co-morbidities

Bone disease and motor disabilities. Whereas cardiovascular disease and infections have proven to be the most life-threatening co-morbidities, chronic fatigue in dialysis patients and motor disabilities as a result of metabolic bone disease are the most frequently reported daily problems of young adults with childhood ESRD [16, 19, 50]. As expected, in a cohort of patients that has grown up in the pre-growth hormone era, more than two-thirds of the LERIC patients were severely growth retarded [16]. A more surprising and more worrisome finding was the extent and severity of clinically manifest metabolic bone disease that we found in the LERIC patients. More than one-third had daily complaints or disabilities related to metabolic bone disease. About 18% were disabled as a result of bone disease [16]. Very few data exist on this evidently underexposed problem. Although conclusive evidence is lacking, most of these problems seem to be related to chronic inactivity, inadequately managed CKD-MBD, a high burden of corticosteroids and an increased total duration of renal

replacement therapy [93]. Bone mineral densities (BMD) are lower than −2.5 SDS in over 50% of adult patients with childhood ESRD [16]. However, in a 10-year extension of the LERIC study, no association of low BMD with clinical bone disease, such as fractures, daily pain or motor disabilities, could be found (unpublished data).

Itching is a frequently mentioned complaint of both dialysis and transplanted patients [94].

Psychosocial Consequences

Cognitive Functioning

Neurocognitive dysfunction is a well-recognized complication of pediatric CKD. Many studies have found evidence of several neuropsychological deficits, including IQ, academic achievement, memory, and executive functioning [95–99]. This deficit is reflected by cognitive dysfunction at adult age. Cognitive and learning impairment is also more prevalent in middle-aged adult patients with childhood ESKD than in the age-matched population [15, 100]. In the LERIC study mean IQ scores of adults aged 20–40 years with childhood ESKD were on average ten points lower than in the aged matched Dutch population, which is in line with the results of IQ studies performed in children [15, 101, 102]. Impaired schooling and cognition appear to be induced by a long period of dialysis during youth. The LERIC study found no difference in intellectual performance between patients who were on dialysis and those who were transplanted by the time of investigation. In theory, chronic aluminum intoxication as a result of chronic use of aluminum-containing phosphate binders could have influenced the cognitive development of our patients. Yet, no evidence was found for this [15]. On the contrary, the compatible results of recent IQ studies in ESRD children indicate that abandoning aluminum-containing phosphate binders has not shown beneficial effects on intellectual development [15, 103]. Most deficits are found in tasks requiring concentration, memory and most of all general knowledge. Consequently, early educational intervention in young patients on dialysis might prevent most of these impairments. In spite of improvements in identification and treatment, CKD causes both direct and indirect insults to a variety of organ systems. In contrast with the Dutch study, the educational attainment of pediatric transplanted patients with a median age of 25.7 years in a Swedish study was in line with the general population. The problem of this study is that 40% of the cohort did not participate and no information about the characteristics of these non-participants was provided [104].

Previous studies of children with CKD suggest that toddlers and children with CKD are at increased risk for delays in neuro-cognitive development [105]. In a large North American study (CKiD) on 386 children with CKD 2–4 (mean GFR 41), the overall neuro-cognitive functioning was within the average range for the entire group, but 21% to 40% of participants scored at least one SD below the mean on measures of intelligence quotient (IQ), academic achievement, attention regulation, or executive functioning. A higher GFR was associated with lesser risk for poor performance on measures of executive function. Significant proteinuria was associated with lower verbal IQ, full-scale IQ, and attention scores [106].

In an accompanying analysis, CKiD cohort participants with hypertension scored lower on visual-spatial organization, planning, constructive abilities, and nonverbal reasoning [107]. Results from these studies highlight the importance of recognizing neurocognitive dysfunction in children with CKD early on because of the significant impact it may have on school performance and the opportunity it presents for prompt intervention [108].

Quality of Life

Transplant Patients

According to most studies, adult patients with a functioning renal graft achieve normal scores on self-assessment of mental and physical health for most domains. In the LERIC study, data on quality

of life were assessed in 2000 in 131 of 186 surviving patients aged 20–40 years with mean onset of RRT at age 11 years. In transplanted patients, only scores on social functioning and general health perception were slightly lower than in the age related general Dutch population. All other scores were within the normal range [17]. Equally good outcomes were found in an Italian follow up study on transplanted patients at aged 18–34 years who were transplanted a median age of 15 years [109]. In contrast, a study from Japan noted lower mean scores, especially of those related to the mental quality of life, in kidney allograft recipients of the same age [110]. Remarkably, in this study, nearly all scores of hemodialysis patients awaiting transplantation appeared to be similar to those of transplanted patients. In contrast, dialysis patients not awaiting transplantation (i.e. not on a transplant waiting list for medical or personal reason) had much lower scores [110]. Most available studies emphasize the improvement of the quality of life after renal transplantation [111–113]. However, for some young adults, transplantation not always had the wellness and health that they hoped it would have, as was shown in a more recent review on adult perspectives of living with kidney failure. Young adults with pediatric ESRD most often have a driving desire to *be* "normal," whereas those diagnosed later in early adulthood often feel an "unbearable loss" and hope *to return* to their previous state of "normal" [114].

Dialysis Patients

Not unexpectedly, in the LERIC study patients aged 20–40 years with pediatric onset of ESRD who were on dialysis at time of assessment indicated more often an impaired quality of life than the general population in all physical domains: activities that require good physical condition (Physical Functioning), social activities that require a good physical condition = Role Limitations due to physical health (Role Physical), social functioning (SF), general health perception (GH) and the so-called physical component summary (PCS). Yet the same patients reported an impaired quality of life for the mental domains equally or even less often than aged-matched Dutch citizens [17].

These results sharply contrast with data derived from dialysis patients with onset of ESRD in adulthood [115–117]. Patients with adult onset of disease appear to have a substantially poorer quality of life, particularly in Physical domains and General Health perceptions, but also in domains related to the mental quality of life.

In the LERIC study, outcomes of patients on dialysis were compared with age-matched dialysis patients with adult-onset ESRD from the NECOSAD-2 study. The latter concerned Dutch patients who were only on RRT since 1 year. In all domains except one, scores of NECOSAD patients were significantly lower than those found in the general population, whereas the LERIC dialysis patients had normal mental scores [17].

The high scores on mental health in LERIC are on the other hand consistent with findings in other studies of adolescents and adults with chronic illness since childhood, including sickle cell patients, cystic fibrosis and asthma [118–120]. Different expectations of life and different coping strategies by children and adults may explain the difference in mental status of patients with pediatric and those with adult onset of disease. Carr et al. have reasoned that health-related quality of life is to a large extent based on the difference between health expectations and health experiences [121].

Effect of Age at Assessment

Quality of Life (QoL) scores seem to be age dependent to some extent. At both late adolescent and very old age, patients score on average lower than at middle age [111, 122–124]. Using another scoring system, a Finnish group assessed QoL at young adult age years in 21 patients transplanted at very young age (mean 2.4 years) and found significantly lower scores than in age-matched controls, contrary to the Dutch and Italian late outcome studies. Significantly lower scores were found regarding mobility, usual activities, mental functioning and vitality [123]. There was a clear tracking effect, as scores per patient were comparable to those measured 10 years before. These outcomes mimic more the QoL scores found in children with ESRD [125].

Trend Over Time

Most favorable outcomes in transplanted patients are reported after at most 10–20 years of follow up. The QoL assessment in the Dutch LERIC study was repeated in 2010 after a mean duration of about 30 years of RRT in surviving patients aged by then 30–50 years. The scores in physical domains were significantly lower in 2010 than in 2000. These concern limitations of daily activities and social participation by physical impairment or pain ('Physical Functioning,' 'Role Physical' and 'Bodily Pain') as well as General Health perception. The decrease of physical QoL over time also accounted for transplanted patients [126]. The deterioration of physical QoL is partly explained by a normal effect of ageing in line with the trend in the general population as older age is an important negative predictor of perceived physical health status [124].Nevertheless, the ongoing decay superposed on lower scores on some domains could become a significant problem for these patients in the near future, adversely influencing their social functioning. In the LERIC study, one transplanted woman aged 32 years illustrated this by questionnaire comments: "Much more often than 10 years ago, even when I was on dialysis, I feel very tired nowadays, reason why I recently have decided to stop working for a while."

According to the same study, all domains relating to psychosocial functioning had remained stable over the last 10 years.

Co-morbidity was associated with an increased risk of impaired QoL in the domains 'Physical Functioning,' 'Role Physical' and 'Bodily Pain.' Having disabilities was associated with an increased risk of impaired QoL regarding Physical Functioning, Vitality and Bodily Pain. Notably, the current RRT modality at time of investigation was not correlated with impaired QoL in any domain. However in case lifetime on dialysis exceeded lifetime on renal transplant, there was an increased risk of impaired participation in social activities by physical strain.

Regarding the socio-demographic variables, being employed appeared to be associated with a lower risk of impaired QoL in the domains of Physical Functioning, Vitality and General Health perception. Having offspring was associated with a lower risk of impaired QoL regarding Social Functioning and an income equal to or above the national average of €2500 (about $ 3200) gross per month was associated with a lower risk of RP [126].

Although in line with positive outcomes on mental health perception for other chronic diseases of childhood with shorter follow-up time, the universally high scores on mental health after 30 years of RRT with a concurrent decline in physical health and subsequently physical QoL are striking. Nearly 80% of patients stated that their disease had brought them something positive in life. The perceived benefits of having ESRD included more satisfaction with (small things in) life, having developed a sense of perseverance and positive responses from friends and relatives [126].

Social Outcome

Employment

A review from a social work perspective of data on late social consequences of pediatric kidney transplantation showed a significant impact on many aspects of social development including education, peer/intimate relationships, employment and overall well-being. Young adults, kidney transplanted at childhood, are more likely to live with their parents and less likely to have a partner. Social isolation, fear to disclose their disease status to peers and difficulties to establish intimate relationships are more prevalent than in the general population [127].

Social outcome was assessed in the LERIC study in 2000 and in 2010. In 2000, 67.4% was employed, about 85% for more than 50% time equivalent [128]. Involuntary unemployment occurred in 19.1% vs. 6.4% in the Dutch population. Most patients (53%) had low skilled and only 10% had high skilled professions, a situation significantly different from the average Dutch population [128]. In 2010, 61.8% were employed of whom 81.8% had at least 50% full time equivalent paid work. However, different from the situation in 2000, there was a very significant difference

between patients on dialysis of whom only 31.3% were still employed, and transplanted patients [126]. Apart from dialysis as RRT modality, having motor disabilities was the most important risk factor for becoming unemployed. Patients also mentioned increasing chronic fatigue as an important reason for becoming unemployed. Some patients, however, reported that their employment contract was not renewed as a direct result of the disclosure of their dialysis patient status to the employer. Unemployment was related to patients' low subjective health perception, an apparent failure to adjust to their disease, rather than to their objective physical condition, or to whether they were transplanted or on dialysis [126]. Among 42 transplanted patients aged 20–38 years in a Swedish long-term follow up study 54% were part-time or full time employed, 14% were unemployed (compared to 5.3% in the general population, $p = 0.059$) and 21% received education [104].

A positive change was a significant trend towards more highly educated occupations. Also the educational level had on average increased over time. Among the patients, 22.1% had completed a high vocational training or scientific degree, compared to 31.2% in the general Dutch population ($P > 0.05$) [126] 34.8% of the patients had an income equal to or above the national modal income of €2500 (about US$ 3200) gross per month, a significantly smaller proportion compared to the general population (61.1%) [126].

In a very recent French outcome study of 624 patients transplanted in childhood, fewer patients than expected had a high-level degree (Q3-year university level: 14.8 vs. 30.2% general population) and fewer women had a baccalaureate degree (49.2 vs. 76.5%), but these differences were less marked than in the Dutch study [129, 130]. Mean incomes were much lower than in the French population [130]. While the distribution of professional occupations was representative of the French society, more patients were unemployed (18.5% vs. 10.4%; $p < 0.01$). Independent factors for poor social outcome with respect to professional career were ESRD onset in infancy, the presence of co-morbidity and disabilities, a low educational level of the parents or patient, female gender and being (again) on dialysis at time of assessment [130]. Interestingly, patients less often had a permanent contract than the average French employee (66.8 vs. 81.8%) [130]. This might reflect the observed reluctance of employers against a long-term professional commitment with renal patients, as also observed in the LERIC study. The relatively good overall outcome of the French study might be slightly biased by the fact that more non-responders than responders had graft failure at time of investigation and that the cumulative duration on dialysis was also higher in the non-responder group [130].

Interestingly, nearly 50% of the French transplanted patients indicated to have suffered from discrimination, either at school (60.8%), from employers (27.8%) or work colleagues (19.9%) and even from friends (19.3%) [130]. In the Dutch cohort, 35.2% of patients lost their job between 2000 and 2010, in 32.3% because they were fired—sometimes as part of a 'reorganization' and in 45.2% for medical reasons. In 12.1%, employers indicated that the disease state of the patient influenced their achievements. About 21% of patients felt that their disease had a significant negative influence on their professional achievements and career [126].

Partnership and Independency

In the first evaluation of the LERIC study in 2000 patients showed to have significant difficulties in finding a partner. Of all 144 patients, 31.9% lived alone, 34% lived with a partner, and 49 (34%) still lived with their parents. The odds ratio of living with parents, as a measure of dependency, vs. living alone or with a partner was 3.3 (95% CI, 2.3–4.7) for LERIC patients compared with age-matched Dutch inhabitants [128]). The odds ratio of living with a partner was 0.3 (95% CI, 0.2–0.4) for LERIC patients compared with age-matched Dutch controls. These figures are in line with those of the French follow-up study of pediatric transplanted patients of the same age as the LERIC study at time of assessment (31.1% partnership, 35.7% living with parents) [130]. In 2010, the situation in the LERIC cohort was completely changed: 67.4% was married or lived with a partner and 28 (31.5%) had offspring compared to respectively 74.4% ($P > 0.05$) and 64.8%

($P < 0.05$) in the general population [126]. This delay in starting a relationship could reflect a genuine delay in sexual maturity or a late 'social maturity' as has been described in patients with a chronic illness [131]. Patients with disabilities and patients from Southern more than from Northern European countries tend to remain living with their parents [25, 101, 126, 129, 130]. In an older study, patients reported on average successful partnerships after pediatric kidney transplantation, but fewer than in the general population had children and 40% reported not to be sexually active [132]. Men appear to have problems finding a life partner more often than women [129, 132].

Conclusions

Although the prospects of end-stage kidney disease in children have improved over the last 30 years, the risk for premature death remains extremely high and adult life comes with considerable physical troubles. Cardiovascular disease has been recognized for more than a decade as most important threat in pediatric ESKD. Recent data indicate that adjusted therapeutic approaches and changes in lifestyle may be very effective in reducing the risk for premature cardiac death in this population. On the other hand, physicians should be aware for a potential increase in life-threatening infections and malignancies as a result of more potent immunosuppressive therapy after transplantation. Co-morbidities, most importantly motor disabilities have an important impact on social life in adulthood, especially with respect to finding a job and a life partner. Despite all physical discomfort, the mental health perception of adult patients with pediatric ESKD is remarkably good. Most patients are highly motivated to fully participate in society and to join the work force, well-adjusted to their situation.

In appreciation of the insights gained from long-term outcome research in patients with childhood-onset ESKD, the following should be the principles of modern pediatric renal replacement therapy in order to optimize late outcomes in these patients: Reduction of RRT time on dialysis to the absolute minimum; propagation of pre-emptive and living-related transplantation; personalization of immunosuppressive therapy; aggressive prevention of CKD bone-mineral disease in order to avoid later motor disabilities; timely start of intensified dialysis regimes (preferably frequent nocturnal home hemodialysis) in patients who are not eligible for transplantation; and finally, active and early stimulation of development towards independency.

A Patient's Story

"I am 41 years old, live with my boyfriend in this beautiful apartment. Currently I am in between 2 jobs, partly because, over the last few years, I often feel quite exhausted, despite the fact that I have a well-functioning renal graft at this moment.

I used to work as a radio reporter at the local radio of Utrecht and as a stand-up comedian. The radio work was very exciting, making documentaries and interviews that was actually my dream as a young girl. My father was photographer. As a Moluccan son of a military servant of the Dutch Indian (former "Dutch India"— currently Indonesia), he moved to Holland in the fifties where he met my mother. She worked as a nursery-class teacher and has Dutch as well as Russian-Jewish blood. Combativeness and urge for moving both run in the family.

I was 3 years old when I turned ill. I remember having a sore throat and getting medicines that didn't work. I felled extremely tired, but our house doctor was not impressed, everybody feels occasionally tired. It took months before they realized that something was wrong. Then, I was hospitalized for 3 months, where I had to stay in bed and live on an awful diet. Later, they transferred me to the Sophia Children's (university) Hospital of Rotterdam

where the doctors told me that my kidneys were very sick and would slowly get ruined. Gradually the troubles came. I blew up like a balloon for which I felt awfully ashamed. At school I was abused, children called me a stupid Chinese. Between my third and 11th year, I was more often in the hospital than at home. At a certain time, my mother quit her job to join me at the hospital as she always did, unlike my father.

I do not know why they have waited so long with starting dialysis, because in a way it came as a relief. I had to dialyze 3 times per week for 4–5 h per time. Fortunately, my doctors were wonderful. They gave me much attention and explained everything to me. After 6 months, I got my first transplantation. That kidney lasted 10 years. Once in a while I had to go to the hospital for treatment of a rejection. At age 22, when I had entered university to study Dutch language, I was back on dialysis. I had never noticed that my kidney function was deteriorating, which is a frightening experience. Even today I am still nervous about my creatinine when I have am at the out clinic.

Then I got problems with my shunt. I had to turn to peritoneal dialysis. Not a big deal, but very tiresome to combine with my study. Luckily, I was rid of my dialysis hang-over. I have never understood how people could drive home themselves after a hemo session. I was always exhausted, even though I nicely kept my diet and fluid restriction. I have always experienced the day of dialysis as a totally wasted day. Actually, you only lived 3 days per week. With PD, things were different; I was freer, changed bags on the train, airplane, airfield, whatever. At first, I felled embarrassed to do so, but very rapidly it became quite normal.

The hospital feels like my second home; it is all very familiar, the smell of the ward, the nurses, the smell of antiseptics, the idea that you can drop down and people will help you immediately. I always thought that if I would get a child, I would call her Sophia, after the name of the hospital. I never regretted to go to the hospital. There were always people that I knew very well and who always paid attention to you. That is a very particular thing. Up to your 18th, people always listen to you and treat you as a person and suddenly at the internal department you become a number and you have to do everything by yourself. Behave as a mature person, but not too clever or candid!

Later I have learned to speak freely about my disease but when I entered university, nobody knew about my illness, even not when I was back on dialysis. I dialyzed in the evening, so I always had to find an excuse for not joining my friends at the pub or the movie. At first this was not a big problem. Many people had a job in the evening, but then friends started asking questions about my scars, so I had to disclose my disease, which actually turned out to be a big relief.

During my school time at home, I always felt extremely tired after a hemo session—there was no EPO at that time -, yet my mother always forced me first to clean my room or do a little job, before she allowed me to lie down. Later I have learned to appreciate her behavior. Many parents treat their sick children as princesses, which I think is disastrous. Most of the times, you are spared anyway. I always got attention from everybody. People were always worried about me, always asked about me, even the most distant acquaintances of my parents. That was a big frustration for my sister. So, even although my parents try to avoid it, I got pampered by my environment. The first time, that I realized this was at work, where nobody new about my situation. I could be heavily upset when people did not understand why I was not willing to work over-time or demanded a day off. I always thought, ‘man, if you would know my situation ...’ Later, I sometimes used by illness when I thought that people were whining.

When I started working at the radio, I told nobody at work about my situation. I just wanted to work for a maximum of 20 h a week and I thought it was of no once business to know why. Unfortunately, I got problems with my knees which was due to bone necrosis caused by long time prednisone use. I had to be operated and to disclose my disease status. My direct colleagues were all shocked and people started to behave differently. You could feel that they thought, that lady will be out for a long time, so we probably have to look for someone else. And with all those medicines, this will of course happen more often. These things were never openly discussed, but suddenly things were taken out of my hands and my contract was not extended. I received a letter in which I was thanked for everything, they wished me good luck and appreciated how I had handled my disease. But I learned my lesson. Never disclose your disease if not absolutely necessary to your employer!

During my time at the university, I first realized how awful it is not to be able to comply with the activities of your friends. They really could feel offended when I turned down their invitations to go out at evening. As in my world, everybody knows that there are good and bad days and that if you feel good, you want to enjoy your day which always means that you have to pay for it the next day.

I think that I am less ambitious than my healthy peers, that I am more realistic and easier pleased with small things. I have no driving license, I am not married, I rent my house, have no children. I have probably consciously avoided all these obligations, yet the thought of never having children is most depressing. Currently my graft function is too bad, my doctor advised against it. I do not see it as a problem to have a child with my disease, even when I would have to start dialysis again.

Getting mad is my biggest fear. When I was 29 years old, I got psychotic. I had visions how my parents must had felt when I got sick and religious delusions. I thought that I had special medical gifts and I was convinced to be pregnant of a twin. I was isolated for 3 weeks in a psychiatric hospital, because I was intruding other patients with my special medical gifts. When I recovered I realized that it was all due to a strange chemical reaction inside my head and not due to some unprocessed emotions. I always thought that to be bull shit. In the seventies, I had to encounter a pediatric psychologist and had to talk about my disease, how I dealt with it. She noticed from my drawings that I drew too many clouds, clearly a sign that I could not express my anger. Me, not able to show anger, that was totally absurd! She showed me that I was observed from a one-way screen by at least 10 people. One of the most terrible things you could do to a child! Every time, I was at the hospital afterwards, I checked the room for one-way screens. Anyway, the point was that I had no anger, I just liked the doctors. I was never sad when I had to go to the hospital. I always stood immediately ready with my suitcase. I was eager to help the doctors and was never afraid of the blood punctures. I always stuck out my arm and told the doctor what I thought would be the best vein to attack with the needle. To some extent, it was nice to return, as it was part of my family, I knew all the nurses and doctors and there were funny things to do on the ward. The worst thing about it was the sadness of my parents.

The psychosis has changed my attitude. I previously thought that I could rationally solve all my problems, now I have learned to be more honest. It has taught me that nothing is certain in life. And then suddenly, I was asked my former employer at the radio if I would be interested to come back. The work at the radio really helped me to overcome my psychosis."

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Part XIV

Drugs and Toxins

Drug Use, Dosing, and Toxicity in Kidney Disease

71

Matthias Schwab, Simon U. Jaeger, and Guido Filler

Abbreviations

ABC	ATP binding cassette
AIN	Acute interstitial nephritis
ATN	Acute tubular necrosis
BSA	Body surface area
CL	Clearance
GFR	Glomerular filtration rate
LSS	Limited sampling strategy
MATE1	Multidrug and toxin extrusion protein 1
MDR	Multidrug resistance
MRP	Multidrug resistance-associated proteins
OAT1	Organic anion transporter 1
OAT3	Organic anion transporter 3
OATs	Organic anion transporters and organic cation transporters
OCTs	Organic cation transporters
PAH	Paraminohippuric
SCr	Serum creatinine
SLC	Solute carrier

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Introduction

The interplay between xenobiotics (including most drugs) and the living organism (in this context the patient) is traditionally viewed from two perspectives, and this chapter is organized along them:

- How does the organism (patient) affect the drug? *Pharmacokinetics* or *drug disposition* are the terms used to describe the body's handling of drugs. This topic includes drug dosing in kidney disease and it will be discussed in the section "Drug Handling by the Kidneys: Principles of Pharmacokinetics/Drug Disposition."
- How does the drug affect the organism (patient)? *Pharmacodynamics* describe the drug's effects, i.e., both desired (therapeutic) and unwanted (adverse) effects. This topic includes most aspects of nephrotoxicity, and it will be discussed in the section "Drug Injury to the Kidney: Nephrotoxicity."

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Drug Handling by the Kidneys: Principles of Pharmacokinetics/ Drug Disposition

Generally, the effects of drugs depend on the concentration at the site of action. The concentration is a result of the way and speed at which the body deals with the drug.

Obviously, drug handling by the organism is time-dependent and quantitative. This is why numbers are useful to describe a drug's pharmacokinetic features and why equations provide the most appropriate tools to describe the underlying processes [1].

Drug handling by the organism includes processes that are usually encompassed by the LADME acronym—*l*iberation, *a*bsorption, *d*istribution, *m*etabolism, and *e*xcretion. While liberation, absorption and distribution are rarely critical for individual variation of pharmacokinetics, elimination is the central process that, with repeated administration of drugs at steady state, determines the relationship between dose (D) and concentration (C_{ss}).

Clearance is the best parameter to describe the body's overall capacity of eliminating a specific xenobiotic. Clearance is defined as the volume (usually plasma) that is cleared of the compound within a particular period of time. The usual units of expression are liter per hour (L/h) or milliliter per minute (ml/min).

$$\frac{D \cdot F}{\tau} = C^{ss} \cdot Cl \qquad (71.1)$$

- D = dose
- F = bioavailability
- τ = dose interval
- C_{ss} = concentration at steady state
- Cl = clearance

Clearance determines the maintenance dose rate required to achieve a target plasma concentration of a drug, and therefore its effect, at steady state.

As a consequence, dose reduction is required to avoid toxic concentrations (C^{ss}) in individuals and situations with impaired clearance (Eq. 71.1). For the selection of the dose that is appropriate for a specific patient, it is important to consider by which route the drug leaves the body and whether or not that particular organ works properly.

The bile and the urine provide aqueous environments for xenobiotics to leave the body from systemic circulation. Xenobiotics need to be water-soluble to take these routes. It is the biological purpose of metabolism, predominantly in the liver, by chemical alteration to facilitate water solubility of xenobiotics. While the majority of drugs first undergo hepatic metabolism, some other drugs, which are water-soluble by themselves, skip metabolism and are excreted unchanged via the bile or urine. Xenobiotics with a molecular weight of less than 400 to 500 g/mol undergo excretion via the kidneys [2–4]. Larger molecules prefer the biliary route for excretion.

While the liver is important for both metabolism and biliary excretion, the kidneys are essential for excretion and thus have a predominant role in disposing xenobiotics. Most drugs, before leaving the body by the kidney route, undergo metabolism. In fact, some drugs (e.g., allopurinol, morphine, meperidine) have active metabolites which are cleared by the kidney.

Kidney drug clearance is the net result of three processes, i.e., glomerular filtration, tubular secretion, and tubular reabsorption (Eq. 71.2).

$$\text{Renal clearance} = \text{glomerular filtration} + \text{tubular secretion} - \text{tubular reabsorption} \qquad (71.2)$$

Glomerular Filtration of Drugs

The extent of a drug's glomerular filtration depends heavily on its binding to plasma proteins. The proteins that are relevant for drug binding, i.e., albumin and α1-glycoprotein, do not cross the glomerular membrane unless the patient is nephrotic. Thus, the protein-bound component cannot be filtered, and only the free fraction (f_u, fraction unbound) undergoes glomerular filtration. Beyond this, functional integrity of the glomeruli, the drug's molecular size, and kidney plasma flow (which is a marker of nephron endowment) determine glomerular filtration.

Glomerular filtration rate (GFR) and Cl are equal for drugs that are not bound to proteins and do not undergo secretion and reabsorption (Eq. 71.2). Inulin is an example of an exogenous substance without plasma protein binding; assessment of its clearance is the gold standard of measuring GFR [5].

For drugs and drug metabolites primarily eliminated by glomerular filtration, drug elimination declines as kidney function decreases and drug will accumulate if the dosing regimen is not adjusted. There are not many drugs that are subject to exclusive glomerular filtration; most drugs are actually excreted through active tubular transport.

Tubular Secretion of Drugs

As outlined above, most drugs excreted by the kidneys are eliminated by active tubular transport. Organic anion transporters (OATs) and organic cation transporters (OCTs) have an important role in the excretion of drugs. They are members of the solute carrier (SLC) transporter family. The energy required for the transport is provided by a gradient of the drug to be transported or by a co-transported ion (e.g., sodium).

In addition to OATs and OCTs, ATP binding cassette (ABC) transporters are important. Hydrolysis of ATP provides the energy. ABC transporters that are important for the renal elimination of drugs are some multidrug resistance (MDR) proteins, i.e., MDR1 (ABC1), multidrug resistance-associated proteins (MRP), i.e., MRP2 (ABCC2), MRP4 (ABCC4) and MRP5 (ABCC5), and BCRP (ABCG2). Figure 71.1 gives an overview of the localization of SLC transporters in human proximal tubule epithelial cells.

The active kidney tubular secretion of drugs and drug metabolites by relatively nonspecific anionic and cationic transport systems in the proximal tubule contribute substantially to the amount of drug eliminated by the kidney. The tubular secretion of basic and acidic drug molecules involves three steps:

1. *Drug uptake from blood by transporters in the basolateral membrane*

   At least two systems perform the basolateral uptake of organic acids into the tubular cell, a Na^+-dependent (linked to Na^+/α--ketoglutarate co-transport and subsequent exchange of α-ketoglutarate with organic anions by OAT1) and a Na^+-independent anion transporter. The basolateral uptake of organic cations with primary, secondary, tertiary, or quaternary amine structure is mediated by OCTs and depends on membrane potential. OCT2 is responsible for the uptake of numerous drugs such as cimetidine, famotidine, ranitidine, metformin, and cisplatin.
2. *Drug diffusion through the cytosol*
3. *Drug transport into the lumen by transporters in the brush-border membrane*

   The efflux of organic acids is performed by OATs such as OAT4. Two organic cation antiporters, OCTN1/2 (organic cation transporter novel), organize the efflux of organic cations. OCTN2 physiologically mediates the reabsorption of L-carnitine and thus organizes the homeostasis of the kidney carnitine pool. MDR1, localized in the luminal membrane of the proximal tubule, is involved in the secretion of lipophilic basic and neutral drugs (e.g., anthracyclines, vinca alkaloids, taxanes, quinidine, and digoxin). Other ABC transporters, such as MRP2 and MRP4, transport glucuronides. Interactions by competition may occur with the simultaneous administration of drugs which are substrates of these transporters.

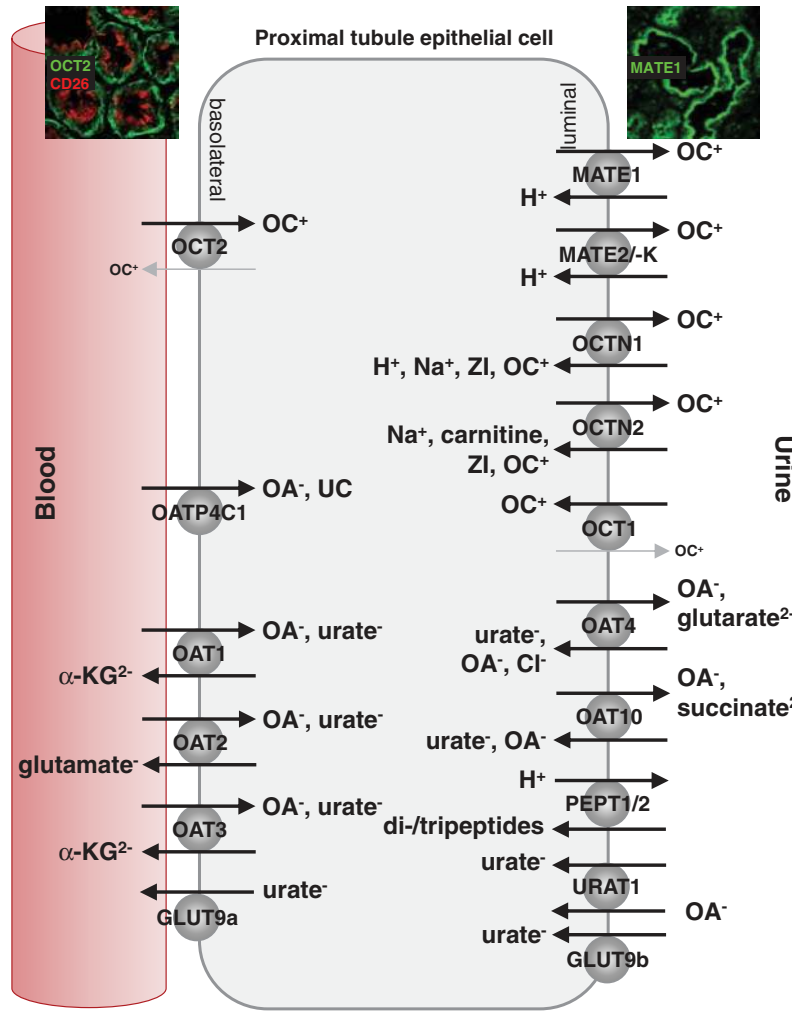

Fig. 71.1 Localization of solute carrier transporters in human proximal tubular epithelial cells implicated in drug-related nephrotoxicity. Representative immunofluorescence pictures show localization of organic cation transporter 2 in the basolateral membrane and *MATE1* in the luminal membrane of proximal tubule epithelial cells in cryosections from human kidney. *Cl* − chloride, *MATE1* multidrug and toxin extrusion proteins 1, *OA* − organic anion, *OC* + organic cation, *UC* uncharged compound, *ZI* zwitterion, *α-KG2* α-ketoglutarate. (Modified from Fisel et al. [6])

Thus, cimetidine, by inhibiting the OCT2-mediated uptake of cisplatin into tubular cells, reduces its nephrotoxicity. This transport system is an elimination pathway for many drugs such as penicillins. For instance, probenecid competitively inhibits the tubular secretion of organic anions such as penicillins. In consequence, plasma levels of weak organic acids such as penicillins will increase.

Tubular Reabsorption of Drugs

As the filtered urine becomes increasingly concentrated in the proximal tubule, a concentration gradient towards the intercellular space and vascular lumen develops. As a result, lipophilic compounds are reabsorbed to a larger degree.

Reabsorption is the passive diffusion of nonionized (non-charged) drug from the filtrate into the kidney tubular cell. Reabsorption of acids and bases depends on their pK_a and urinary pH. Basic urine (e.g., urine pH > 7.5) favors the ionized form of acidic drugs and limits their reabsorption, whereas reabsorption of basic drugs is enhanced in basic urine because the nonionized form of the drug is favored. Alteration of urinary pH thus affects the renal clearance of acidic and basic drugs because predominance of ionized drug reduces their tubular reabsorption. This intervention, together with increasing urinary flow ("forced diuresis"), has

been used in the past to treat intoxications. With hemodialysis and hemofiltration, safer and more effective methods of elimination are available now.

Kidney Failure Induced Alterations in Drug Disposition

Kidney dysfunction affects the disposition of renally cleared drugs. The clinical significance of decreased kidney function on a drug dosing regimen is a function of:

- the therapeutic index (TI), i.e. the amount of a therapeutic agent that causes the therapeutic effect relative to the amount that causes toxicity. The TI varies widely among drugs. Drugs with a high TI such as penicillins have a wide therapeutic window, have less side effects and are considered to be less toxic. Drugs with a low therapeutic index such as aminoglycosides or vancomycin require therapeutic drug monitoring to achieve plasma concentrations in the therapeutic window and limit toxicity and adverse effects of the drug.
- the percentage of total drug elimination that is due to renal clearance. E.g., the angiotensin converting enzyme inhibitor fosinopril is subject to both kidney and liver clearance and will not accumulate in kidney failure, whereas Enalapril is exclusively cleared by the kidneys and will accumulate with worsening kidney function [7].
- the degree of kidney failure.

The most difficult task is obtaining an accurate assessment of kidney function, e.g., GFR [5]. The GFR is estimated by measuring the rate at which the kidney removes a substance from the blood (e.g., kidney clearance). Endogenous compounds (e.g., creatinine, cystatin C) [5, 8], exogenous compounds that are specifically administered to measure the GFR (e.g., inulin), or compounds primarily eliminated by glomerular filtration that are administered as part of clinical care (e.g., gentamicin or vancomycin) are used for measurement.

Age-Related Development of Kidney Function

The developmental increase in GFR involves active nephrogenesis, a process that begins at 9 weeks and is complete by 36 weeks of gestation, followed by postnatal changes in blood flow, both to the kidney and inside the kidney [9, 10] (Fig. 71.2). In the postnatal period, the mean arterial pressure increases, consequently altering the glomerular hydraulic pressure and increasing the GFR. When GFR is indexed to body surface area, GFR increases rapidly after birth in term neonates from 10 to 15 ml/min/1.73 m^2 in the first days of life to values of 90–110 ml/min/1.73 m^2 at the age of 12–18 months [2, 3]. In preterm infants GFR rises more slowly and reaches adult values only by 2 years of age [11, 12].

Similarly, to the above-mentioned factors, tubular secretory pathways are immature at birth and gain full capacity during the first year of life [14]. In preterm neonates sodium excretion for example is inversely correlated to gestational age, mostly due to tubular immaturity [15]. Also, loss of bicarbonate and glucose may necessitate supplementation in preterm neonates. Other tubular functions are impaired such as organic cation transporters (OAT); these have been reported to mature functionally over the first weeks of life and reach adult levels around 7–8 months post-partum. For example, para-amino hippurate (PAH), a substrate of OAT1, is used as a marker for blood flow as the tubular secretion is approximately 100% at first pass through the nephron. PAH is therefore used to test for drug-induced inhibition of OAT1 that would lead to a reduced uptake of PAH. The expression of the luminal drug transporter P-glycoprotein changes significantly during maturation, as illustrated by the threefold difference in digoxin dosing between children and adults. Of note, the inter-individual variation in tubular maturation seems to be much greater than the variations seen in GFR maturation. As these tubular transport mechanisms play an important role in drug excretion, preterm birth can be expected to have an impact on kidney drug handling, although different mechanisms are impor-

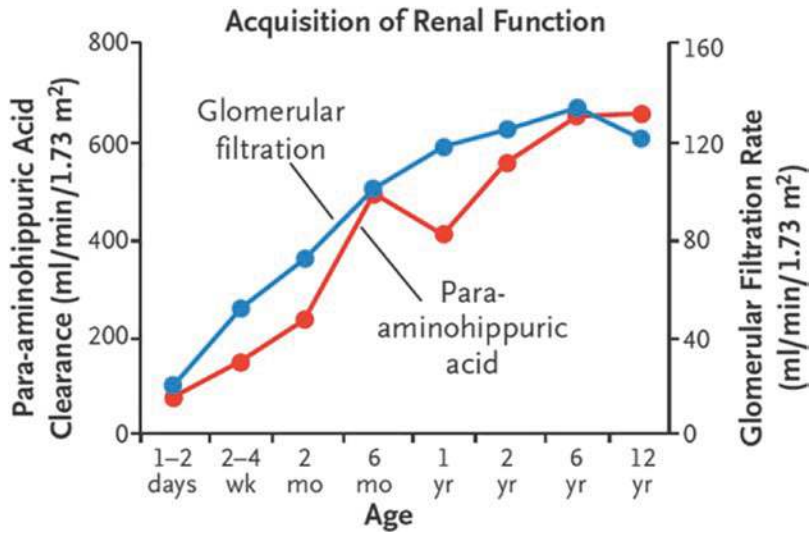

Fig. 71.2 Summary of the effect of postnatal development on the processes of active tubular secretion—represented by the clearance of para-aminohippuric acid and the glomerular filtration rate, both of which approximate adult activity by 6–12 months of age. (From Kearns et al. [13])

tant for different drugs and the actual impact may be difficult to predict.

Dialysis

The impact of dialysis on drug disposition is determined largely by the extent of drug removal by the dialysis procedure. During dialysis, drug clearance is a composite of ongoing drug removal by kidney, hepatic, and other intrinsic clearance pathways and the additional clearance provided by dialysis.

The physicochemical properties of a drug such as molecular size, protein binding, and volume of distribution largely determine its dialyzability [16, 17]. Additional factors specific to the dialysis prescription such as the type of dialyzer and blood/dialysate flow rates can also impact drug removal.

In general, drug removal is considered clinically significant when >25% of the administered dose is removed by dialysis. Only a drug present within the systemic circulation in the unbound form is available for removal by dialysis. Uremic solutes that accumulate in CKD are known to increase the free fraction of some drugs, making them more easily dialyzable.

High-flux dialyzers can remove drugs with molecular weights of up to 40,000 Da. Failure to recognize the extent of dialytic drug removal and provide supplemental dosing is needed can result in underdosing and therapeutic compromise. For instance, vancomycin (~1450 Da) is cleared minimally with conventional low-flux dialysis but is extensively removed by high-flux dialyzers (dialytic clearance between 45–131 ml/min with high-flux polysulfone dialysis membranes) [18, 19]. Other drugs that exhibit the same characteristic include carbamazepine, cisplatin, daptomycin, fluorouracil, ranitidine, and valproic acid.

Drugs cannot be directly removed from tissue stores but must be redistributed from the tissue sites into the vascular space to be available for elimination by any dialysis procedure. Drugs that have a small V_d (e.g. <0.7 L/kg) are generally restricted to the blood compartment and therefore more accessible for removal during dialysis. In contrast, drugs with a large V_d are distributed into the tissues and are generally minimally impacted by dialysis. Although drug usually moves from the blood compartment to dialysis fluid, drug can also be absorbed from the dialysis fluid into the blood compartment when the dialysis fluid drug concentration exceeds the serum concentration. The bidirectional movement of drugs is exploited for the antibiotic treatment of peritonitis in patients on peritoneal dialysis where therapeutic blood concentrations can be achieved by intraperitoneal administration.

Drugs with a large V_d are also more susceptible to the rebound effect following dialysis.

Examples of drugs with a substantial rebound effect include gentamicin and vancomycin. Gentamicin concentrations rebound by up to 25% several hours following the completion of a dialysis session [20]. Vancomycin exhibits a similar rebound as plasma concentrations decrease by 38% immediately following dialysis but are only 16% lower than the pre-dialysis concentration 5 h post-dialysis [21].

The efficiency of drug removal is greatest for hemodialysis, followed by continuous kidney replacement therapies (CKRTs), and least by peritoneal dialysis. Although drug removal by CKRT and peritoneal dialysis is less efficient than hemodialysis, the total drug removal may be equivalent because CKRT and peritoneal dialysis are performed for a longer period of time.

Guidelines for Drug Dosing in Children with Kidney Failure

The optimal drug prescription for a child with kidney failure considers the multiple factors impacting drug disposition and response and is best achieved by using an individualized approach. Although drug lists and dosing tables can be helpful to identify those drugs that require attention in children with kidney failure (Table 71.1), such guidelines fall short when it comes to providing dosing recommendations because optimal therapy must be individualized according to the degree of kidney failure, concurrent medications, and developmental factors—all of which can impact the disposition of a drug. Table 71.1 should be interpreted with caution since systematic clinical data from trials in children of different age groups and across wide GFR ranges are still limited.

Thus, the provision of safe and effective therapy in children with kidney failure is best accomplished using an individualized systematic approach (Fig. 71.3). The design of a successful therapeutic regimen begins with an estimate of the child's residual kidney function and an estimate of the relative contribution of kidney elimination to the total drug elimination obtained from the literature. Reference books such as the *Pediatric Drug Handbook*, *Physicians' Desk Reference*, and *Micromedex* are excellent sources for information on drug disposition—and most references are available as electronic documents that can be used on hand-held devices for point-of-care therapeutic decisions.

Although children receiving dialysis by definition have very poor kidney function, it is inappropriate to assume that there is no kidney elimination because many children maintain a significant amount of residual kidney function. Failure to account for the continued kidney elimination of drug may result in insufficient drug dosing and therapeutic failure. If one assumes that drug protein binding, distribution, and metabolism are not altered to a clinically significant degree in kidney failure (an assumption likely true for most drugs), a dosing adjustment factor Q can be estimated using the following equation (Eq. 71.3):

$$Q = 1 - \left[\text{fraction renal elimination} \cdot \left(1 - \frac{\text{child's } Cl_{cr}}{\text{normal } Cl_{cr}} \right) \right] \tag{71.3}$$

- Cl_{cr} = creatinine clearance [ml/min/1.73 m^2]

The appropriate dose amount or dosing interval for a child with reduced kidney function is generated by applying the dosing adjustment factor Q to either the normal dose amount (Q × normal dose = adjusted dose) or normal dosing interval (normal dosing interval + Q = adjusted dosing interval). The dosage adjustment factor estimates the change that occurs in elimination associated with kidney failure but does not account for any additional clearance by dialysis. If appropriate, supplemental doses may be required to replace the dialysis-related drug losses.

Whether a change is made in the dose amount or dosing interval depends on the therapeutic goal

Table 71.1 Drug dosing guidelines for common therapeutic agents according to Filler et al. [22]

Drugs	Normal DOSE/day Not to exceed adult dose	Dose at GFR 50–30	Dose at GFR 30–10	Dose at GFR < 10	MW	Plasma protein binding	%eliminated by kidney	Elimination t ½ with normal GFR
Aminoglycosides								
Amikacin	15–22.5 mg/kg div Q8 h	Q12–18 h	Q18–24 h	Q48–72 h	585.6	<11%	>95	2–3 h
Gentamicin	6–7.5 mg/kg div Q8 h	Q12–18 h	Q18–24 h	Q48–72 h	477.6	<30%	>95	5–>100 h
Tobramycin	6–7.5 mg/kg div Q8 h	Q12–18 h	Q18–24 h	Q48–72 h	467.5	<30%	>95	2–3 h
Carbapenems								
Imipenem + cilastin	60–100 mg/kg div Q6 h	7–13 mg/kg/ dose Q8 h	7.5–12.5 mg/kg/ dose Q12 h	7.5–12.5 mg/kg/ dose Q24 h	Imipenem: 299.3	Imipenem 13–21% cilastin 40%	70	1 h
Meropenem	30–100 mg/kg div Q8 h	20–40 mg/kg/ dose Q12 h	10–20 mg/ kg/dose Q12 h	10–20 mg/kg/ dose Q24 h	383.5	2%	70	1 h
Cephalosporins								
Cefaclor	20–40 mg/kg div Q8–12 h	Normal	Normal	50% dose	367.8	25%	80	40 min
Cephalexin	25–100 mg/kg div Q6–8 h	Normal	Q8–12 h	Q12–24 h	347.4	10.60%	~100	1–1.5 h
Cefazolin	50–150 mg/kg div Q8 h	60%, Q12 h	25%, Q12 h	10%, Q24 h	454.5	74–86%	80–100	2 h
Cefixime	8 mg/kg div Q12–24 h	75%	75%	50%	453.4	76–91%	20–35	3–4 h
Cefotaxime	100–200 mg/kg div Q6–8 h	35–70 mg/kg, Q8–12 h	35–70 mg/ kg, Q12 h	35–70 mg/kg, Q24 h	619.6	31–50%	80	1.4–1.9 h
Cefotiam	50–100 mg/kg				525.6	76–91%	80	0.9–1.2 h
Ceftazidime	100–150 mg/kg div Q8 h	50 mg/kg Q12 h	50 mg/kg Q24 h	50 mg/kg Q48 h	546.6	17%	80–90	1.8–2.2 h
Ceftriaxone	50–100 mg/kg Q24 h	Normal	Normal	Normal	554.6	85–95%	67	6–9 h
Cefuroxime	75–150 mg/kg div Q8 h	Normal	Normal dose Q8–12 h	Normal, Q24 h	424.4	33–50%	95	1–1.5 h

Drugs	Normal DOSE/day Not to exceed adult dose	Dose at GFR 50–30	Dose at GFR 30–10	Dose at GFR < 10	MW	Plasma protein binding	%eliminated by kidney	Elimination t ½ with normal GFR
Cefuroxime axetil	20–30 mg/kg div Q12 h	Normal	Normal dose Q24 h	Normal Q48 h	510.5	33–50%	95	1–1.5 h
Glycopeptides								
Teicoplanin	10 mg/kg/dose Q12 h × 3 then 3–10 mg/kg Q24 h	50% ?	Avoid ?	Avoid ?	1893.7	71.9–80.5%, higher in older children	42–58	21–58 h
Vancomycin	40–60 mg/kg div Q6 h	10 mg/kg Q12 h	Q18–24 h	Single dose of 10 mg/kg, then follow levels	1449.3	55%	80–90	4–11 h
Ciprofloxacin	20–30 mg/kg div Q12 h	Normal	Q18 h	Q24 h	331.3	16–43%	30–50	3–5 h
Macrolides								
Azithromycin	10 mg/kg × 1 day, then 5 mg/kg Q24 h × 4 days	Normal	Normal	Normal	749	7–51%	6	54.5 h
Clarithromycin	15 mg/kg div Q12 h	Normal	8 mg/kg Q12 h	4 mg/kg Q24 h	748	42–70%	20–40	3–7 h
Erythromycin	30–50 mg/kg PO div Q6–8 h	Normal	Normal	50–75% Q6–8 h	734	73–81%	2–5	1.5–2 h
Nitromidazoles								
Metronidazole	30 mg/kg div Q6–8 h	Normal	Normal	4 mg/kg/dose Q8 h	171.2	<20%	60–80	6–14
Penicillins								
Amoxicillin	20–50 mg/kg div Q8 h	Normal	8–20 mg/kg/ dose Q12 h	8–20 mg/kg/dose Q24 h	365.4	17–20%	60	1–2 h
Amoxicillin + clavulanic acid (dosed by amoxicillin component)	Variable, dependent on formulation 25–50 mg/kg div Q8 h	Normal	8–20 mg/kg/ dose Q12 h	8–20 mg/kg/dose Q24 h	602.7	Amoxicillin 17–20% Clavulanate 25%	Amoxicillin 50–70 Clavulanate 25–40	1–1.5 h
Ampicillin	50–400 mg/kg div Q6 h	Normal	Q8–12 h	Q12–24 h	349.4	10–18%	90	1–1.8 h

(continued)

Table 71.1 (continued)

Drugs	Normal DOSE/day Not to exceed adult dose	Dose at GFR 50–30	Dose at GFR 30–10	Dose at GFR < 10	MW	Plasma protein binding	%eliminated by kidney	Elimination t ½ with normal GFR
Other antibiotics								
Clindamycin	PO 20–40 mg/kg div Q6–8 h	Normal	Normal	Normal	425	60–95%	10	2–3 h
Doxycycline	2–4 mg/kg div Q12–24 h	Normal	Normal	Normal	444.4	80–85%	23	12–15 h
Trimethoprim/ sulfamethoxazole TMP/SMZ Dosed by TMP component	Variable PO 6–12 mg/kg div Q12 h	Normal	GFR15–30 50% dose	GFR < 15 avoid	543.6	TMP 44% SMX 70%	TMP IV 17–42.4 TMP PO 66.8 SMX IV 7–12.7 SMX PO 30	TMP 4–8 h/ SMX 9–12 h
Nitrofurantoin	5–7 mg/kg div Q6 h	Avoid	Contrain-dicated	Contrain-dicated	238.2	~40–60%	40	0.3–1 h
Antifungal agents								
Amphotericin B	0.25–1.5 mg/kg Q24 h	Normal	Normal	Q24–36 h	924.1	90%	2–5	12–40 h
Fluconazole	3–12 mg/kg Q24 h	50%	50%	50%	306.3	11–12%	80	15–25 h
Itraconazole	5–10 mg/kg div Q12–24 h	Normal	Normal	50%	705.6	99%	0	Parent: 36/ metabolite: 18
Antituberculosis agents								
Ethambutol	PO 15–25 mg/kg Q24 h	Normal	Q24–36 h	Q48 h	204.3	20–30%	50	2.5–3.6 h
Isoniazid	10–15 mg/kg Q24 h	Normal	Normal (use with caution)	Normal (use with caution)	137.1	10–15%	75–95	2.3–4.9 h
Pyrazinamide	15–30 mg/kg Q24 h	Normal	Normal	50–100%	123.1	50%	4	6.7 h
Rifampin	10–20 mg/kg div Q12–24 h	Normal	Normal	Normal	822.9	80%	Up to 30%	3–5 h
Antivirals								
Acyclovir	30–60 mg/kg div Q8 h	Normal dose Q12 h	Normal dose Q24 h	50% dose Q24 h	225.2	9–33	60–90	2–3 h

Drugs	Normal DOSE/day Not to exceed adult dose	Dose at GFR 50–30	Dose at GFR 30–10	Dose at GFR < 10	MW	Plasma protein binding	%eliminated by kidney	Elimination t ½ with normal GFR
Ganciclovir	IV 5–10 mg/kg div Q12–24 h	50% dose Q24 h	25% dose Q24 h	25% dose 3 times week post-hemodialysis	255.2	1–2%	80–99	2.5–6.5 h
Indinavir	1050–1500 mg/m^2 div Q8 h	Not studied	Not studied	Not studied	613.8	60%	<20%	1.4–2.2 h
Lamivudine	8 mg/kg div BID	Normal Q24 h	50% dose Q24 h	25% dose Q24 h	229.3	<36%	70	0.5–4 h
Valacyclovir	60 mg/kg div Q8 h	Normal Q12 h	Normal Q24 h	50% dose Q24 h	324.3	13.5–17.9%	88%	1.3–2.5
Valganciclovir	Once daily using formula	Formula	Formula	Formula	354.4	1–2%	80–90%	2–7 h
Zidovudine	Varied	Normal	Normal	50% dose Q8 h	267.2	25–38%	63–95	1–2 h
Anticonvulsants								
Carbamazepine	10–30 mg/kg	Normal	Normal	75%	236.3	75–90%	1–3%	Single dose 40 h/ maintenance 36 h
Clonazepam	0.05–0.5 mg/kg	Normal	Normal	Normal	315.7	85%	Majority	23–36 h
Ethosuximide	15–40 mg/kg div Q12 h	50–75% (use with caution)	50–75% (use with caution)	No data (use with caution)	141.2	<10%	10–20	30 h
Phenobarbital	4–8 mg/kg div Q12–24 h	No data	No data	No data	232.2	35–50%	≤ 75	
Phenytoin	5 mg/kg	Normal	66%	50%	252.3	90–95%		
Sodium valproate	10–100 mg/kg (plasma levels 5–100 mg/L)	Normal	Reduce or avoid	Avoid	166.2	80–90%	~100	13–17
Antihypertensive drugs								
ACE-inhibitors								
Captopril	0.3–3.15 mg/kg	50%—Normal	25–50%	25–50%	217.3	25–30%	~65	4–5 h
Enalapril	0.1–0.3 mg/kg	25–50%	12.5–25%	6.25–12.5%	376.4	50–60%	61	35 h

(continued)

Table 71.1 (continued)

Drugs	Normal DOSE/day Not to exceed adult dose	Dose at GFR 50–30	Dose at GFR 30–10	Dose at GFR < 10	MW	Plasma protein binding	%eliminated by kidney	Elimination t ½ with normal GFR
Ramipril	0.1–0.2 mg/kg Q24 h	>40 ml/ min = no adjustment; <40 ml/ min = 25% dose	25%	25%	416.5	73%	60	>50 h
ARB-inhibitors								
Irbesartan	75–300 mg	Normal	Normal	Normal	428.5	90%	~20	11–15 h
Losartan	0.7–1.4 mg/kg	Normal	Normal	Normal	422.9	>98%	~35	1.5–2.5 h
B-blockers								
Atenolol	0.5–2 mg/kg	Normal	50%	25%	266.3	6–16%	100	6–10 h
Bisoprolol	0.2 mg/kg	Normal	50%	Normal	325.4	30%	50	10–12 h
Carvedilol	Low-dose: 0.2–12.5 mg/kg High-dose: 0.4–25 mg/kg	Normal	Normal	Normal	406.5	>98%	~35	7 h
Metoprolol	1–6 mg/kg	No data	No data	No data	267.4	12%	~95	3–4 h(though 2–9.5 reported)
Propanolol	1–4 mg/kg div Q8–12 h	Normal	Normal	Normal	259.3	93%	<1	4–6 h
Ca-antagonists								
Amlodipine	0.1–0.5 mg/kg	Normal	Avoid	Avoid	408.9	93%	~95	10–36 (with repetitive dosing, 45)
Nifedipine	0.5–2 mg/kg	Normal	Normal	Normal	346.3	92–98%	70–80	4 h

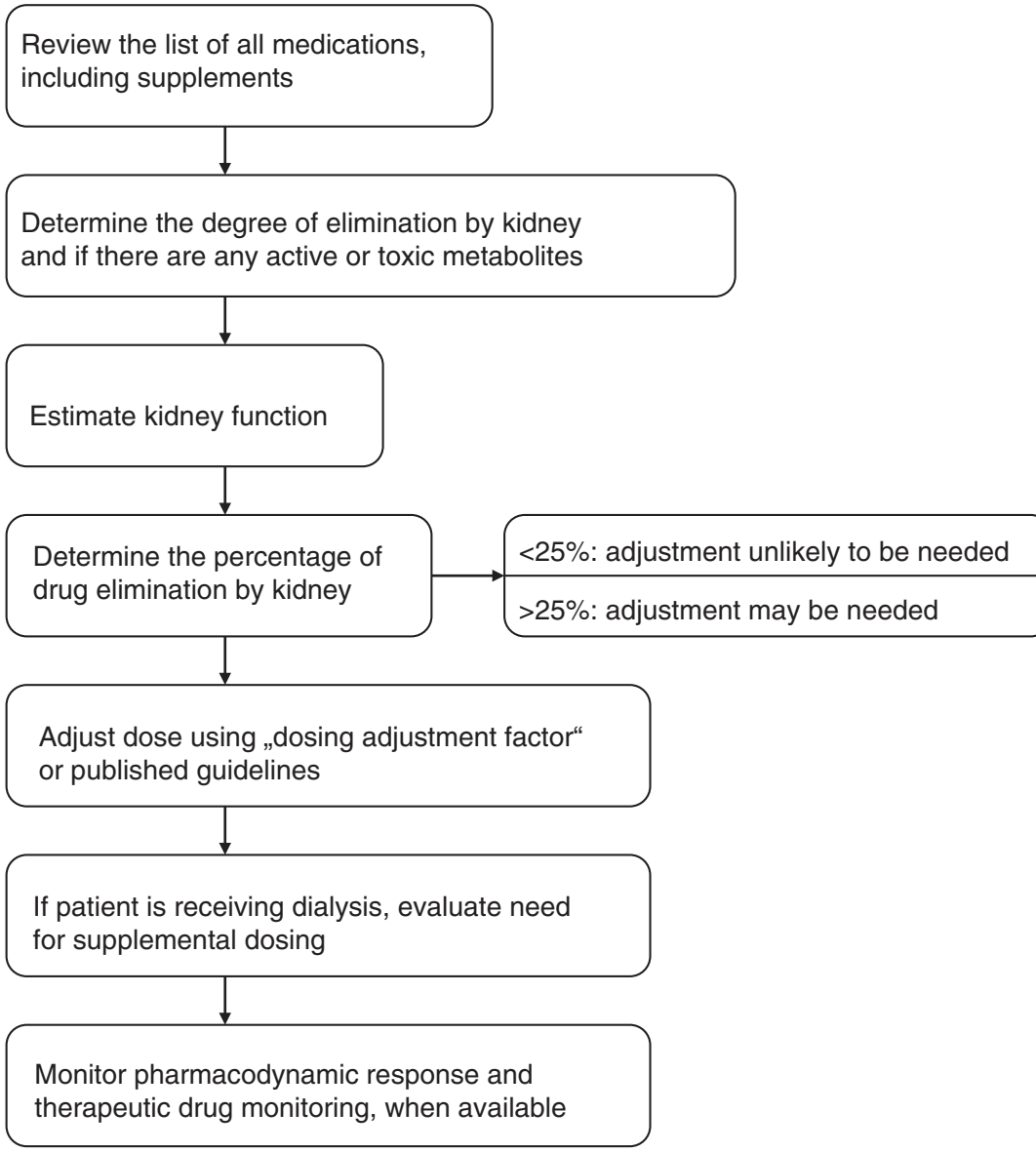

Fig. 71.3 Guidelines for drug dosing in children with kidney failure

and relationships between drug concentrations and clinical response and toxicity. In general, increasing the dosing interval will increase the variation between peak and trough blood concentration and would be most appropriate for drugs whose effects are based on achieving a certain peak drug level (e.g., aminoglycosides). In contrast, a decrease in variations between peak and trough blood concentration will be observed when a normal dosing interval is maintained but the dose amount is decreased. This dosing adjustment would be most appropriate for drugs that should be maintained at a relatively stable blood concentration, such as cephalosporins or blood pressure medications.

Once the prescribed drug dosing schedule has been adjusted for kidney failure, a supplemental dose or dosing adjustment may be required for children receiving dialysis when >25% of a drug is removed during the dialysis procedure.

Supplemental dosing is given to replace the amount of drug removed by dialysis and may be achieved as a partial or full dose administered after hemodialysis, or an increase in the dosing amount or frequency in children receiving peritoneal dialysis or CRRT. When possible, routine maintenance drugs should be provided after hemodialysis. Guidelines for drug dosing during dialysis are available in selected references [16].

The determinants of drug disposition and action in children with kidney failure and on dialysis are frequently altered such that changes in the dosing regimens are necessary to avoid toxicity or inadequate treatment. In view of the many factors capable of altering both the disposition and action of a given drug, it is important to individualize drug therapy for the known alterations associated with age, kidney failure, and dialysis.

Therapeutic Drug Monitoring

Humans are not equal, and it is increasingly appreciated that this inequality pertains to pharmacokinetics also. Much of what carries the label "personalized medicine" is related to pharmacokinetics.

Target concentration strategy is a concept where pharmacokinetics plays a critical role. This strategy is useful as an adjunct in initiating and monitoring drug therapy when certain criteria are met. Most important is a close concentration-response relationship, which means the plasma concentration of a drug must correlate quantitatively with the intensity or probability of therapeutic or toxic effects across the patient population. The strategy becomes particularly attractive when a therapeutic endpoint is difficult to quantify, i.e., with the non-occurrence of epileptic seizures. The strategy is ideal when the aim is to maintain a therapeutic effect for which a systemic exposure within a given range is necessary. Drug administration in a constant-rate input or multiple-dose regimens is possible when the potential for organ toxicity or rejection may be predicted from the trough or peak concentration.

Multiple dosing regimens are usually based on the total body clearance such that the dosing rate is taken as the product of the clearance and the desired steady-state plasma concentration of the drug. For drugs eliminated by the kidneys, it is widely accepted that the kidney clearance is proportional to GFR [23].

For drugs with a narrow therapeutic index and poor correlations of trough levels with efficacy and adverse effects, limited sampling strategy (LSS) models have been developed to estimate the area under the concentration time curve. Ting et al. published different LSS approaches [24]. Many LSS studies utilize blood sampling within the first 4 h (C2 and C4) or the first 8 h (C1, C4, and C8) postdose with multiple regression analysis.

Drug Injury to the Kidney: Nephrotoxicity

Kidney dysfunction and toxicity secondary to medications are common, but the real incidence of drug-induced nephrotoxicity is difficult to determine. Approximately 18% of acute tubular necrosis (ATN) or acute interstitial nephritis (AIN) cases can be attributed to nephrotoxic medication. The incidence of nephrotoxic injury due to antibiotics such as aminoglycosides is even higher, up to 36% [25].

Fortunately, most episodes of drug-induced kidney injury, if diagnosed and treated early, are reversible, with *a restitution ad integrum* after cessation of the causing medication. Chronic kidney damage may occur with prolonged exposure to drugs such as analgesics and calcineurin inhibitors and will lead to chronic tubulointerstitial inflammation, papillary necrosis or prolonged proteinuria. A close follow-up is necessary to avoid kidney failure [26].

Nephrotoxic drugs require therapeutic drug monitoring (TDM) to maintain a balance between efficacy and toxicity. This is particularly important if a drug has a narrow target range, significant pharmacokinetic variability, a reasonable relationship between plasma concentration and clinical effects, an established therapeutic win-

dow, and availability of a cost-effective assay. Most places offer TDM for aminoglycosides (acute tubular necrosis), vancomycin (acute interstitial nephritis), calcineurin inhibitors (cyclosporine: acute tubular necrosis, chronic interstitial nephritis, thrombotic microangiopathy; tacrolimus: acute tubular necrosis), antifungal drugs (amphotericine: acute tubular necrosis, distal tubular renal acidosis), antiepileptic drugs (mostly not nephrotoxic), lithium (chronic interstitial nephritis, glomerulonephritis, rhabdomyolysis), and digoxin (not nephrotoxic) [27]. For some drugs like acyclovir (acute interstitial nephritis, crystal nephropathy) [28] or ganciclovir (crystal nephropathy), which should have TDM, this is not widely available. It is important not note that toxicity may be more related to the area under the time concentration curve (AUC) than the pre-dose trough level. For instance, switching from BID dosing of cyclosporine to TID dosing may substantially reduce the AUC [29]. Some other drugs may not be increasing the active drug, but rather metabolites, which then account for toxicity. An example is mycophenolic assay, where the main metabolite mycophenolic acid glucuronide (MPAG) accumulates with decreased kidney function, rendering it intolerable with low eGFR [30].

An increasing number of observational studies report on early drug-related nephrotoxicity in humans. The investigations concern nephrotoxicity from antibiotics (particularly aminoglycosides), angiotensin-converting enzyme (ACE) inhibitors, non-steroidal anti-inflammatory drugs and antifungal agents [31]. Also, there is growing interest in the long-term effects of drugs on the neonatal kidney. A recent up-to-date summary of nephrotoxic drugs with particular impact on children highlighted additional underlying pathophysiological mechanisms [32]. Figure 71.4 summarises the clinically relevant information. In the following paragraph we describe selected mechanisms of drug nephrotoxiciy and include some examples of nephrotoxic drugs.

Regarding toxic effects on nephrogenesis drugs administered to pregnant women and to neonates born preterm may influence kidney development. Nephrogenesis ceases at approximately 36 weeks gestation in humans so that most toxic effects on nephrogenesis may be expected in treatment with drugs during pregnancy. In premature-born neonates nephrogenesis has not completed at time of birth. Many of them will be treated with drugs in the vulnerable phase of kidney development. An adequately functioning RAS is essential for kidney development. The use of angiotensin converting enzyme inhibitors (ACEIs) in pregnancy can therefore negatively influence nephrogenesis and lead to neonatal kidney failure (ACEI fetopathy). Mutations in genes coding for renin, ACE and AT1 cause autosomal recessive kidney tubular dysgenesis and fetal hypotension [33]. Both inherited and acquired defects of the RAS can alter kidney hemodynamics with deleterious effects on kidney development.

Aminoglycosides are well-known for their nephrotoxic effect on the developing fetal kidney. These drugs will result in tubular alterations and low nephron number. Offsprings of pregnant rats treated with aminoglycoside are born with lower nephron numbers and subsequently develop glomerulosclerosis with aging. Unfortunately these drugs are still commonly used as first-line treatment of infections in premature neonates although alternative treatment options with less adverse effects on nephrogenesis are available, such as cephalosporins and carbapenems [34, 35].

Pathophysiologic Mechanisms of Drug Nephrotoxicity

Direct Tubular Cell Toxicity

This damage is at least in part dose-dependent, is generally of surreptitious onset (with symptoms often undetected in the early stages), and is characterized by acute tubular necrosis (with loss of a proportion of kidney proximal tubular cells). This mode of toxicity is typical for the aminoglycosides, where it has been reported in 20–35% of exposed children [25], and for antivirals such as aciclovir, foscarnet, cidofovir, adenovir and tenofovir [36]. Cisplatin induced nephrotoxicity is

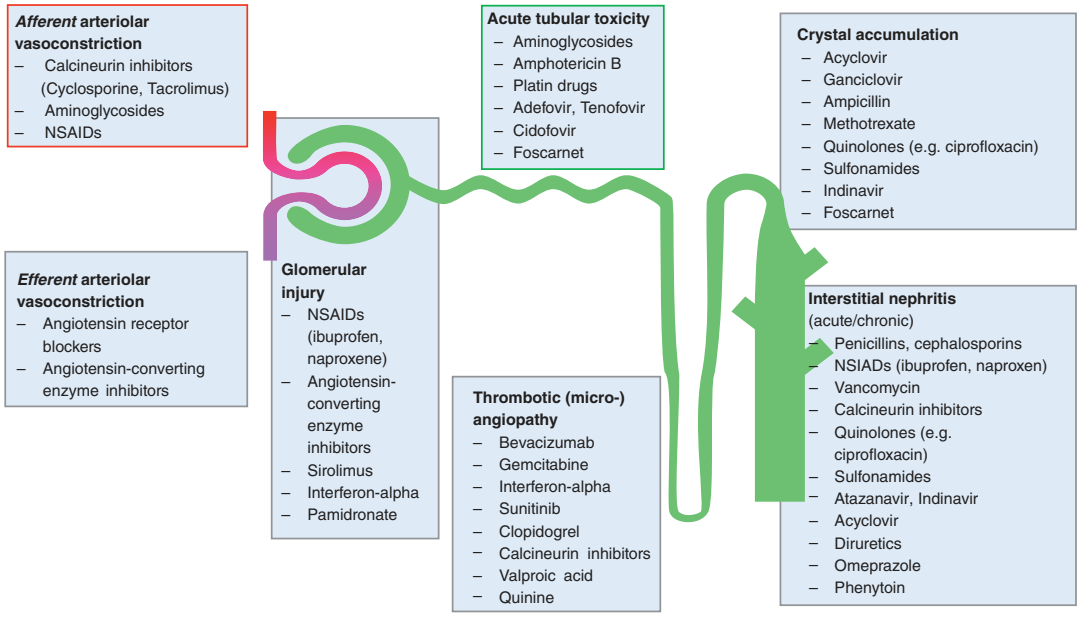

Fig. 71.4 Nephrotoxic drugs in children with related pathophysiological mechanisms. Modified from Tjon and Teoh [32]

frequent (up to 80%) [37] and linked to the accumulation of toxic metabolites with subsequent inflammation and cellular damage. The cyclophosphamide analogon ifosfamide causes proximal tubular dysfunction (Fanconi syndrome) in up to 30% of exposed children and the toxic damage is conferred by the metabolite chloroacetaldehyde. Other drugs associated with this type of damage are amphotericin B, calcineurin inhibitors, methotrexate, pamidronate, pentamidine, and cocaine.

Generally, transient enzymuria and a Fanconi-like syndrome are early signs of proximal tubular damage. These often remain undetected but may be followed by urine sediment disorders (granular, hyaline, and cellular casts) and kidney failure.

Acute Interstitial Nephritis and Immunologically Mediated Kidney Toxicity

Immunologic kidney toxicity is mediated by inflammation of the interstitium and tubules. It occurs on an allergic basis in an idiosyncratic, dose-independent manner with a predominance of lymphocytes, monocytes, eosinophils and plasma cells within the interstitium, and active urinary sediment [38]. Clinical and laboratory signs such as fever, rash and eosinophiluria are rare (<10%) and therefore, if indicated, the diagnosis can be confirmed with certainty only by kidney biopsy. Interstitial nephrotoxicity is typical of antibiotics such as beta-lactams, quinolones, rifampin, macrolides, sulfonamides, vancomycin, most NSAIDs, diuretics (thiazides, loop diuretics, and triamterene), anticonvulsants (phenytoin), cimetidine and ranitidine, allopurinol, and antivirals (acyclovir, indinavir, atazanavir), etc.

If acute interstitial nephritis is diagnosed, potential nephrotoxic drugs should be discontinued immediately.

Chronic interstitial nephritis is more complex and associated with drugs such as calcineurin inhibitors (cyclosporin A, tacrolimus) and carmustine. In contrast to acute interstitial nephritis, the prognosis of chronic interstitial nephritis is rather poor. Data from post-transplant patients treated with cyclosporine indicate a high frequency of cyclosporine-induced interstitial nephrotoxicity resulting in chronic rejection [39].

Arteriolar Vasoconstriction

Decreased kidney blood flow and GFR due to dose-dependent reversible vasoconstriction affecting primarily the afferent but also the efferent arterioles (prerenal dysfunction with intact tubular function) is the main pathophysiogical mechanism of acute kidney toxicity typical for calcineurin inhibitors, NSAIDs, angiotensin-converting enzyme (ACE) inhibitors, and angiotensin receptor blockers. The synthesis of strong vasoconstrictors such as angotensin II, leukotrienes and endothelin is stimulated and endothelial nitric oxide concentrations are reduced.

Prostaglandins contribute to the regulation of kidney perfusion and GFR—with their vasodilating properties opposing the action of vasoconstrictive substances. Thus, subjects suffering from conditions associated with high levels of vasoconstrictive substances (such as hypovolemia, cardiac failure, sepsis, and hypertension) may develop kidney damage when treated with NSAIDs which induce a reduction of prostaglandin synthesis.

Kidney dysfunction associated with antihypertensive therapy is a result of excessive lowering of blood pressure. Since kidney blood flow and kidney perfusion are maintained during treatment with ACE inhibitors despite the adverse effects on glomerular function, kidney function readily returns to pretreatment levels when the drug is discontinued.

Crystal Accumulation

The pH-dependent precipitation of insoluble crystals in the distal tubular lumen is a nephrotoxic mechanism occurring mostly with antiviral drugs (e.g. acyclovir, ganciclovir), sulphonamides, methotrexate, indinavir, etc. Crystalluria after fluoroquinolone administration is rare, and may occur in the condition of dehydration. Among the factors that increase the likelihood of kidney crystal deposition, severe volume contraction is the most important. Uric acid and calcium phosphate crystals contribute to tumor lysis associated nephrotoxicity after chemotherapy for malignancies.

Proteinuric Glomerular Dysfunction

Some drugs can cause nephrotic range proteinuria due to podocyte toxicity. These include NSAIDs, captopril, interferon-alpha, pamidronate, MTOR inhibitors (sirolimus, everolimus), tiopronin, etc. Discontinuation of the drug usually leads to resolution of proteinuria, although irreversible lesions have also been reported.

Vascular Injury/Thrombotic Microangiopathy

Thrombotic microangiopathy has been associated with a wide range of drugs, including cyclosporin A, tacrolimus, gemcitabine, clopidogrel, sunitib, valproic acid, bevacizumab, etc. The main pathologic finding is presence of hyaline thrombi in the microvessels of many organs, including glomerular thrombosis. The manifestations can include fever, hemolytic anemia, thrombocytopenic purpura, kidney dysfunction, central nervous system involvement (thrombotic thrombocytopenic purpura, TTP), and predominance of kidney failure with anemia and thrombocytopenia (hemolytic uremic syndrome).

Alterations of Fluids and Electrolytes

Examples of alteration of fluids and electrolytes are excess of sodium and water removal; metabolic alkalosis and hypokalemia induced by excessive use of diuretics; and hyperkalemia and metabolic acidosis secondary to pharmacologic hyporeninemic hypoaldosteronism as induced by NSAID and tacrolimus.

Other Mechanisms

Additional specific mechanisms of drug nephrotoxicity include osmotic nephrosis (related mainly to mannitol and immunoglobulins) and rhabdomyolysis (e.g. codein, corticosteroids). Osmotic nephrosis is characterized by vacuoliza-

tion and swelling of renal proximal tubular cells as consequence of glucose and sucrose reabsorption by pinocytosis. Thrombolytic agents often disrupt or dissolve protective thrombi covering ulcerated plaques, thereby releasing cholesterol plaques into the circulation leading to occlusion of small-diameter arteries of the kidney (i.e., arcuate and interlobular arteries, terminal arterioles and glomerular capillaries). Cholesterol embolization with anticoagulants, such as warfarin and heparin, or thrombolytic agents (streptokinase and tissue-plasminogen activator) is sometimes observed weeks or months after initiation of therapy.

Ten Rules for Prevention of Drug-Induced Kidney Damage

- **Rule 1**: **Alternatives**. *Do not use nephrotoxic drugs if alternatives are available*.

- The simplest way to prevent drug-induced nephrotoxicity is to avoid the use of these drugs. Knowledge of drug safety is a useful element for preventing iatrogenic dysfunction. Lack of nephrotoxicity is one of the parameters to be considered in the choice of drug therapy. Before prescribing a potentially nephrotoxic drug, the risk-to-benefit ratio and the availability of alternative drugs should be considered.

- Nephrotoxic drugs should be avoided whenever possible, especially in high-risk situations. Critically ill children are not ideal candidates for potentially nephrotoxic drug treatment, although pediatricians may not always have valid alternative choices. E.g., aminoglycosides have two unique pharmacodynamic properties: their postantibiotic effect and concentration-dependent killing. However, aztreonam is a reasonable alternative to aminoglycoside therapy in children and low-birth-weight infants with gram-negative infections at risk of nephrotoxicity.
- **Rule 2: High-Risk Patients**. *Do not use nephrotoxic drugs in high-risk patients.*
- Chronic kidney failure and a preexisting kidney injury are major risk factors for most nephrotoxins. Diabetes mellitus increases vulnerability to aminoglycosides, nonsteroidal anti-inflammatory drugs (NSAIDs), and ACE inhibitors. Sepsis itself is a major risk for nephrotoxicity. Significant volume depletion is a risk factor for NSAID-induced nephrotoxicity.
- Volume management is essential for prevention. Generally, pretreatment hydration can reduce the nephrotoxic potential of many drugs, including amphotericin B, aminoglycosides, NSAIDs, cisplatin, and indinavir. A "diuretic holiday" (a period off diuretics) is suggested before starting an ACE inhibitor. Modifiable risk factors should be corrected. In many cases, patients are routinely prehydrated before the administration of a nephrotoxic drug. In such cases, it is mandatory to accurately monitor urine output and avoid intravascular volume overload.
- Novel biomarkers such as kidney injury molecule-1 (KIM-1) may be helpful to early identify children with aminoglycoside-induced tubular toxicity (see Chap. 51) [25].
- **Rule 3: Choice of Compound**. *Choose the least nephrotoxic compound.*
- For the kidney, netilmicin is better than gentamicin, teicoplanin is better than vancomycin, and lipid formulations of amphotericin B are better than conventional formulations. Ceftazidime seems the safest cephalosporin for the kidney. Analgesics other than NSAIDs are preferred in children with compromised hemodynamic status or volume depletion.
- **Rule 4: Dosage and Monitoring**. *Use correct dosage and therapeutic drug monitoring, if required*.
- The correct drug dosage should be prescribed. For many years, it has been debated whether TDM of aminoglycosides and vancomycin will decrease toxicity—especially at the kidney level. Probably it depends on the patient population, with high-risk patients benefiting more. However, a tailored TDM is generally associated with lower nephrotoxicity.
- **Rule 5**: **Concomitance**. *Do not use concomitant nephrotoxic drugs*.

- Specific combinations of drugs (such as cephalosporins and aminoglycosides, cephalosporins and acyclovir, and vancomycin and aminoglycosides) may result in synergistic nephrotoxicity. The combination aminoglycoside-vancomycin is believed to increase the nephrotoxic risk up to sevenfold.
- **Rule 6**: **Duration**. *Limit the duration of treatment*.
- Prolonged duration of treatment has been associated with increased aminoglycoside and amphotericin B nephrotoxicity. In adult studies, aminoglycoside-related nephrotoxicity may reach approximately 55% of cases according to the duration of the treatment (high risk with duration >10 days). Moreover, repeated courses of aminoglycoside therapy a few months apart can enhance nephrotoxicity.
- **Rule 7**: **Diagnosis**: *Seek to diagnose kidney damage early*.
- Kidney function (and particularly the GFR) should be frequently monitored during the administration of a potentially nephrotoxic drug. Cystatin C, a marker of glomerular function in the "creatinine blind range" (GFR 60 to 90 ml/min/1,73m^2), and urinary biomarkers of nephrotoxicity (microglobulins, enzymes, and growth factors) can be used for early noninvasive identification of kidney damage occurring in the course of drug therapy. Moreover, they are helpful in establishing its extent and monitoring its time course.
- **Rule 8**: **Damage**. *Stop drug administration if damage occurs*.
- In many cases, the most important first step in treating drug-induced nephropathy is to stop the offending drug. This is true in many cases, such as for acute interstitial nephritis, nephritic syndrome, drugs associated with TTP-HUS, and obstructive nephropathy.
- **Rule 9**: **Pediatric Drugs**. *Use caution when using new drugs in pediatrics*.
- It is important to be cautious in administering drugs to children outside the terms indicated in the product license (off-label use as regards the dose, age group, route of administration, different indication) or in an unlicensed manner (formulations modified, extemporaneous preparations, imported medicines, chemicals used as drugs).
- The lack of approval for pediatric use does not imply a drug is contraindicated or disapproved. It simply means that insufficient data are available to grant approval status and the risks/benefits balance (including nephrotoxicity) cannot be evaluated.
- This suggestion should be extended to obstetricians because increasing reports of kidney damage in newborns are related to drugs administered to the mother.
- **Rule 10**: **Kidney Failure Dosing**. *Modify dose and/or interval dosing in kidney failure*.

- Normal kidney function is important for the excretion and metabolism of many drugs. Kidney failure alters drug clearance and requires modification in dosage regimens to optimize therapeutic outcome and minimize the risk of toxicity. This is a key point for prevention and is recommended as an essential component of any computer-based prescription system to minimize medical errors and adverse drug events, including drug-induced nephrotoxicity.

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Complementary Therapies for Renal Diseases

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Cecilia Bukutu and Sunita Vohra

Abbreviations

b.i.d.	Bis in die (twice a day)
BUN	Blood urea nitrogen
CAM	Complementary and alternative medicine
cfu	Colony-forming unit
CI	Confidence interval
CKD	Chronic kidney disease
CKD	Chronic kidney disease
Cr	Creatinine
CrCl	Creatinine clearance
dl	Decilitre
ESKD	End stage kidney disease
ESWL	Extracorporeal Shockwave lithotripsy
FDA	Food and Drug Administration
g	Grams
GFR	Glomerular filtration rate
GTP	Guanosine triphosphate
Hz	Hertz
IgA	Immunoglobulin A
IgAN	Immunoglobulin A nephropathy
IU	International unit
kg	Kilogram
MD	Mean difference
mg	Milligram
ml	Millilitres
Mol/L	Moles per liter
n-3 LCPUFA	n-3 Long-chain polyunsaturated fatty acid
NHP	Natural health product
O3FA	Omega-3 fatty acids
PAC	Proanthocyanidin
RCT	Randomized Control Trial
RR	Relative risk
SD	Standard deviation
TCM	Traditional Chinese medicine
TEAS	Transcutaneous electrical acupoint stimulation
TENS	Transcutaneous electrical nerve stimulation
TNF-α	Tumor necrosis factor-alfa
TwHF	Tripterygium wilfordii Hook F
US	United States
UTI	Urinary tract infection
VAS	Visual analog scale
μg	Microgram

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Introduction

Complementary therapies have been described as "health care approaches that are not typically part of conventional medical care or that may have origins outside of usual Western practice" [1]. Thus complementary therapies are a term that includes a diverse range of products and practices (e.g., natural health products [NHPs], massage therapy, acupuncture, etc.) used in the prevention or treatment of illness or the promotion of health and well-being.

The distinction between conventional medicine and complementary therapies is not always well defined or fixed [1]. As complementary therapies do not have a well-demarcated border, the inclusion or exclusion of dietary modifications, vitamins, and prayer/spirituality as complementary therapies remains unresolved [2]. This chapter focuses on evidence regarding NHPs, traditional Chinese medicine (TCM), and massage therapy. First, we review core relevant concepts, including definitions, epidemiology of complementary therapy use (including reasons for use and lack of disclosure), NHP quality and regulation, and legal and ethical issues related to the use of complementary therapies.

Natural Health Products, TCM, and Massage Therapy

In Canada, NHPs are defined in the *Natural Health Products Regulations* as vitamins and minerals, herbal medicines, homeopathic remedies, traditional medicines such as TCMs, probiotics, and other products like amino acids and essential fatty acids [3]. In the United States (US), these products are more commonly referred to as "dietary supplements" [4].

TCM is an ancient medical system that includes acupressure, acupuncture, breathing exercises, NHPs, moxibustion, oriental massage, qi gong, and tai chi [5]. An important concept in TCM is qi, a life energy with various mental, physical, and spiritual manifestations. This energy is said to flow throughout the body, along the meridian system, allowing for the integration of internal organs and other body structures. If one's qi is flowing in an orderly fashion, the person is healthy. Disorderly flow causes disease. One of TCM's aims is to restore the orderly flow of qi (Table 72.1) [9].

Yin-yang is another important concept in TCM, in which yin and yang represent opposing but complementary qualities [10]. The philosophical and physiologic implications of yin-yang imbalance in TCM theory are complicated and beyond the scope of this chapter. It can be confusing; for example, the term *kidney* in TCM has a very different meaning than it does in conventional Western medicine. In TCM theory, it does not imply the physical organ; instead, in TCM theory, the kidney's main function is to store energy that governs conception, growth, development, sexual maturation, reproduction, and pregnancy [10].

Table 72.1 Principles of selected Traditional Chinese Medicine (TCM) therapies

Therapy	General principles
Acupressure	Pressing and/or massaging various acupuncture points (acupoints) using fingers, palms, elbow, feet or special devices on the body's meridians [6].
Acupuncture	The Chinese art of stimulating the pathways of energy (14 main meridians plus branches) by puncturing, pressing, heating, using electrical current, or using herbal medicines [7].
Moxibustion	Moxibustion is the process where dried moxa herb (a mugwort) is burned usually just above but sometimes directly on the skin over acupuncture points. The herb may be in the form of incense sticks or wool [8].
Oriental massage	A wide range of therapeutic techniques involving the manipulation of muscles and soft tissues, including kneading, rubbing, tapping, friction, vigorous or relaxing, deep or superficial [7].
Qigong	Exercises aimed at bringing about harmony, as well as improving health and longevity. Healing methods involve breathing, movement, the mind, and the eyes [7].
Tai chi	Balanced gentle movements, incorporating a combination of meditation and breathing, are designed to dissolve physical and karmic layers of tension in both the physical body and the energy body, and to open up the spiritual space inside [7].

In massage therapy, pressure is used to manipulate the body's soft tissues to impact the circulatory, lymphatic, musculoskeletal, and nervous systems, and in so doing enhance the body's self-healing ability [11]. Aromatherapy generally refers to the use of massage therapy in conjunction with aromatic plant extracts, also known as essential oils [12].

Epidemiology of Complementary Therapy Use

There is widespread use of complementary therapies worldwide [13] with varying prevalence rates: 10–40% in different European countries; 40–60% in the USA [14]; 49% in Australia; 75% in Africa, and between 21.6–90% in Saudi Arabia [15, 16]. A US survey of complementary therapies use in the past 12 months found that 4 in 10 adults and 1 in 9 children and youth had used complementary products or therapies [17]. In a Canadian 2011 survey (n = 2001), 71% of respondents reported using one or more NHPs [18], while in a 2016 survey 56% of Canadians reported having used at least one complementary therapy in in the last 12 months [19]. The prevalence of complementary use tends to be higher among individuals with conditions that are chronic, serious, or recurrent [20–22]. A systematic review reported that prevalence rates for complementary therapy use among children/adolescents range from 10.9% to 87.6% for lifetime use and from 8% to 48.5% for current use [23]. The review also found variance in lifetime prevalence by type of complementary therapy: homeopathy 0.8–39% (highest in Germany, United Kingdom, and Canada) and herbal medicine use 0.8–85.5% (highest in Germany, Turkey, and Brazil) [23].

There is a high prevalence of complementary therapy consumption among adult renal patients. The use of complementary therapies in the previous 6 months among patients (N = 278) at a nephrology outpatient clinic in India was 66.3% [24]. A Saudi cross-sectional study (N = 315) reported that 54.9% of chronic kidney disease (CKD) patients were current complementary therapy users [15]. An earlier survey in Egypt reported a similar complementary therapy prevalence rate (52%) among outpatient nephrology clinic attendees (N = 1005) [25]. In a German survey, regular complementary therapy use was reported by 57% of dialysis patients (n = 119) and 49% of kidney transplant patients (n = 45) [26]. In a Thai survey of 421 adults with CKD, 45% had used NHPs in the past year, and a 2004 US survey found 29% of CKD patients used NHPs (n = 250) [27]. A Canadian study reported that 45% of patients (n = 100) with CKD had used NHPs [28], and 25.2% of 206 CKD patients in a Turkish study reported having used complementary therapies at least once following their renal diagnosis [29].

Parents who use complementary therapies are likely to offer these to their children. Although no complementary therapies prevalence studies among pediatric kidney patients were identified, studies show the main predictors of complementary therapy use to be higher parental income and education, older children [23], female children and the existence of comorbid medical condition [30]. The prevalence of complementary therapy use increases to over 50% in children who have chronic [31, 32] recurrent or incurable conditions [22]. For adults and children, complementary therapy use often occurs in combination with conventional care [32].

Reasons for complementary therapy use may include a desire to improve and cure the disease [31]; having an expectation of benefits of complementary therapies; and dissatisfaction with conventional medicine due to lack of cure, side effects, or higher costs [27, 33, 34]. Some adults mention long wait times and insufficient time spent with their physician as reasons for using complementary therapies. Many complementary therapy users value the long appointments that complementary therapy practitioners provide and feel their concerns can therefore be addressed more thoroughly [34, 35]. Other commonly cited reasons for complementary therapy use include increased personal control regarding treatment, and the perception of complementary therapies

as more natural, and therefore safer, than Western medical treatments [27, 33].

Despite common use, many physicians remain unaware about their patients' complementary therapies use. Only 20% to 65% of families discuss their complementary therapies use with their physician [22]. A US complementary therapies user study (N = 7493) found that 42.3% (n = 3094) did not discuss complementary therapies use with their primary care physicians [36]. Among adults with kidney disease, non-disclosure of complementary therapy use can be as high as 72% [37]. Lack of disclosure usually occurs because patients may not think it is relevant, healthcare providers do not ask, concerns about the healthcare provider's knowledge regarding complementary therapies or patients feel their healthcare provider would disapprove of their complementary therapies use [36, 38, 39].

Regulation of complementary therapy practices varies significantly within and among countries [13, 40]. For example, the regulation of complementary therapy practices within the US is inconsistent (some complementary therapy practices are regulated in some states, and not others). Different states can have different requirements for practitioners or different regulations regarding scope of practice [41]. In the United Kingdom, with the exception of osteopaths and chiropractors, most complementary therapy providers are largely unregulated [41, 42]; conversely, in Canada, regulation of chiropractors, naturopaths, homeopaths, osteopaths, TCM practitioners, acupuncturists, and massage therapists often varies by province [41]. In Canada, various provincial professional medical bodies have produced guidelines that address how physicians should deal with complementary therapies and practitioners [43, 44].

Natural Health Product Quality and Regulation

NHPs have complex product quality issues. Heterogeneity in product quality is common for various reasons. For example, for any given herbal product (e.g., *Echinacea*) there may be several plant species (*Echinacea purpurea, Echinacea pallida, Echinacea angustifolia)* with different phytochemical constituents and different physiologic effects [9]. Moreover differences in growing conditions, time of harvesting, parts used (e.g., aerial vs. root), and extraction methods used to prepare the herbal product may vary, compounding differences within and among manufacturers [45]. Some manufacturers have tried to reconcile this problem by standardizing some herbal products to a marker compound specific to that particular plant [46].

NHP product quality suffers from issues related to species misidentification, adulteration, and contamination. Various techniques, including microscopy, mass spectrometry and, more recently, DNA barcoding have been used to identify NHP quality issues [47]. Using DNA barcoding techniques, Newmaster and colleagues [46] found that 59% of herbal products sold on the North American herbal market were contaminated, and McCutcheon's [48] examination of Chinese herbal medicines found that 7% to 23.7% were adulterated [48]. Other reports of NHP quality concerns include a case of neonatal androgenization reported in Toronto where a pregnant mother consumed what she believed to be Siberian ginseng, but was actually Chinese silk vine [48].; herbal products being contaminated with bacteria, fungi, herbicides, and heavy metals; and cases of adulteration of NHPs with pharmaceutical agents [49]. Some herbal products have been found to be mislabeled, a problem that is often associated with the absence of regulation [48]. A 2003 US study found that 10% (6 of 59) of products marketed as *Echinacea* actually contained no *Echinacea* [50, 51].

Different regulatory approaches have been adopted internationally with regard to NHPs, In the US, the 1994 Dietary Supplements and Health Education Act reclassified NHPs as dietary supplements (i.e., neither food nor drug), and thereby exempted them from the usual safety and regulatory rules set by the Food and Drug Administration (FDA).[41, 52] As such, dietary supplements can be marketed without proven safety or efficacy, and it is the responsibility of the FDA to demon-

strate that a supplement is unsafe, which can be challenging. In Canada, Health Canada's Natural and Non-prescription Health Products Directorate regulates NHPs under the NHPs Regulations. Although Health Canada has not attempted to standardize NHPs, its regulations demand that manufacturers meet label claims and eliminate contamination and adulteration from products sold in the Canadian marketplace [3]. In order to help consumers to select and safely use NHPs, Health Canada has proposed regulation amendments to make NHP labels easier to read and understand [3]

Legal and Ethical Considerations

A number of legal and ethical considerations surround the use of complementary therapies. While many patients report that complementary therapy use helps them feel better, use of complementary therapies may delay the use of known effective treatment. Physicians should always inquire about complementary therapy use, monitoring high-risk patients such as those on dialysis, and consider the possibility of NHP-drug interactions as many patient mix NHPs with prescription medications. Clinicians may be hesitant to encourage the use of complementary therapies, as it is unfamiliar [53]. A survey of clinicians (N = 195) in Quebec found that 86.7% believed it was their role to advise patients on complementary therapies, but only 33.1% reporting being able to do so [54] Studies show that most medical students believe that integrative medicine information and education is important to their future practice and improves patient care [55].

Medical school curriculum is evolving to include more information about complementary therapies. For example, a Thai survey found that 50% of medical schools had integrated traditional, complementary, and alternative medicine training in their curriculum [56]. In the US over 50% of medical schools now include some complementary therapies education in their curriculum, with the majority of courses offered as electives [57–59]. At the same time, the number of US hospitals that offer complementary and integrative medicine as an ancillary clinical service has increased, with different hospitals addressing issues related to scope of practice, licensure and malpractice liability differently in their models of integrative health care [57]. There are over 12 residency programs that offer board-certified fellowships (by the American Board of Integrative Medicine or the Academic Consortium for Integrative Medicine and Health) in Integrative Medicine, with opportunities for elective residency experiences in the US [60]. Across North American, there has also been an increase in academic pediatric integrative medicine programs that develop and promote evidence-based integrative approach within Children's hospitals [22]. In Canadian medical programs, few formal courses on complementary therapies are offered as part of medical training [55]. Established in 2008, the Academic Consortium for Integrative Medicine and Health is a group of more than 70 universities, many of which offer educational courses for students and continuing education courses for practicing providers [55].

If a patient expresses interest in using complementary therapies for his or her illness, the physician should review the literature and advise accordingly. If there are sufficient safety and efficacy data, physicians may choose to recommend complementary therapies or refer their patient to a licensed complementary therapy provider [21] (see Fig. 72.1). If efficacy is uncertain, but the therapy is likely to be safe, patients may choose to try the therapy while their clinician continues to monitor them. If a therapy is felt to be unsafe, the patient should be advised to discontinue its use [9].

This chapter reviews the best available evidence with regard to NHPs, TCM and massage therapy. When pediatric evidence is unavailable, evidence from studies conducted among adults is discussed. Because limiting research by language can promote bias, our searches were conducted without language restriction.

It is important to highlight that the methodological quality of randomized controlled trials (RCTs) examining complementary therapies have been assessed and found equivalent to that

Fig. 72.1 Potential malpractice liability risk associated with complementary and integrative medical therapies [61]. (Used with permission from Cohen MH, Eisenberg DM. Potential physician malpractice liability associated with complementary and integrative medical therapies. Ann Intern Med. 2002;136(8):596–60)

of conventional interventions, while complementary therapies' systematic reviews have tended to be of better quality than those of conventional therapies [62, 63]. While this is reassuring regarding the overall quality of complementary therapies evidence, there have been calls for improvement in specific areas, such as Chinese TCM studies [64].

Nephrotic Syndrome

Various studies have examined complementary therapy treatments in children with nephrotic syndrome, including TCM and dietary modification. We identified several RCTs and systematic reviews of Chinese herbal medicines in the treatment of children with nephrotic syndrome.

TCM

Deng and colleagues [65] have published a systematic review that investigates the effect of Chinese medicine prescription on nephrotic syndrome.

Feng and associates [66] performed a Cochrane review that examined the benefits and harms of administering Huangqi or Huangqi formulations alone (oral solution or intravenous injection) or in addition to other drug therapies in treating nephrotic syndrome [66]. Haungqi is a traditional Chinese herbal medicine and is a root of *Huangqi membranaceus* (Fisch) Bge. var. *mongholicus* (Bge) Hsiao or *Huangqi membranaceus* (Fisch) Bge. or *Hedysarum polybotrys* Hand. –Mazz (fam. *Leguminoseae)* [66]. Haungqi and its formulations have been used commonly for nephrotic syndrome in China [66]. In the review, nine Chinese studies (n = 461 participants) were included; five included children only, one study was of adults only and three studies included both adults and children. Compared to control interventions, Huangqi and Huangqi formulations were found to have positive effects in treating nephrotic syndrome by increasing plasma albumin and reducing urine albumin excretion, blood cholesterol, triglycerides. At 3 months, more patients who had taken Huangqi showed improvement (relative risk (RR) 0.41, 95% confidence interval (CI) 0.20 to 0.84). However, due to small sample sizes and other methodological concerns including lack of blinding and unclear randomization, the evidence was insufficient to support the use of Huangqi formulations for the treatment of nephrotic syndrome. No adverse effects of Huangqi formulations were reported.

A previous systematic review [67] examined various interventions for preventing infections in children with nephrotic syndrome. Twelve studies (n = 762), all carried out in China, were included. Nine studies assessed prophylactic pharmacotherapy (e.g., intravenous immune globuli, Bacillus Calmette-Guerin) compared to placebo, no or other treatment. Three studies investigated the efficacy of the Chinese medicinal herbs tiaojining (one study) and Huangqi (astragalus) granules (2 studies). Tiaojining is a compound of six primary Chinese medicinal herbs (Shengdi, Zhimu, Zexie, Shanyurou, Xianlinpi, and Baihuasheshecao) associated with immunomodulation effects. *Huangqi astragalus* granules contain astragalus polysaccharides, astragaloside, amino acids and various microelements associated with improved immune function. The authors noted that the trials included in the review were of very poor methodological quality because most studies did not describe the methods used for randomization, blinding or withdrawals.

We describe here the three RCTs included in the Wu et al. [67] review that investigated the efficacy of Chinese medicinal herbs for nephrotic syndrome, one about tiaojining and two about Huangpi (astragalus) granules. The first study evaluated the efficacy of tiaojining for reducing the risk of infection among children with nephrotic syndrome (n = 60; aged 1–13 years) [68]. Children in the treatment group received 8 weeks of prednisone combined with various doses of tiaojining 3 times/day based on their age. Children in the control group received prednisone for the same duration. At the end of treatment tiaojining was effective in preventing infection, with a relative risk (RR) of 0.59 (95% CI 0.43 to 0.81, p = 0.001). No adverse events or safety data were presented.

The second study was a parallel group RCT in which 92 children (aged 2–13.7 years) were assigned to either prednisone in combination with Huangpi granules (dose between 7.5 and 15 g b.i.d. based on age) or control (prednisone) for 3 months [69]. The third study was a smaller parallel RCT, which allocated 38 children (aged 1.5–7 years) to either prednisone with 15 g of Huangqi granules for 3–6 months or control (prednisone only) [70]. When both studies were combined, Huangqi granules showed a significant beneficial effect (RR 0.62, 95% CI 0.47 to 0.83) in reducing the risk of infection in children with nephrotic syndrome. Both studies reported that no adverse events were observed. Although these findings seem promising, these studies had methodological limitations and no recommendations for use can be made without larger and better designed studies.

Tripterygium wilfordii Hook F (TwHF) is a traditional herbal medicine that has been used as an immunosuppressive agent to decrease proteinuria and preserve kidney function [71]. A systematic review published in 2013 evaluated the benefits of taking two standardized types of TwHF (ethanol-ethyl acetate extract and chloroform methanol extract only) alone or in combination with other drug therapies in primary nephrotic syndrome patients. The review included 10 Chinese RCTs (9 involving adults only and one involving both adults and children) with 630 patients. The main outcomes measured were complete or partial remission. Treatment with TwHF was administered for 4 to 8 weeks at dosages ranging from 0.5 mg/kg/day to 2.0 mg/kg/day, while the follow-up period ranged from 3 to 18 months. In 4 trials (n = 293) comparing TwHF to control, TwHF significantly increased complete or partial remission. The evidence was not statistically significant when comparing TwHF to prednisone or cyclophosphamide. No serious adverse events of TwHF were observed. The authors concluded that TwHF may have an add-on effect on remission in patients with primary nephrotic syndrome. The authors had major concerns regarding the poor quality of the included studies and called for better studies with larger sample size and adequate follow-up.

Standardised TwHF preparations are purported to be less toxic and have fewer serious adverse effects than non-standardised preparations [72]. Some common adverse events associated with standardised TwHF include gastrointestinal tract disturbances, leukopenia, thrombocytopenia, rash, skin pigmentation, and malfunction of the reproductive system [73]. These adverse effects reportedly resolve after adjusting the dose or discontinuing TwHF treatment [66, 74]. In Chinese clinical practice, many physicians are unwilling to use TwHF in children due to potential reproductive system complications [75]. Serious adverse events associated with use of non-standardized TwHF include severe liver dysfunction, aplastic anaemia, and death [72]. More evidence is needed regarding long-term use and safety of TwHF in children with nephrotic syndrome before recommendations can be made for its clinical use.

An earlier RCT carried out by Wang et al. (2005) [76] compared the effects of Tripterygium glycosides with that of cyclophosphamide in 80 children (aged 1–13 years) with relapsing primary nephrotic syndrome. Children in the experimental group (n = 39) received 1 mg/kg of tripterygium glycosides orally 2 or 3 times each day for 3 months. The control group (n = 41) received 10 mg/kg/day of cyclophosphamide by intravenous pulse over 3–6 months. All children also received tapering doses of prednisone over a period of 12–18 months. After follow-up for 3–7 years, no significant differences in the relapse rates in the two groups were observed ($p > 0.05$). The researchers concluded that treatment with tripterygium glucosides and prednisone was as effective as cyclophosphamide and prednisone, although this study was not designed as an equivalence trial. More side effects were reported in the control (n = 21) than the experimental group (n = 2), but in both groups symptoms resolved after treatment was discontinued. The control group side effects included one case of rising guanosine triphosphate (GTP) levels, 3 cases of transient leukocytopenia, 11 cases of alopecia, and 6 cases of gastrointestinal disturbance. The experimental group had a case of transient leukocytopenia and another of rising GTP. Safety information regarding tripterygium glycosides beyond this trial are unknown; therefore, more studies are needed.

The efficacy of Chai-Ling-Tang (Sairei-to), a preparation of 10 Chinese medicinal plants, was evaluated in 69 children (aged 5–12 years) with steroid-dependent nephrotic syndrome [77]. Over 3 weeks, children in the experimental group (n = 37) received tapering doses of prednisone until they had protein-free urine and consistent doses of Chai-Ling-Tang for 1.5 years. The control group (n = 32) received tapering doses of prednisone plus 2.5 mg/kg/day of cyclophosphamide for 8 weeks. All study participants were monitored for at least 2 years. No significant differences were reported between the experimental and control group in terms of outcomes measured, including relapse time, time to absence of

proteinuria, amount of prednisone intake, and side effects. Chai-Ling-Tang was therefore equivalent to cyclophosphamide in treating steroid-dependent nephrotic syndrome. The authors suggested that Chai-Ling-Tang could be used as an alternative where patients are either non-responsive or have severe side effects from cyclophosphamide. Beyond this RCT, the safety of Chi-Ling-Tang is not known. More research is needed before any considerations for its routine clinical use in pediatric patients with nephrotic syndrome.

Immunoglobulin A (IgA) Nephropathy

There has been a substantial amount of research conducted on the effectiveness of omega-3 fatty acids (O3FA) as a therapy for individuals with IgA nephropathy (IgAN). Most of the research comes from studies involving adult patients, with a few that included children. There are also RCTs that assessed the usefulness of TCM and Vitamin E in IgAN patients.

Omega-3 Fatty Acids

A 2017 meta-analysis [78] of 9 RCTs (N = 444) evaluated the effects of O3FA on renal function and subsequent end-stage kidney disease (ESKD) events in patients with IgAN (7 RCTs) and CKD (2 RCTs). Two trials were in the US, 2 in Japan, 3 in Europe and 2 in Australia. Participants ranged from 23 to 106 patients, and follow-up ranged from 2 to 76.8 months. O3FA supplementation was significantly associated with both a lower risk of proteinuria (SMD: −0.31; 95% CI: −0.53 to −0.10; p = 0.004) and ESKD (RR: 0.49; 95% CI: 0.24 to 0.99; p = 0.047). There was no evidence to suggest that O3FA has an effect on creatinine clearance (CrCl) and estimated glomerular filtration rate (eGFR). There was no mention of adverse effects in this meta-analysis. The limitations of the meta-analysis included pooled data (due to unavailability of data), and not having sufficient relevant trials to conduct subgroup analyses for ESKD or examine for publication bias [78].

Chou et al. (2012) [79] conducted a meta-analysis to evaluate the effects of O3FA on GFR and proteinuria in IgAN. The review included 5 RCTs reported between 1989 and 2009 and included 233 adults. One hundred and sixteen patients received O3FA and 117 patients received no treatment (3 studies) or placebo (2 studies). Patients received therapy for 6–48 months. There was no significant difference in renal function between O3FA and control groups. No significant differences in renal function or proteinuria were observed when analysis was based on comparing patients who received high (> 3 g/day) and low (≤ 3 g/day) O3FA doses. In addition, no dose-effect relationships between O3FA and renal function or proteinuria were observed. This meta-analysis included a small number of RCTs with study design limitations that included small sample size, short duration and variable stages of renal disease. The authors concluded that there was no evidence to suggest that O3FA has significant effects on renal function compared to controls.

An earlier meta-analysis published in 2009 [80] combined evidence from 17 RCTs to determine the effectiveness of n-3 long-chain polyunsaturated fatty acid (n-3 LCPUFA; also known as OSFA) supplementation on change in urine protein excretion and GFR in 626 adults with various chronic kidney conditions [80]. The underlying conditions in the patients were IgA nephropathy (5 trials), diabetes (7 trials), lupus (1), and kidney disease of mixed etiology (3). One trial included in the review did not report the underlying kidney disease of the study participants. Four of the IgAN trials included in this review were the same studies included in the 2012 meta-analysis [58]. The dose of n-3 LCPUFAs administered ranged from 0.7 to 5.1 g/day and follow-up was 6 weeks to 48 months. Supplementation with n-3 LCPUFA significantly reduced urine protein excretion compared to control, but there was no effect on GFR. Side effects were reported in 5 of the 17 trials and included; gastrointestinal side effects including nausea, fish aftertaste, and smell/taste of fish on eructation [80].

In a US based double blind RCT, Hogg et al. [81] compared the efficacy of prednisone or O3FA

to placebo in 96 children and young adults with IgAN. A total of 23 patients were randomly assigned to receive O3FA 4 g/day (1.88 g/day of eicosapentaenoic acid, 1.48 g/day docosahexaenoic acid) for 2 years. Another 33 patients received alternate day prednisone at 60 mg/m^2 for 3 months, then 40 mg/m^2 for 9 months and 30 mg/m2 for 12 months. The last group of 31 patients received placebo. The main outcome measure was time to failure, defined as an eGFR ≤60% of baseline. The investigators found no differences with respect to time to failure between the three groups. Significantly more patients in the prednisone group compared to the placebo group experienced heartburn (48 vs. 16%; p = 0.018), and increased appetite (73 vs. 32%; p = 0.001). Other adverse events such as weight gain and anxiety were not statistically different between the two groups.

The current body of evidence assessing the efficacy of fish oils for the treatment of IgAN is from relatively small trials. More evidence from large, randomized, double-blind, placebo-controlled studies is required. Fish oils are considered extremely safe for adults when taken in recommended doses. Common adverse effects of fish oil supplements include fishy after-taste and gastrointestinal complaints such as nausea and dyspepsia [82]. Fish oil ingestion above 3 g/day may result in an increased risk of bleeding, especially in patients taking warfarin [82]. Based on this safety information, the inclusion of fish oils at recommended dosages in the treatment of adult IgA patients may be considered. The potential use of fish oils in children warrants further examination.

TCM

An RCT conducted in pediatric patients (n = 62) found that treatment with both TCM and Western medicine improved symptoms in pediatric patients with renal biopsy diagnosed IgAN [83]. Children (aged 5–14 years) were randomized to receive TCM and Western medicine (n = 34) or Western medicine only (n = 28) for 6 months. The Western medicine group took dipyridamole, captopril, common threewingnut, prednisone or cyclophosphamide according to their illness condition. Based on the clinical features, a range of TCM preparations such as Huang Qi (RadixAstragali seu Hedysari), Jian Qu (Massa Medicata Fermentata Fujianensis), fresh Mao Gen (Radix Rubi Parvifolii) and Lu Gen (Rhizoma Phragmitis) were prescribed, leading to considerable variability in TCM preparations and dosing used between patients. Eight children dropped out from the study; 2 from the TCM group and 6 from Western medicine group. After 6 months of treatment, there was no significant difference in efficacy between the two groups. The cure plus marked effect rate in the group treated with both TCM and Western medicine was higher compared to the group treated with Western medicine alone. One adverse event occurred in a patient who received TCM and Western medicine: the patient had increased alanine aminotransferase, which returned to normal after 1 week of "liver-protecting treatment" (not specified).

Safety information regarding TCM beyond the adverse effect described in the study is unknown. More studies and safety information are required before TCM herbals can be recommended for routine clinical use.

Vitamin E

A pilot double-blind, placebo-controlled trial found reduced protein/creatinine ratios in 55 children with early or mild IgAN (biopsy-proven) who were given vitamin E as antioxidant therapy [84]. Children were randomized to receive placebo (n = 28) or vitamin E capsules (n = 27). The dose of vitamin E amount was 400 IU/day if they weighed less than 30 kg and 800 IU/day if they weighed more than 30 kg. Thirty-eight patients completed 1 year of the follow-up, with no side effects reported in either the vitamin E or the placebo group. At study conclusion, there was no significant difference in GFR or in hematuria. The urine protein to creatinine ratio was significantly better in the vitamin E group (0.61; 1.37 mg/mg) vs. the placebo group (0.24; 0.38 mg/mg; p = 0.013). This small pilot study supports the use of vitamin E in pediatric patients with mild IgAN. However, larger studies investigating the long-term treatment effects of taking vitamin E in patients with mild to severe IgAN are needed.

Vitamin E is generally considered to be a safe, inexpensive product, with no clinically relevant side effects [84] when taken at recommended dosages for short periods of time.

Urolithiasis

A number of studies have assessed complementary therapies for urolithiasis and renal colic. The research has mainly focused on adults and the efficacy of acupuncture, with one study each investigating the efficacy of massage and probiotics in treating urolithiasis.

Acupuncture

Acupuncture involves the insertion of fine sterile needles into specific acupuncture points on various parts of the body.

A systematic review investigated the role of complementary therapies (including acupuncture) in decreasing analgesia requirement and alleviating anxiety during extracorporeal shockwave lithotripsy (ESWL) [85]. The systematic review included only studies published in English; they had sample sizes from 35 to 100 patients. In the five studies (4 RCTs and 1 prospective design without a control), 235 adult kidney stone disease (KSD) patients received different types of acupuncture: electro-acupuncture (2 studies), sham acupuncture and electro-acupuncture (2 studies), sham acupuncture, electro-acupuncture and auricular acupuncture (2 studies), and unspecified acupuncture (one study) d. The primary outcome measures across the studies were visual analogue scale (VAS) for pain and the State-Trait Anxiety Inventory (STAI) for anxiety. Four studies reported a statistically significant lowered pain and/or anxiety score. Two studies also reported a decrease in analgesia or opiate use. No major or minor side effects were noted with the use of acupuncture. The reviewers concluded that acupuncture reduced pain as well as anxiety and should be considered for use in outpatient urological procedures [85].

An RCT [86] in Turkey compared the efficacy of diclofenac, acetaminophen and acupuncture in treating urolithiasis-driven renal colic. Adult patients (N = 121) were divided into 3 groups: Group A (n = 40) was treated with 1 g of intravenous acetaminophen over 15 min, Group B (n = 41) was treated with acupuncture applied to the urinary bladder meridian points the side with acute renal colic pain (UB-21 to 24, UB-45 to 48), and Group C (n = 40) was treated with a 75-mg intramuscular injection of diclofenac sodium. VAS and verbal rating scale (VRS) were used to assess drop in pain intensity after 10, 30, 60, and 120 min. After 10 min, the largest decrease in VAS and VRS scores was observed in patients who received acupuncture ($p < 0.05$). At subsequent intervals (30, 60, and 120 min) either diclofenac or acetaminophen had higher decreases in VAS and VRS scores compared to acupuncture. Adverse effects were reported in individuals who received acetaminophen (one patient had an allergic reaction and another reported dizziness and vomiting) and diclofenac (one patient had rash, and 2 patients had abdominal burning/pain). No adverse effects were reported in the patients that received acupuncture treatment. The authors concluded that acupuncture is a viable alternative treatment modality for renal colic.

A RCT conducted in Tunisia [87] randomized 115 renal colic patients into two groups. The first group (n = 61) received 0.1 mg/kg intravenous morphine every 5 min until the pain score as measured using the VAS dropped by at least 50% of its baseline value. The second group (n = 54) received a 30-min acupuncture session where needles were inserted to urinary bladder meridian points on the side of the pain (UB21–24, UB26, UB45–49). VAS was used to assess pain intensity at baseline and at 10, 20, 30, 45, and 60 min after treatment. From the tenth minute until the end of the intervention, acupuncture was associated with a faster and higher analgesic effect compared to morphine ($P < 0.05$). Forty-two side effects (namely, dizziness, nausea and vomiting and drowsiness) were reported in the morphine group compared to 3 (1 needle blockage and 2 reports of itching/rash/bleeding at needle insertion point) in the acupuncture group ($P < 0.001$). The investigators suggested that acupuncture rep-

resents an effective alternative treatment for patients with high risk of adverse events due to morphine and its use be further assessed [87].

A Chinese RCT evaluated the clinical effects of body and auricular acupuncture compared to medication in treating renal colic in 60 participants (aged 16–45 years) [88]. Renal colic patients in the intervention group received body acupuncture (acupoints: bilateral Zusanli (ST36), Yanglingquan (GB 34), and Ashi points on the affected side) and auricular acupuncture (ear points: Shenmen (TF 4), Kidney (CO 10), and Bladder (CO 9) for 30 min). Patients in the medication group received an intramuscular injection with pethidine 50 mg and atropine 1 mg. Pain relief and analgesic effects were measured using a patient numeric rating scale. Patients in both groups reported reduced pain. The total effective rate was higher in the acupuncture group (89.7%) than in the medication group (77.4%); ($p < 0.05$). The researchers concluded that acupuncture could be used as an alternative to pethidine and atropine in treating renal colic.

Hodzic et al. (2007) [89] investigated whether acupuncture could lower or replace the need for analgesics in ESWL of kidney stones in adult patients (aged 17–85 years) in Germany. The control group (n = 78) received 50 mg pethidine plus 10 mg diazepam. Patients randomized into the treatment group (n = 86) received acupuncture at various acupoints. The main outcome was self-rated pain scores. Pain sensation was rated prior to ESWL and for every minute until 21 min after the therapy started, and for 10 min after therapy stopped. All patients who reported a pain sensation higher than 5, received analgesics delivered intravenously. Twenty percent of recruited patients refused acupuncture treatment and were excluded from the study. There was no mention of adverse events. The mean pain score throughout the treatment was significantly less for the treatment group (2.6) vs. the control group (3.2) ($p < 0.0001$). Acupuncture was reported to provide significantly more effective analgesia than pethidine and diazepam. It is important to note that 23% (n = 20) of patients in the acupuncture group needed additional pain medication [89].

Another RCT assessed the clinical effectiveness of electro-acupuncture compared to a combination of tramadol and midazolam in relieving pain during outpatient ESWL [90]. Thirty-five adults with kidney stones were allocated to undergo lithotripsy with a third generation lithotriptor following treatment with either electro-acupuncture (treatment group; n = 17) or tramadol/midazolam (control group; n = 18) for sedation and analgesia. For patients in the treatment group, the same licensed acupuncturist administered 20-min electro-acupuncture stimulation with 2–4 Hz frequency for 30 min prior to ESWL. Patients in the control group received treatment with tramadol (1.5 mg/kg) 30 min before the start of lithotripsy and midazolam (0.06 mg/kg) 5 min before ESWL. The main outcome, pain intensity, was measured using a VAS. Although the electro-acupuncture group was found to have lower VAS compared to the medication group, this finding was not statistically significant. Similarly, there was no significant difference in stone-free rates between the two groups. There were no adverse effects in the electro-acupuncture group. Participants in the control group experienced moderate adverse events such as orthostatic hypotension and dizziness, which did not warrant their removal from the study. The authors concluded that electro-acupuncture is an effective alternate pain relieving method without any demonstrable side effects.

In an earlier RCT, Lee and colleagues [91] investigated the effect of acupuncture compared to a conventional analgesic (Avafortan, which has since been discontinued) in the treatment of 38 adult males with renal colic from urolithiasis [91]. Patients were randomized to either receive acupuncture treatment (n = 22), or an intramuscular injection of Avafortan (n = 16). Renal colic pain scores were evaluated before treatment and 30 min following treatment. There was no significant difference in the reduction in mean pain score between the two groups, but acupuncture had a significantly faster analgesic onset than Avafortan ($p < 0.05$). Nearly half (n = 7) of the Avafortan group experienced side effects; 3 cases of skin rash, 2 cases of tachycardia and one case

of facial flushing and drowsiness. Patients in the acupuncture group did not experience any adverse effects. The authors contended that acupuncture could be a good alternative for the treatment of renal colic.

Serious adverse events associated with acupuncture are rare. In a prospective survey of 229,230 patients (mean age 46 years) 8.6% patients reported experiencing at least one adverse event [92]. Bleeding (6.1% of patients) and pain (1.7% of patients) were the most common adverse events reported. A systematic review evaluating the safety of pediatric needle acupuncture calculated a mild adverse event incidence per patient of 168 in 1422 patients (11.8%; 95% CI: 10.1% −13.5%). In the review, adverse events included bleeding, pain, bruising and worsening of symptoms. Although rare, serious adverse events associated with acupuncture in children have occurred such as infections, thumb deformations, cardiac rapture (a fatal consequence of myocardial infarction) and hospitalization [64]. Some of the acupuncture related serious adverse events may have been avoidable as they were likely caused by substandard practice. The evidence suggests pediatric acupuncture is safe where it is performed by appropriately trained acupuncture practitioners [64, 93].

Massage

A retrospective RCT assessed the use of vibration massage therapy after ESWL in 103 adults with lower caliceal stones [94]. Patients in the experimental group (n = 51) received ESWL and 20- to 25-min sessions of vibration massage therapy (applied at a speed of 3800 rpm) in 2-day intervals for 2 weeks. The control group (n = 52) received ESWL alone. The stone-free rates in the experimental and control groups were 80% and 60%, respectively ($p = 0.003$). The rate of stone recurrence was significantly higher in the control group than in the experimental group ($p = 0.0006$). However, there were more reports of renal colic in the experimental group ($p = 0.03$) than in the control group. No trials investigating the use of vibration massage therapy in children were identified.

While therapeutic massage generally carries a low risk of complications, information pertaining to the safety of vibration massage is unknown.

Probiotics

Probiotics have been described as live microorganisms which can provide a health benefit to the host when ingested in adequate amounts [95].

A small pilot study investigated whether a mixture of freeze-dried lactic acid bacteria could reduce oxaluria in 6 adults with idiopathic calcium oxalate urolithiasis and hyperoxaluria [96]. During a 4-week period, all patients received 8×10^{11} freeze-dried lactic acid bacteria (*L. acidophilus, L. plantarum, L. brevis, S. thermophilus, B. infantis*) and did not eat foods rich in oxalate (e.g. spinach, chocolate, peanuts, cocoa and rhubarb). There was a significant decrease in 24-h oxalate excretion compared to baseline ($p < 0.05$). The treatment was associated with a mean reduction in oxaluria of approximately 30 mg/day [96]. The reduced levels were sustained for at least 1 month after the end of treatment.

Probiotics are generally safe in healthy individuals, and some probiotics (*L. acidophilus*, *Lactobacillus* GG, *Saccharomyces* sp.) have been found safe for use in children, when administered in appropriate doses [97]. Case reports of serious infections including bacteremia, fungemia, endocarditis, liver abscess, septicemia and meningitis have been associated with probiotics [95, 98]. The risk of adverse effects associated with probiotics is high in patients that are immunocompromised, including those with indwelling medical devices [95, 98].

Urinary Tract Infections

Various NHPs have been used as a prophylaxis for urinary tract infections (UTIs) including cranberry, probiotics and vitamin A. The evidence evaluating the efficacy of cranberry juice in pediatrics is increasing, while the number of pediatric trials evaluating probiotics and vitamin A remains limited. Studies often have small sample sizes and other methodological limitations.

Cranberry

In vitro evidence suggests that proanthocyanidins (tannins) and fructose found in cranberries have antibacterial activity [98] via preventing bacteria from adhering to the walls of the bladder and thus decrease the development of UTIs [99].

In 1998 Jepson and colleagues [100] published a systematic review of cranberry trials for the prevention of UTIs which they updated in 2004, 2008 and in 2012 [101]. The 2012 update included 24 studies which compared the effectiveness of cranberry in different forms and combinations (concentrate juice, tablets, liquid concentrate syrup and capsules/tablets) to placebo, no treatment, water, methenamine hippurate, antibiotics or lactobacillus. Due to design and data shortcomings, 11 studies were excluded, leaving 13 studies (2462 participants) for the meta-analyses. Studies were analysed together and separately by participant subgroups (e.g., children with first or subsequent UTI, participants with a history of recurrent lower UTI, and pregnant women). Cranberry products did not significantly reduce the risk of repeat UTI across the combined 13 studies (overall RR 0.86, 95% CI 0.71 to 1.04) or any subgroup populations analysed. Although not significant, the pediatric subgroup analysis, which compared cranberry juice to placebo/control, suggested the greatest effect (RR 0.48, 95% CI 0.19 to 1.22). Many of the studies reported low compliance and a high number of withdrawal/dropout due to problems with the palatability/acceptability of the cranberry product, mainly the cranberry juice. The authors concluded that cranberry products compared to placebo was not effective in most population groups, and that any benefit in sub-groups is likely very small.

The three pediatric RCTs included in the review are described. The largest pediatric RCT investigating the efficacy of cranberry juice in preventing UTI recurrences was performed in Finland and involved 263 children (1–16 years) with a verified UTI in the previous 2 months [102]. Children were randomized to receive 1 or 2 daily doses of up to 300 ml per day of cranberry juice containing 41 grams cranberry concentrate (n = 129) or placebo (n = 134) for 6 months and monitored for UTI recurrences over 12 months. Nearly 45% of participants in this study were toddlers, the mean age being 3.8 years (standard deviation (SD) 2.5) in the cranberry group and 4.5 years (SD 2.9) in the placebo. Eight children were excluded from the study due to protocol violations leaving 255 children included in the analysis. There were no significant differences observed in the proportion of children that had at least one UTI after entering the study; 20 vs. 28 children in the intervention and controls, respectively. The UTI incidence per person-year at risk was 0.16 episodes lower in the cranberry group (95% CI, 2.31 to −.01; p = 0.035) [102]. Twenty-seven children dropped out: 16 from the cranberry group and 11 from the placebo group. The authors only cited the main reason for withdrawal, which was reluctance to drink the juice (7 from the intervention and 6 from the placebo groups).

The second RCT [103] compared the effectiveness of cranberry juice vs. *Lactobacillus* in preventing the recurrence of UTIs in 84 girls (aged 3–14 year; mean age 7.5) in Italy. The girls were randomized to receive, for a 6-month period, either: a daily 50 ml dose of cranberry juice containing 7.5 g of cranberry concentrate and 1.7 g of lingonberry concentrate juice in 50 ml of water, without sugar additives (n = 28); 100 ml of *Lactobacillus* drink containing 4×10^7 cfu of *Lactobacillus* GG/100 ml on 5 days a month (n = 27); or control (n = 29). Four children dropped out due to poor compliance to the protocol, two from the control group and one from each of the other groups. During the 6 months of observation, reduction in UTIs was significant ($p < 0.05$) for the cranberry group compared to the other groups: UTI occurrence was 5/27 (18.5%) in the cranberry group; 11/26 (42.3%) in the *Lactobacillus* group; and 18/27 (48.1%) in the control group.

The third RCT [104] recruited children (aged 1 month to 13 years) with more than two UTIs in the last 6 months. Children (n = 192) in the treatment group received a nocturnal dose of 0.2 ml/kg of cranberry syrup (5 ml of the syrup contained 36 mg of highly bioactive proanthocyanidin (PACs); n = 75). Children in the control group

ingested 0.2 ml/kg of a color-masked suspension of trimethoprim at a concentration of 8 mg/ml just before the evening meal (n = 117) for a year. The incidence of UTIs was 18.9% (n = 18) in the trimethoprim group (95% CI:11%–26.3%) and 8.4% (n = 8) (95% CI: 2.8%–13.9%) in the cranberry group. However, there was no significant difference between the two groups. Adverse reports included: gastrointestinal intolerance (5 in the trimethoprim group and 2 in the cranberry group) and a case of rash in each group. The authors concluded that cranberry syrup was non-inferior to trimethoprim in preventing recurrent UTIs.

In 2015 Durham and colleagues evaluated the use of cranberry products for the prevention of UTIs in pediatric patients in a literature review. Of the eight trials reviewed: 3 were in healthy children [102, 103, 105] and 5 in children with underlying urogenital abnormalities [104, 106–109]. The literature review concluded that cranberry products may be an effective option for preventing recurrent UTIs in healthy children, while in children with anatomical abnormalities the findings were inconclusive. In two trials that compared the effectiveness of cranberry juice to antibiotics in children with underlying urogenital abnormalities, cranberry juice was found to have comparable efficacy to antibiotics [104, 108]. This finding is important as cranberry juice could be an alternative to the use of antibiotics at a time when overuse of antimicrobial agents and antibiotic resistance is on the rise [110].

A Taiwanese RCT [111] not included in the previously described reviews evaluated the effects of highly concentrated cranberry juice in preventing repeat UTI's in boys aged 6–18 years (55 uncircumcised and 12 circumcised). Over a 6-month period, uncircumcised boys in group A (n = 28) drank 120 mL of cranberry juice daily and in group B (control) drank placebo. A third control group C of circumcised boys also drank a placebo juice. The main outcome was a confirmed urine culture of symptomatic UTI. The results showed that recurrent UTI's was 25%, 37% and 33.3% in groups A (cranberry), B and C, respectively. No adverse effects were observed in the study. The findings support the use of concentrated cranberry juice to reduce the number of repeated episodes of UTI in uncircumcised boys compared to placebo (in circumcised and uncircumcised boys).

Cranberry products (mostly as juice) have been observed to decrease the risk of UTIs in healthy children, and in children with urogenital abnormalities cranberries appear to be just as effective as antibiotic prophylaxis. The primary adverse effects reported by children following cranberry juice intake are mild: sour taste and a lingering aftertaste [99]. No serious adverse effects have been reported from cranberry fruit; however, large intake of cranberry products should be used with caution to prevent the potential for gastrointestinal distress and diarrhea. The use of sweetened cranberry products should be used with caution in diabetic and overweight individuals as it unnecessarily exposes them to carbohydrates and calories [110].

It is safe to use cranberry in clinical practice; however, it is important to note that information regarding the amount and concentration of proanthocyanidins (PACs) in commercially available cranberry juice products may not be known. In addition, PACs are degradable molecules, which means the manufacturing data and storage practices are crucial factors in determining the bioavailability of PACs.

Probiotics

A RCT compared the effect of probiotic and placebo in preventing UTIs in children (4 months to 5 years) with uncomplicated UTI [112]. Children (N = 181) were randomized to receive a probiotic mixture of Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium bifidum, and Bifidobacterium lactis (n = 91) or placebo (n = 90) for a total of 18 months of therapy. The primary outcome measure was being UTI free (composite cure) during the study period. At 18 months, compared to children who received placebo, the composite cure was significantly (p = 0.02) higher in children treated with probiotics (96.7% vs 83.3%, respectively). The researchers reported that there were no specific adverse events among the participants who received the probiotic mixture during the course of therapy.

The findings by Sadeghi-Bojd (2020) [112] are consistent with those reported in an RCT [113] that compared probiotic to antibiotic prophylaxis for the prevention of UTIs in 129 children with persistent primary vesicoureteral reflux (VUR) for 1 year prior to the study [113]. Children were randomized to receive probiotics twice daily (*Lactobacillus acidophilus* 10^8 colony forming units (cfu) twice a day, n = 60) or once a day antibiotic (trimethoprim/sulfamethoxazole 2/10 mg/kg, n = 60) at bedtime. The incidence of UTIs was 18.3% in the probiotic group and 21.6% in the antibiotic group, but the difference was not statistically significant. Two children from the probiotic group and one from the antibiotic group dropped out due to noncompliance. The authors concluded that probiotics prophylaxis was as effective as antibiotic prophylaxis in preventing UTIs in children with persistent primary VUR [113].

An earlier double blind RCT assessed whether probiotics could prevent UTIs in 585 preterm infants (birth weight < 1500 g or gestational age < 33 weeks) [114]. Newborns were randomized to receive standard milk enriched with 6×10^9 cfu of *Lactobacillus* GG (n = 295) or standard milk with placebo (n = 290) once a day beginning with the first feed and continued until they were discharged (mean 48 days). The occurrence of UTIs in the babies receiving probiotic-enriched milk was less compared to that observed in the control group (3.4% vs. 5.8%). This difference was, however, not statistically significant.

Unlike the above studies a meta-analysis that included 10 studies (N = 2865; 61.5% boys) found no beneficial effect of probiotics in preventing UTIs (RR = 0.94; 95% CI 0.85–1.03; p = 0.19) and recurrence (RR = 0.93; 95% CI 0.85–1.02; p = 0.14) in children and adolescents [115]. When probiotics were used as adjuvant therapy to antibiotics, the incidence of UTI was reduced (RR = 0.92; 95% CI 0.85–0.99; p = 0.02). The studies in the meta-analysis included children with the following characteristics: admitted into intensive care (4 studies, n = 1681); with vesicoureteral reflux (3 studies, n = 333); first-time UTI (1 study, n = 80), preterm (1 study, n = 585) and preterm with acute pyelonephritis (1 study, n = 186). The outcomes measured were incidence of UTI (5 studies), recurrence of UTI (4 studies), and both were evaluated in one study. The meta-analysis had several limitations (7 studies were of poor quality) that prevent generalizing the findings. Moreover, most studies were conducted in preterm infants or children that were admitted into intensive care.

An earlier 2015 [116] systematic review (N = 725) that included 9 trials (4 included children) comparing the effectiveness of probiotic use to no treatment, placebo or antibiotics in children and adults with complicated UTI also reported no significant benefit for probiotics. Adverse effects reported in the studies included diarrhoea, nausea, vomiting, constipation, and vaginal symptoms. The reviewers noted that most studies had small sample sizes and poor methodological reporting limiting rigorous evaluation [116].

Current evidence on the efficacy of probiotics in preventing or reducing the recurrence of UTI in children is inconclusive. Additional research with larger sample sizes is needed to provide more conclusive evidence and to answer many unresolved questions such as what is the most effective probiotic strain, the ideal combination of strains, effective doses, and the safety of long-term use.

Vitamin A

Vitamin A has anti-inflammatory properties, which in animal studies have been demonstrated to reduce the damage to kidneys after glomerulonephritis [117].

An Iranian RCT evaluated the efficacy of vitamin A supplementation in combination with antibiotics for improving UTI symptoms and preventing renal scarring in girls (N = 90, aged 2–12 years) with acute pyelonephritis (APN) [118]. Children were randomized to receive 10 days of oral vitamin A (n = 36) or placebo (n = 38) in addition to antibiotics during the acute phase of infection. Sixteen girls were lost to follow-up. The main outcomes were duration of UTI symptoms during trial treatment period and

renal scarring as measured by 99 mTc-DMSA scan. The duration of fever (vitamin A: 1.8 days, placebo: 3.1, p = 0.0026), urinary frequency (1.3 vs. 2.8, p = 0.003) and poor feeding (2.3 vs. 4.2, p = 0.005) were significantly less in the vitamin A group than in the placebo group. The vitamin A group showed renal lesion improvement on DMSA scan compared to the placebo group: 63.8% (23 patients) vs. 21% (8 patients), respectively (P < 0.0001) [118] No vitamin A intolerance or adverse effects were observed in the study. The researchers concluded that vitamin supplementation was effective in improving UTI clinical symptoms and reducing scarring following APN, but called for larger studies with longer follow-up.

A meta-analysis [119] of four studies assessed the efficacy of vitamin A administration in addition to antibiotic therapy on renal damage in children aged 1–144 months (N = 248) after APN (120 in the experimental group and 128 in the control group). Three studies were performed in Iran and one was performed in China. The doses and duration of vitamin A were slightly different across studies. Vitamin A was inversely associated with renal damage (relative risk 0.53, 95% confidence interval 0.43–0.67) when compared with placebo after an average follow-up of 5 months. Although vitamin A may have a preventive effect on renal damage in children with APN, these findings need to be further investigated because the few studies in the meta-analysis were of low methodological quality (risk of selection and attrition bias) [119].

A small Turkish RCT not included in the meta-analysis [119] examined the effectiveness of a high dose of vitamin A in preventing UTIs in 24 children (mean age 6.3 ± 1.09 years) with non-complicated lower recurrent UTI [120]. All children received 10 days of antimicrobial therapy and were also randomly assigned to vitamin A (n = 12) or placebo (n = 12). After eradication of infection, children in both groups received antimicrobial prophylaxis and were followed for 1 year. Among children who received vitamin A supplementation, the recurrence rate of UTIs was reduced from 3.58 to 0.75 (p = 0.002) in the first 6 months. During the same period, UTI recurrence rate in the placebo also decreased, but was not statistically significant. There was no mention of adverse events. The authors concluded that vitamin A may have a beneficial role as an adjuvant for treatment of recurrent UTI [120].

Vitamin A is safe at usual doses; however, when taken at more than 25,000 IU, toxicity may include elevated intracranial pressure, severe liver injury (cirrhosis), bone and cartilage damage, and diarrhea [121]. Because vitamin A is fat soluble, it can be stored in the human body and released long after it is taken. There have been two reports of vitamin A toxicity in two hemodialysis patients who had consumed large quantities of vitamin A. The two patients had serum vitamin A levels of 220 ug/dl and 380 ug/dl respectively (normal vitamin A is between 30–95 ug/dl) [122].

Larger and adequately designed studies are needed to confirm whether vitamin A is an effective prophylaxis that alleviates renal scarring (damage) and to determine what dosage is safe for long-term use in the prevention of UTIs in children.

Vitamin D

Vitamin D is known to have an effect on bone and mineral homeostasis in the human body. What is not yet known is its immunoregulatory role in enhancing the body's defenses against bacterial and viral infections [123].

A 2014 small RCT [124] in Iran investigated the effect of Vitamin D supplementation on prevention of recurrent UTI in 68 children and adolescents. Study participants either received Vitamin D (1000 IU/daily) (n = 33) or placebo (n = 32) for 6 months. The differences in the frequency of UTIs was not significantly different between the two groups studied (P = 0.72). There was no mention of adverse effects. The author concluded that vitamin D at the dose levels taken in this study had no significant effect on preventing recurrent UTI, and suggested that more research is required with higher doses of vitamin D and longer follow-up [124].

Chronic Kidney Disease

Complementary therapies that have been used to treat symptoms associated with CKD include NHPs such as folic acid, L-arginine, rhubarb, and various TCM herbal medicines. More conventional approaches have included evaluations of a low-protein diet.

Folic Acid

One of the leading causes of death among CKD patients is cardiovascular disease. Some research has been conducted to evaluate the effects of folic acid supplementation on endothelial function and homocysteine levels. Bennett-Richards et al. [125] performed a crossover RCT involving 25 children (aged 7–17 years) with CKD, who over an 8-week period received 5 mg/m^2 of folic acid per day, followed by an 8-week washout period, and 8 weeks of placebo [125]. Twenty-three children completed the study. During the folic acid phase, homocysteine levels decreased (10.3 mol/L to 8.6 mol/L, p = 0.03), while no decrease in levels was observed during the placebo period. In addition, during the folic acid phase, endothelial-dependent flow-mediated dilatation improved significantly from 7.21 (2.8%) to 8.47 (3.01%) (p = 0.036). The authors speculated that, although folic acid supplementation in adults with CKD have largely been negative, the positive finding in children may be linked to the timing of treatment because atherosclerosis in children is at an earlier stage of its natural history. Folic acid is safe when taken at recommended dosages. More studies in pediatric populations are needed with relevant clinical outcomes before folic acid supplementation can be recommended in pediatric CKD.

Oral L-Arginine

In a crossover RCT, Bennett-Richards et al. [126] examined the effect of dietary supplementation with oral L-arginine on the response of the endothelium to shear stress in 21 children (aged 7–17 years) with CKD and documented endothelial dysfunction. During the treatment phase, each child received 2.5 g/m^2 or 5 g/m^2 of oral L-arginine three times a day for a 4-week period. This was followed by a rest period of 4 weeks and then 4 weeks of placebo. Twenty-one children completed the study as 4 children withdrew (2 due to L-arginine taste complaints, one due to L-arginine related nausea and another received a renal transplant). After the treatment phase, a significant rise in levels of plasma L-arginine was observed; however, there was no significant change in endothelial-dependent dilation. Hence, dietary supplementation with L-arginine was not useful in the treatment of children with CKD. L-arginine at the dosage used resulted in some children experiencing metabolic side effects, namely increased urea and extracellular acidosis [126]. The authors suggested that this could have also contributed to the negative results observed in the study.

Rhubarb

A systematic review that included 18 randomized and quasi-randomized trials (n = 1322) assessed the use of rhubarb in adult patients with CKD [127]. The included trials compared different forms of rhubarb, including tablets and decoctions, to conventional medicine (e.g. captopril) and TCM herbs. The doses and parts of the plant used were not specified. Rhubarb was found to be significantly more effective in treating CKD than conventional medicine alone. However, there was no significant difference in effectiveness between rhubarb and other TCM herbs for treating CKD. Although rhubarb was effective in reducing the symptoms of CKD, it was not possible to determine whether it could slow or stop long-term progression due to the small number of patients. Half the included trials reported adverse events, but these were not described.

Rhubarb is possibly safe for most people when used for short periods (i.e., less than 8 days) and in low doses [128]. Rhubarb has been associated with side effects such as abdominal pain and diarrhea [128]. Long-term use of rhubarb can result in several adverse effects, including electrolyte depletion, edema, colic, atonic colon, and hyperaldosteronism. Rhubarb leaves contain oxalic acid and are considered toxic if ingested [128]. Patients with renal disorders should be cautious and monitored closely when using rhu-

barb as potential electrolyte disturbances may occur [128]. Individuals with renal stones are not advised to consume rhubarb because of its high oxalate content [129]. Rhubarb safety and dosing information for children is limited and not conclusive.

TCM Herbs

The effectiveness of TCM herbs as a supplement treatment to conventional drugs in the treatment of CKD was assessed in an RCT including 248 adults [130]. For a year, the TCM group (n = 120) received conventional drugs (including prednisone and furosemide) in combination with 5 different herbal decoctions which were individualized in terms of type used and dosage. The TCM herbs were selected to supplement the kidney (as described by TCM theory) and invigorate blood flow. Details related to the composition of the TCM herb decoctions can be found in the original article. The control group (n = 128) received conventional medicine for a year. Significant differences between the two groups were found in improved symptoms (92.5% for TCM vs. 49.2% for control group, $p < 0.01$) and in improved CRCL (56 ml/min for TCM vs. 37 ml/min for control group, $p < 0.01$). There was no mention of side effects in the study, and safety information regarding the various TCM herbs used is unknown. Further research is required to determine if the results can be replicated in children.

Protein Restriction

A Cochrane review [131] assessed the effectiveness of a protein-restricted diet in delaying the start of maintenance dialysis and maintaining nutrition in children with CKD. The review included 2 studies (250 children) which compared outcomes for 124 children receiving a protein restricted diet to 126 children on a control diet. The protein-restricted diet given to children was equivalent to the lowest safe protein intake recommended by the World Health Organization (0.8 to 1.1 g/kg/day). The reviewers found no significant differences in the number of renal deaths (RR 1.12, 95% CI 0.54 to 2.33), progression of kidney disease as measured by CRCL at 2 years (mean difference 1.47, 95% CI −1.19 to 4.14) or growth. Thus, the authors concluded that reducing protein intake does not appear to have a significant impact in delaying the progression to ESKD in children. A major limitation for this review was that only two studies were identified. Of these, one had a small sample size (n = 24) and the larger study (n = 226) had significant loss to follow-up [131].

The larger study included in the above systematic review was a multicenter randomized trial involving 226 children (aged 2 to 18) with CKD from 25 pediatric nephrology centers across Europe [132]. After a 6 month run in period, children were first stratified into one of two groups (progressive or non-progressive disease). They were then further stratified into 3 renal disease groups. Children were then randomly assigned into either a diet or control group. Over a 2-year period, the intervention group received a low protein, 0.8 to 1.1 g/kg of protein a day, with adjustments made for age, while the control group had no protein intake restrictions. After 2 years in the study, 112 participants agreed to continue for an additional year. GFR was estimated every 2 months by CRCL. No statistically significant differences in the decline of CRCL were found, suggesting little value in protein restriction in pediatric CKD. There was significant loss of follow-up. No adverse effects were reported, including growth impairment due to the protein-restricted diet.

The smaller study included in the protein diet restriction systematic review was a multicenter RCT which evaluated the effect of a low-protein intake in a group of 24 infants (aged 8 months) with CKD [133]. Infants were randomized to receive a low protein (1.4 ± 0.3 g/kg/day, (protein: energy ratio 5.6%) or control protein (2.4 ± 0.4 g/kg/day, protein: energy ratio10.4%) formula for 10 months. During the 2-month run-in period prior to randomization, all infants were fed formula with intermediate protein (protein: energy ratio of 8%). An assessment of GFR over time could not be conducted due to the short follow-up period and lack of progression to ESKD in patients. At 18 months, the low protein group had significantly lower standard deviation scores

for length compared to the control group (−2.6+ 1.2 vs. −1.7 + 1.4),raising safety concerns of a low protein diet in this young population. It is recommended that extra caution be employed when considering any protein restrictions until larger prospective RCTs provide more data on efficacy and safety in children. Although there were no dropouts due to adverse events, 2 infants from each group had possible protein deficiency (based on poor weight gain) or excess protein (defined as blood urea nitrogen (BUN) greater than 80 mg/dl or BUN/serum creatinine ratio greater than 60). These infants were given their individual group formula combined (50:50) with the intermediate (8%) protein: energy ratio formula until the end of the study.

Rheum Officinale

Rheum officinale (Da Huang, a medicinal herb) is a type of rhubarb from the family *Polygonaceae*, and has been used by TCM practitioners for its strong cathartic action, and more recently, to delay progression of CKD [134].

A systematic review [134] which examined the effectiveness of *Rheum officinale* (Da Huang) in treating or preventing the progression of CKD found no evidence to recommend its use. The systematic review included 682 patients from 9 studies. Seven of the trials compared *Rheum officinale* with no treatment and 2 compared it to captopril. The main outcome was changes in two blood markers that indicate progression of CKD: serum creatinine (Cr) and BUN. Compared with no treatment, *Rheum officinale* had a positive effect on Cr (MD −87.49 μmol/L, 95% CI −139.25 to −35.72) and BUN (MD −10.61 mmol/L, 95% CI −19.45 to −2.21). The studies had various methodological shortcomings that included lack of reporting on group allocation or blinding (all studies) and small or relatively small sample size (all studies). Only one small trial (n = 30) reported on adverse effects: two-thirds of the study participants experienced diarrhea when taking more than 3 g/day of *Rheum officinale*. Despite the seemingly positive results, the authors highlighted that there was no high quality evidence to indicate that treatment with *Rheum officinale* can improve CKD or delay its progression. Well-designed RCTs are needed to better assess if there are benefits from *Rheum officinale* for CKD patients. More safety information about *Rheum officinale* is also needed.

Topical Herbal Medicine

Zhang H et al. [135] evaluated if the topical application of herbal medicine delays the progress of renal disease and improves its complications in CKD patients. The review included 23 trials, all published in Chinese, that compared external use of herbal medicine (e.g. herbal paste, herbal body/foot bathing or fuming) with no treatment, placebo, or conventional treatment. Commonly prescribed ingredients in the herbal paste and bathing formulas were Radix et Rhizoma Rhei (Da huang), Radix Salviae Miltiorrhiza (Dan shen), Rhizoma Chuanxiong (Chuan xiong), Radix Angelicae Sinensis (Dang gui), and Radix Astragali (Huang qi). The authors suggested that herbal paste and bathing or fuming treatment may be effective in delaying renal disease progression, improving kidney function, and improving some kidney complications in CKD patients. However, because of the low quality and poor reporting practices of the included trials, the authors were unable to reach a more definitive conclusion. Instead, they expressed a need for the current findings to be confirmed through larger, well-designed clinical trials with longer follow-up (greater than the 2 weeks to 2 months employed in the majority of studies included in the review) and appropriate primary outcome measures (GFR, ESKD, and all-cause mortality).

Bicarbonate

An open label RCT found that supplementation with bicarbonate was effective in slowing progression of CKD and in improving the nutritional status of 134 adults attending a predialysis clinic in the United Kingdom [136]. Patients were randomized to receive 1.82 ± 0.80 g/day oral supplementation with sodium bicarbonate (n = 67) or usual care (n = 67) for 2 years. The main primary and secondary outcomes were change in CrCl and dietary protein intake, respectively. Side effects were similar between the two groups, and

included worsening of hypertension and edema. A small proportion (6.5%) of patients in the supplementation group did not like the taste of bicarbonate. Compared with the control group, decline in CrCl was significantly slower in patients who received bicarbonate supplementation (5.93 vs. 1.88 ml/min 1.73 m^2; $p < 0.0001$). Fewer patients supplemented with bicarbonate developed ESKD (6.5 vs. 33%; relative risk 0.13; 95% confidence interval 0.04 to 0.40; $p < 0.001$). Compared to the control group, nutritional status, as measured by dietary protein intake, also improved significantly in the bicarbonate supplementation group ($p < 0.007$). The observed benefits and tolerability of bicarbonate supplementation, inexpensive and simple strategy, warrants further investigation of efficacy and safety through multi-centre double-blind RCTs in adults and children.

Traditional Chinese Medicine (TCM)

A meta-analysis of 39 Chinese RCTs investigated the efficacy and safety of TCM alone or in combination with Western medicine in reducing the risk of kidney damage in children (N = 3643) with Henoch-Schönlein Purpura (HSP) [137]. The RCT had a follow-up period between 2 weeks and 1 year and interventions included TCM self-preparation, TCM differentiation, Xijiao Dihuang Decoction, and Chinese patent medicines. In 7 RCTs (n = 582), adverse effects were significantly ($p < 0.05$) higher in children in the control 7.6% (20 cases of 263 patients) than treatment group 3.5% (12 of 319 patients). TCM significantly ($P < 0.01$) improved the treatment effect (OR: 4.31, 95% CI [3.34, 5.57], reduced the risk of kidney damage (RR: 0.36; 95% CI [0.21, 0.61], and rate of HSP recurrence (RR: 0.43, 95% CI [0.34, 0.54]. Although this study showed that TCM is an effective treatment for HSP, the RCTs included had several limitations, including small sample size, inconsistent follow-up, and poor to moderate quality of some of the studies. Large multi-centre trials are required to confirm these results.

A prospective, controlled, open-label study [138] of 150 children (aged 5–16 years) with HSP and proteinuria examined the efficacy and effectiveness taking a Traditional Chinese herbal decoction Qingre-Lishi-Yishen Formula (QLYF). All (N = 150) children received oral glucocorticoid and cyclophosphamide intravenous pulse regimen (Western medicine), and 100 of the 150 also received QLFY (integrated therapy). Children were followed up for 2 years. The main outcome measures were: adverse events and short- and long-term clinical effects. Compared to children that received the Western medicine only, children who received the integrated therapy had lower adverse event rates of respiratory infection, urinary infection, hepatoxicity, poor appetite, cardiotoxicity, and neutropenia($p < 0.05$). The integrated therapy group also had lower levels of 24-h urine protein, urine blood cell count, occurrence of secondary TCM syndrome, and recurrence rate ($p < 0.05$).

Zhang et al. [139] and Ding et al. [140] published a protocols for a large multi-centre prospective studies to investigate the effectiveness and safety of Chinese Herbal Medicine (CHM) for the treatment of HSP in children [139, 140]. These findings are not yet available for inclusion in this review.

Uremic Pruritus

Although no pediatric studies were identified, various complementary therapies, such as acupuncture, acupressure, thermal therapy, homeopathy and aromatherapy, have been evaluated in the treatment of uremic pruritus in adults undergoing dialysis.

Acupuncture

Kim and associates [141] examined the effectiveness of acupuncture for uremic pruritus in patients with ESKD. Their review included three parallel RCTs, a controlled clinical trial, and two uncontrolled observational studies (case series). Needle acupuncture was assessed in four studies and electro-acupuncture in 2 studies. All studies used standardized acupuncture protocols, and primary endpoints such as the pruritus score and symptom relief were measured. Although all the included trials reported beneficial effects of acupuncture, the majority of studies had high risk of

bias as measured by the Cochrane criteria. The authors also cited publication bias as a potential limitation to the review and considered the evidence insufficient and inconclusive to support the usefulness of acupuncture as effective treatment for uremic pruritus in patients with ESKD.

Evidence from the three RCTs included in the above systematic review will be described, starting with the most recent study which investigated the efficacy of applying acupuncture at a single acupoint in treating refractory uremic pruritus in 40 hemodialysis patients (mean age of 62 years) in China [142]. Patients allocated to receive intervention had a 1-inch 34-gauge acupuncture needle inserted at Quchi (LI11) for an hour three times a week for a month. Patients in the control group had a 1-inch 34-gauge needle inserted 2 cm lateral to the Quchi (LI11) acupoint, for the same time period. The main outcomes captured through a pruritus score questionnaire included severity, distribution and frequency of uremic pruritus and related sleep disturbance. Outcomes were measured before and after 1 month and 3 months of treatment. Within the acupuncture group compared to baseline (38.3 ± 4.3), pruritus scores significantly decreased after acupuncture at 1 month (17.3 ± 5.5) and 3 months (16.5 ± 4.9; $p = 0.001$). Pruritus scores were not significantly different in the control group at 38.3 ± 4.3, 37.5 ± 3.2 and 37.1 ± 5, respectively. Two patients from the acupuncture group and one in the control group experienced elbow soreness which resolved after 1 day, and three patients in the acupuncture group had minimal bleeding. The authors concluded that acupuncture at the Quchi (LI11) acupoint was an easy, safe and effective means of relieving uremic pruritus.

An earlier study compared the effects of needle acupuncture to oral antihistamine for uremic pruritus in 68 adult hemodialysis patients (mean age 43.6) [143]. Patients in the intervention group (n = 34) received 30-min acupuncture sessions twice a week for 4 weeks. The control group (n = 34) received 4 mg of chlorpheniramine and a topical dermatitis ointment (no ingredient details were provided) three times daily for 2 weeks. Patients were then observed for the alleviation of pruritus. A significantly higher effective rate of 95% was observed in the acupuncture group, compared to an effective rate of 70.6% in the control group ($p < 0.01$). In the acupuncture group, this improvement was maintained in 16 patients for 3 months and 18 patients for 1 month. Once treatment administration stopped, uremic pruritus recurred in all patients in the control group.

The largest study included in the systematic review compared the efficacy of acupuncture to oral calcitriol in treating uremic pruritus in 150 hemodialysis adult patients [144]. Patients in the intervention group (n = 80) received acupuncture for 20 min, twice or three times per week for a total of 16 weeks. During the same period, the control group received oral calcitriol. Within group changes in the acupuncture and control group were similar, with an 88% response rate. No adverse events occurred in this study.

Adult efficacy and safety data for acupuncture are promising and some clinicians may want to consider including acupuncture for patients with uremic pruritus.

Acupressure

Acupressure is part of TCM and involves pressing and/or massaging various acupuncture points on the body [145].

A non-randomized control trial published in 2013 investigated the effect of acupressure on uremic pruritus in 78 adult patients receiving hemodialysis [146]. Using a transcutaneous electrical nerve stimulation (TENS), acupressure apparatus patients in the intervention group (n = 38) received acupressure at the SP6, ST36, SP10, and LI11 points three times/week for 6 weeks and a total of 18 sessions. Patients in the control group received no acupressure. Outcome points were captured using a VAS and a pruritus score. The presence or absence of adverse effects was not reported. Mean VAS and pruritus scores significantly decreased at week 6 ($p < 0.001$) in the acupuncture group compared to the control group. This decrease showed a stable trend in weeks 12 and 18 ($p > 0.05$). Throughout the study, patients in the acupuncture group were also observed to use less medication than the control ($p < 0.001$). Acupressure was concluded

to be effective in reducing pruritus in hemodialysis patients.

An earlier RCT also showed that acupressure decreases pruritic symptoms in 60 adult dialysis patients [145]. Patients in the treatment group (n = 30) received 15–20 min sessions of acupressure three times a week for 5 weeks, either immediately before or after dialysis. The control group (n = 30) received no other treatment. Between the acupuncture and control groups, significant differences in mean pruritus scores at 6, 12 and 18 weeks after baseline were found, with the acupressure group having lower scores ($p < 0.0001$).

Acupressure is believed to be quite safe as it is a non-invasive procedure that does not involve needle insertion. Some clinicians may choose to consider it in treatment in their patients with uremic pruritus.

Thermal Therapy

A RCT that employed convenience sampling [147] compared the effectiveness of thermal therapy to non-thermal therapy on uremic pruritus and biochemical parameters in 49 hemodialysis patients. For various reasons, 8 patients (thermal group = 3, non-thermal group = 5) dropped out of the study, leaving 41 patients. The intervention group (n = 21) was treated with 40 °C thermal therapy with far infrared rays, a type of electromagnetic wave with a wavelength of 4 ~ 1000 μm [147], at the Sanyinjiao (SP6) acupoint once a day for 15 min on 2 days a week for a total of 18 sessions. The control group (n = 20) had a plain adhesive patch placed on the same acupoint plus routine care. Uremic pruritus improved in both groups, with a larger decrease in pruritus scores in the thermal group ($p < 0.001$) as compared with the non-thermal group. There was, however, no difference in pruritus scores between the two groups. No side effects related to the intervention were observed. The effectiveness of thermal therapy for uremic pruritus treatment warrants further investigation.

Homeopathy

Homeopathy is the use of substances that cause a particular symptom (e.g., rash) in healthy individuals to treat unwell patients with the same symptom (e.g., "like cures like"), with the belief that progressive dilution of the substance strengthens the remedy. Cavalcanti et a1 [148] performed an RCT to evaluate the effect of individualized homeopathic treatment on uremic pruritus in 20 adults from 5 dialysis centres. The experimental group (n = 11) received a homeopathic treatment and the control group (n = 9) were given placebo. All patients were assessed individually by a homeopath that was free to change the prescription based on reassessments of the patients after treatment had begun. During the study, 40 homeopathic medications were prescribed and each patient in the homeopathic group received more than one type of homeopathic remedy. At each point of observation, that is after 15, 30, 45, and 60 days of follow-up, pruritus scores were found to have decreased significantly ($p < 0.05$) in the treatment group. However, at the end of the study, post-treatment pruritus scores between the two groups were not significantly different. The authors concluded that homeopathy may be a valuable option in relieving uremic pruritus.

Side effects associated with homeopathy are generally rare and not severe. Some side-effects reported include allergic reactions and symptom aggravation [149].

Aromatherapy

A pre and post-clinical study [150] using convenience sampling assessed the effect of aromatherapy on pruritus relief in 24 adult patients receiving hemodialysis. Over a 2-week period, participants received 6 sessions of 7 min of hand massage in the non-fistulated hand with 3–5 ml of lavender, mint, and tea tree oils at 5% concentration. Four patients withdrew from the study; two objected to the oil odor, one had incontinence linked to the greasy oil sensation, and one patient was lost to follow-up. In the twenty patients that completed the study, the average pruritus score decreased significantly from 7.40 (1.18) at pre-intervention to 5.85 (1.69) at post-intervention ($t = 5.43$, $p < 0.001$), suggesting that aromatherapy can significantly relieve pruritus in hemodialysis patients.

A previous study also showed positive results when using aromatherapy and massage for the treatment of uremic pruritus in 29 adult dialysis patients [151]. Patients in the experimental group (n = 13) received aromatherapy (lavender or tea tree oil) using massage 3 times a week for 4 weeks. The control group (n = 16) received no treatment. In the experimental group, the mean pruritus score decreased significantly from 5.69 (*SD* = 1.25) before treatment to 2.69 (*SD* = 1.03) after treatment ($p < 0.001$). Pruritus scores were significantly lower in the experimental group vs. the control group ($t = 6.60$, $p = 0.001$) after the treatment period. The investigators suggested that the massage component of the aromatherapy may have confounded the findings and recommended future studies also provide massage to the control group.

Some aromatherapy oils have been linked to adverse effects such headache, nausea, and allergic reactions [12]. Although aromatherapy was found to be useful in the studies discussed with mild adverse effects, generalization and application of this therapy for uremic pruritus still requires further investigation using better designed studies with larger sample sizes and longer follow-up periods.

Chinese Herbal Bath Therapy

A meta-analysis [152] of 17 RCTs (N = 970; study sample size ranged from 24 to 156 adult patients) assessed the efficacy of Chinese herbal bath therapy (CHBT) in the treatment of uremic pruritus in adult hemodialysis (HD) patients. In CHBT, decoction or extract of Chinese medicine is poured into warm water and then patients bathe in it [152]. Patients in the treatment group bathed in baths that included 11 Chinese herbs for a period ranging between 2 weeks and 3 months (mean = 4.7 weeks). During the same time period, the control group used standard medical treatment alone or in combination with sham CHBT, clean hot water bath, or calamine lotion. At the end of the treatment period, the outcomes measures were pruritus level (measured using the VAS or the symptom score scale) and the total effective rate. None of the RCTs mentioned adverse events. CHBT plus basic treatment significantly ($p < 0.00001$) reduce the VAS score (MD = −2.38; 95% confidence intervals [CI], −3.02 to −1.74) and the symptom score (MD = −8.42; 95% confidence intervals [CI], −12.47 to −4.36) and had a higher total effectiveness rate (risk ratio [RR] = 1.46; 95% CI, 1.31 to 1.63). The authors noted that the quality of RCTs was poor to moderate. The majority of studies suffered from unclear randomization and concealment, lack of blinding and selective reporting [152].

Although findings suggest CHBT improves pruritic symptoms in individuals with uremic pruritus further investigation, with better designed (large sample size, randomization) studies is warranted to determine efficacy and safety.

End Stage-Renal Disease (ESKD)

Studies have investigated the efficacy of complementary therapies such as O3FA, acupuncture and acupressure, in reducing fatigue and depression and improving the quality of sleep and life among adult patients with ESKD. The findings from these studies suggest some positive effects and warrant further examination in pediatric populations.

O3FA

A multi-center clinical trial conducted in Iran investigated the efficacy of O3FA on inflammatory markers, namely C-reactive protein and tumor necrosis factor-alfa (TNF-α), in 45 patients with ESKD (aged 15–63 years) [82]. For 2 months, study participants received 3 g of O3FA per day (1 g omega-3 Pearl 3 times a day). Nine patients withdrew from the study: 5 for personal reasons; 2 underwent kidney transplantation and one died. After 2 months, TNF-α serum levels decreased significantly from 6.91 ± 15.25 to 2.35 ± 8.02, $p = 0.038$ among the 37 patients who completed the study. Adverse effects associated with O3FA included nausea, diarrhea and an unfavorable smell. The authors concluded that O3FA has a positive effect in reducing inflammatory markers in ESKD patients, but acknowl-

edged that a larger study with a longer duration of treatment was needed. Future studies would benefit from the addition of a control group, randomization, and blinding.

Acupressure

A recent double blind RCT conducted in two hospitals in Iran found that acupressure in conjunction with routine care improves the sleep quality of ESKD adult patients [153]. Over 4 weeks, patients in the intervention group (n = 22) received routine care and acupressure on the Shenmen (He7) and He Gu (Li4) points in the hands and Sanyingjao (sp6) point in the feet, while the control group (n = 22) received routine care only. After the intervention, significant differences between the acupressure group and the control group were recorded for the global Pittsburgh Sleep Quality Index score ($p < 0.001$) and all sleep quality indices: subjective sleep quality ($p < 0.001$), sleep latency ($p < 0.001$), sleep duration ($p < 0.001$), sleep efficiency ($p = 0.006$), sleep disturbance ($p < 0.001$), the use of sleeping medication ($p = 0.028$), and daytime dysfunction ($p < 0.001$). Although these preliminary findings support the effectiveness of acupressure in improving sleep quality in ESKD adult patients, the study was small and would benefit from replication.

Kim et al. [154] conducted a systematic review of the evidence from 7 RCTs of acupressure in the management of symptoms in patients with ESKD. In the studies, acupressure was used to alleviate sleep disorder, muscle cramps, uremic pruritus, fatigue, and depression experienced by ESKD patients. Acupressure treatment was compared to usual care (n = 3), sham acupressure (n = 2), transcutaneous electrical stimulation (n = 1), sleep medication (n = 1) or an undefined control (n = 1). Across 6 RCTs the follow-up period ranged between 4–18 weeks from baseline, while in one study only the immediate effects of acupressure were reported. Although in 5 trials there were some suggested benefits of acupressure compared to usual care (n = 3), sleep medication (n = 1), and undefined control intervention (n = 1), the authors could not draw any definitive conclusions regarding the efficacy of acupuncture until larger trials with clearer methodology and better reporting were conducted. None of the studies included in the review reported any adverse events. Six of the 7 trials included in the systematic review will be briefly described. One trial which evaluated acupressure for uremic pruritus was described in an earlier section of this chapter.

Dai and associates [155] investigated the therapeutic effect of lower extremity point massage for improving quality of sleep in 82 ESKD patients with sleep disorders. Patients in the treatment group (n = 42) received 20 to 30 min lower extremity point massage, once a day, for 4 weeks. During the same time period, patients in the control group (n = 40) took 1 mg of estazolam tablets orally half an hour before sleep. Patients who received acupressure reported significantly better sleep quality and lower rates of sleep disturbance-related disorders than patients in the control group.

A double-blind RCT by Tsay et al. [156] allocated 105 adult ESKD patients experiencing sleep disturbances to either receive manual acupressure (n = 35), placebo (n = 32), or control (n = 31). The acupressure group received 14 min of acupoint massage during hemodialysis three times a week for 4 weeks. The placebo group received sham acupressure, which involved massage on non-acupoints 1 cm from the meridian, at the same frequency and duration as the acupressure group. The control group received only standard care. Sleep quality and quality of life scores as measured by the Pittsburgh Sleep Quality Index in the acupressure group were significantly improved compared to the usual care (control) group, but not when compared to the placebo (sham acupressure) group.

Tsay et al. [157] conducted another RCT, this time to examine the effect of acupressure, transcutaneous electrical acupoint stimulation (TEAS) and routine care on sleep quality, depression and fatigue in 108 adult patients with ESKD. Over 4 weeks, patients in the acupressure (n = 36) and TEAS group (n = 36) received manual acupressure for 20 min 3 times weekly plus usual care for a total of 12 sessions. The control group received usual care only. Sleep quality,

fatigue and depression were significantly improved in the acupressure and TEAS group compared to the control group. No differences were observed between the acupressure and the TEAS group.

In yet another study, Tsay [158] compared the effect of acupressure, placebo and control on fatigue in 106 ESKD patients. Patients in the acupressure group (n = 35) received acupressure massage 3 times a week for 4 weeks, and the placebo group (n = 35) received a massage at locations with no acupoints at the same frequency as the acupressure group. The control group (n = 36) received no intervention. Patients in the acupressure group had significantly lower scores of fatigue than patients in the control group, but no difference was observed between the acupressure and placebo group. The authors concluded that acupressure provided an alternative method for health care providers to use to manage ESKD patients with fatigue.

A smaller study also found positive effects of acupressure on fatigue and depression in 58 hemodialysis patients [159]. Over a 4-week period, patients either received 12 min of acupressure (n = 28) plus 3 min of lower limb massage three times a week or routine care (n = 30; control group). After the 4-week study period, fatigue ($p = 0.04$) and depression ($p = 0.045$) were significantly improved among patients that received acupressure compared to patients who received routine care (control). The authors noted that the lack of a sham acupressure group meant that a placebo effect could not be ruled out.

Another study included in the review was a small RCT which examined the effects of acupressure plus routine care (n = 23) or routine care alone (control; n = 21) on pain associated with muscle leg cramps in 44 hemodialysis patients [160]. Routine care included stopping ultrafiltration and providing hypertonic solution. Patients in the acupressure group received acupressure at acupoints for 1 to 2 min. The rate of treatment response (pain resolving time ≤ 8 min) was significantly ($p < 0.01$) greater among the acupressure group compared to the control group.

Summary

A substantive body of evidence regarding the use, efficacy and safety of complementary therapies exists and is growing. This chapter examined current research evidence on interventions used to prevent or treat symptoms associated with kidney conditions with a specific focus on pediatric patients. The information provided allows for better informed discussions between medical/health care providers and patients or families with an interest in using complementary therapies for their children. The information provided can be used as a guide or resource by pediatric health care providers as they treat patients with kidney disease and consider seeking and referring patients to relevant local qualified complementary therapists.

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Environmental Nephrotoxins

73

Jie Ding and Ruth A. Etzel

There are a large number of environmental chemicals that are potentially toxic to the kidneys [1]. Children may be exposed to these chemicals in toys, consumer products, household pesticides, and as contaminants of food and drink. This chapter will provide information about some of the chemicals that the pediatrician should consider when a child presents with renal injury of unknown etiology. In order to determine the possibility of exposure to nephrotoxic chemicals, a careful environmental history should be performed as part of the complete history and physical examination [2, 3]. Table 73.1 lists agents that should be considered in the environmental history. Table 73.2 shows the most likely site of renal injury for selected toxicants [4].

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Table 73.1 Environmental toxicants and renal dysfunction

Ammonia	Acute nephrotoxicity, oliguria, hematuria
Arsenic	Cortical necrosis, hematuria, leukocyturia, glucosuria
Barium	Renal insufficiency, hemoglobinuria, degeneration of kidney, acute renal failure
Cadmium	Acute renal failure, necrosis of tubular cells, Fanconi syndrome
Carbon tetrachloride	Acute renal failure, aminoaciduria, oliguria
Chloromethane	Albuminuria, proteinuria, anuria, increased serum creatinine and serum BUN
Chromate and chromium (VI)	Necrosis of tubular cells
Copper sulfate	Necrosis of tubular cells
Fluoride	Interstitial nephritis
Lead	Chronic nephropathy, Fanconi syndrome, renal insufficiency
Methyl parathion	Acute nephrotoxicity
Mercury	Glomerular dysfunction, acute nephritic syndrome
Naphthalene	Tubular necrosis, oliguria, anuria, proteinuria, hemoglobinuria, increased serum creatinine and serum BUN
Pentachlorophenol	Renal tubular degeneration, metabolic imbalance
Thallium	Proteinuria, decreased creatinine clearance, increased blood urea
Uranium	Chronic nephritis, renal sclerosis
1,2 dibromomethane	Acute nephrotoxicity
1,2 dichloromethane	Acute nephrotoxicity
1,2 dichloropropane	Acute nephrotoxicity

Source: Adapted from: Agency for Toxic Substances and Disease Registry, Priority Health Conditions: An Integrated Strategy to Evaluate the Relationship between Illness and Exposure to Hazardous Substances. Atlanta, Georgia: U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, 1993, page 98

Table 73.2

Site affected/mechanism	Short-term toxicity	Long-term toxicity
Prerenal failure/hemodynamic alteration	Cocaine; animals	
Kidney failure	Ethylene glycol; potassium bichromate; animals Mushrooms; melamine; creatine; licorice	*Cortinarius* intoxication
Glomerular damage/ proteinuria	Mercury; animals	Mercury; gold; bismuth Glycol ether solvents
Hemolytic uremic syndrome/ thrombotic Microangiopathy	Animals	
Proximal tubular injury (Fanconi syndrome)	Lead, cadmium, mercuric chloride; aristolochic acid Ecstasy; *l*-lysine	Heavy metals; *l*-lysine
Interstitial nephritis	Traditional herbal medicines, cat's claw, creatine	Aristolochic acid; lead, *l*-lysine
Tubular necrosis (acute) or degeneration (chronic)	Heavy metals (mercury); potassium bichromate; animals; fish gallbladders; traditional herbal medicines; chromium; yellow oleander	Germanium; pennyroyal
Crystalliation/nephrolithiasis	Ethylene glycol; melamine; silica-containing milk Thickener; star fruit	Cranberry; ephedra; vitamin C
Immune vasculitis/lupus		Silica; yohimbe

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Cadmium

Cadmium is nephrotoxic [5]. Cadmium has a biological half-life of 10–30 years in humans. At birth, cadmium is present only at very low levels, but the whole-body cadmium burden can reach 20–30 mg by the age of 50 years and in people with occupational exposure it can reach 200–300 mg. Cadmium concentrates in the kidneys. Increased proteinuria is the earliest sign of cadmium nephropathy. A study of children 6–17 years of age living near a previous zinc smelter in Pennsylvania showed that urine concentrations of *N*-acetyl-β-D-glucosaminidase, alanine aminopeptidase and albumin were positively associated with the children's urine cadmium concentrations, but the findings did not remain statistically significant after adjusting for urinary creatinine and other potential confounders [6]. Food is the major source of cadmium for children. A study in France showed that a high proportion of children exceeded the tolerable weekly intake of cadmium in the diet during the first 3 years of childhood [7]. A study in Mexico documented an association between early-life dietary cadmium exposure and kidney function in 9-year-old children [8]. Cadmium is estimated to contribute, along with other metals, to the global burden of foodborne disease [9]. Children may also be exposed to cadmium by drinking water, inhalation of cadmium-containing particles from ambient air or cigarette smoke, ingestion of contaminated soil and dust, or use of inexpensive metal jewelry containing cadmium [10, 11].

The primary route of excretion is through the urine. The rate of excretion is low, in part because cadmium binds tightly to metallothionein, a transport and storage protein synthesized in response to cadmium and zinc exposure, preventing excretion into the tubules. In addition, the majority of filtered cadmium is reabsorbed in the renal tubules.

Clinical Effects

Acute and Short-Term Effects

Large oral exposure can produce renal toxicity. A tragic episode of industrial dumping of cadmium into the environment occurred in the Jinzu and Kakehashi river basins in Japan, leading to contamination of locally grown rice. This event produced widespread human exposure and a syndrome of renal disease coupled with brittle bones referred to as "Itai-itai" (ouch-ouch) disease. The disease was particularly common among women [12, 13], perhaps because of their higher prevalence of iron deficiency and, therefore, greater cadmium absorption.

Chronic Toxicity

Cadmium is well recognized as a toxicant in occupational exposures. Chronic occupational exposures most commonly produce renal toxicity; microproteinuria is one of the earliest signs.

Epidemiological studies among people living in cadmium-polluted areas of Japan have documented that ß2-microglobulinuria regularly occurs following a lifetime accumulation of 2000 mg cadmium or more [14]. Cui et al. reported early renal effects from cadmium exposure in children and adults living in the tungsten-molybdenum mining areas of South China [15]. Kidney damage also may occur at lower levels of cadmium; a study by Wang et al. in China suggested that adverse tubular renal effects (increased levels of *N*-acetyl-β-D-glucosaminidase and β2-microglobulin) occur in children even at the current low cadmium levels in the Chinese general population [16].

Diagnosis

While the cadmium concentration in blood reflects recent exposure, urinary cadmium concentration more closely reflects total body burden because cadmium accumulates in the kidney and is considered the gold standard measure of cumulative exposure. Twenty-four–hour urine collections are standard, but spot urine measures in conjunction with urinary creatinine to adjust for urine volume can also be used to assess exposure. However, the kidney is also a prime target of cadmium toxicity, and if renal damage from cadmium exposure occurs, the excretion rate may increase sharply and urinary

cadmium concentrations will no longer reflect body burden.

In adults, 24-h urinary cadmium excretion should be <10 μg/g of creatinine. There is no child-specific standard. On the basis of data from the National Health and Nutrition Examination Survey, the geometric mean urinary cadmium concentration in children 6–11 years of age is 0.075 μg/L per gram of creatinine. From occupational monitoring studies, the first signs of renal abnormalities in adults typically occur at 2 μg/g of creatinine and include microproteinuria—in particular, β_2-microglobulin and α_1-microglobulin are spilled. At urinary cadmium concentrations of 4 μg/g of creatinine, enzymes such as *N*-acetyl-B-glucosaminidase (NAG) are found in urine; signs of more significant glomerular damage (such as albumin in the urine and decreases in glomerular filtration rate) are seen. In the final stages of cadmium nephropathy, glycosuria, wasting of calcium and phosphate, and altered calcium metabolism are seen [17].

Prevention of Exposure

There is no effective treatment for cadmium toxicity or exposure, thus prevention of exposure is important. Chelation therapy has been shown to mobilize tissue cadmium and increase renal cadmium concentrations, increasing renal toxicity. Children younger than 6 years should not be given or allowed to play with inexpensive metal jewelry. Cadmium should also not be used in consumer products unless absolutely necessary, particularly not in products designed to be used by or with children. Reducing children's exposure to secondhand smoke has obvious health benefits beyond reducing cadmium exposure. Consumption of liver and kidney from exposed animals are potential sources of high dietary cadmium. Exposure to environmental cadmium can be prevented by reducing environmental levels in soil, in water used to irrigate food crops, and by reducing drinking water levels of cadmium. Cadmium concentrations in drinking water supplies are typically less than 1 μg/L (1 part per billion).

Lead

Exposure to high levels of lead causes kidney damage. Children may be exposed to lead in a variety of ways in and near their homes [18]. Studies of adolescents working in auto shops in low and middle income countries and children living near lead smelters have documented significant increases in urinary biomarkers of kidney tubular dysfunction such as *N*-acetyl-β-D-glucosaminidase, retinol binding protein, and α-1-microglobulin [19–23].

Chronic exposures to low levels of lead also cause proximal tubular injury characterized by proximal tubule nuclear inclusion bodies that progresses to tubulo-interstitial disease and fibrosis. Lead accumulation eventually results in decreased renal clearance, tubular reabsorption and glomerular filtration rate [24]. The association between blood lead and lower estimated GFRs has been documented in a representative sample of US adolescents who participated in the third National Health and Nutrition Examination Survey (1988–1994). More than 99% of the adolescents had blood lead levels below 10 μg/dL. Adolescents with lead in the highest quartile (>3 μg/dL) had 6.6 mL/min/1.73 m^2 lower estimated GFR compared to those in the first quartile (<1 μg/dL) [25].

Diagnosis

The exposure history is essential to making a diagnosis of lead poisoning. When taking the history of exposure, it is important to document the occupation and hobbies of the parents and all home occupants, age of the home, and use of ceramic dishes and fold medicines. Essential questions in the environmental history are shown in Table 73.3. The physical examination should include careful evaluation and documentation of hearing, language, and other developmental milestones. It is rare to see a purplish line on the gums (lead line), but if present it usually indicates severe and prolonged lead poison-

Table 73.3 The Environmental History—focus on lead

When obtaining the environmental history of a child with suspected lead poisoning, the following questions and actions should be included:
• What is the age and general condition of the residence or other structure (school) in which the child spends time?
• Is there evidence of chewed or peeling paint on woodwork, furniture or toys?
• How long has the family lived in that residence?
• Have there been recent renovations or repairs to the house or building?
• Are the windows new?
• Are there other sites at which the child spends significant amounts of time?
• What is the condition or composition of indoor play areas?
• Do outdoor play areas contain bare soil that may be contaminated?
• How does the family attempt to control dust and dirt?
• Does smoke or dust come from external sources close to the building?
• Are there any point sources near the home, such as smelters, metallurgic industries, battery recycling activity (even inactive) or open burning of waste?
• What was the previous use of the land before the building was constructed?
• To what degree does the child exhibit hand-to-mouth activity?
• Does the child exhibit pica?
• Are the child's hands washed before meals and snacks?
• Has anyone in the household ever had lead poisoning?
• What are the occupations of adult household members?
• Are the clothes and shoes used for working activities brought into the house or washed with the home laundry?
• Is the family or any member of the family involved in scavenger activities?
• Is there any work done with lead—For example, car battery recycling, radiator repairs or recuperation of metals—In or around the home?
• What are the hobbies of household members? For example, do they include fishing and preparing weights, working with ceramics or stained glass, hunting and preparing shots for guns, or handicraft activities that use tin or lead solders?
• Are painted materials or waste materials burned in household fireplaces or used as combustibles?
• Are there any local idiosyncratic sources or uses of lead?
• Does the child receive or have access to imported food, food of unsecure origin, cosmetics or folk remedies?
• Is food prepared or stored in glazed pottery or metal vessels?
• Does the family use foods stored in soldered cans?

ing [26]. The best index of exposure is a measurement of blood lead concentration [26]. Suggested laboratory tests to evaluate lead poisoning include a blood lead concentration, CBC with peripheral smear, BUN and creatinine level, and urinanalysis (looking for proteinuria, glucosuria, and aminoaciduria, seen in acute poisoning). In 2012 the US Centers for Disease Control and Prevention defined a reference level of 5 μg/dL to identify children with exposure to lead [27].

Management

Management of lead poisoning includes finding and eliminating the source of the lead, instructing the parents in proper hygienic measures (personal and household), and following up closely. Assessing the nutritional status of the child is important because iron and calcium deficiencies enhance the absorption of lead and aggravate pica [26]. Because most children in the United States with higher blood lead concentrations live in or frequently visit a home with lead paint, successful therapy depends on eliminating the child's exposure. Management that does not control environmental exposure to lead is inadequate. A thorough investigation of the child's environment and family lifestyle for sources of lead should be undertaken.

Deteriorated lead paint is the most common source of exposure in the United States. Other sources that should be considered include table-

ware, cosmetics such as surma and kohl, home remedies, dietary supplements of calcium, tap water, and parental occupation. Some children have elevated blood lead levels without access to lead paint. Blood lead levels should fall after the child reaches 2 years of age, and a stable or increasing blood lead level after that age is likely to be due to ongoing exposure. Specific attention should be paid to treating iron deficiency and ensuring adequate calcium and zinc intake.

Chelation therapy for children with blood lead levels of 20–44 μg/dL may reduce blood lead concentrations but has not been documented to reverse or diminish cognitive impairment or other behavioral or neuropsychological effects of lead [28]. If the blood lead level is greater than 45 μg/dL and the exposure has been controlled, treatment should begin. A pediatrician experienced in managing children with lead poisoning should be consulted [29].

Mercury

Mercuric salts are usually colorless or white crystals or intensely colored yellow or red powders; they include mercuric oxide (antiseptic and disinfecctant), mercuric cyanide and mercuric oxide (topical antiseptics), and mercuric nitrate (used in working with felt). Mercurous salts are typically colorless, white or light yellow powders; they include mercurous acetate (antibacterial agent), mercurous chloride or calomel (cathartic, diuretic, antiseptic, and antisyphilitic agent), mercurous nitrate (used to blacken brass) and mercurous oxide (used to make electrical batteries) [30].

Children may be exposed to mercury through uses of mercury compounds at home or in school, or in working environments such as small scale artisanal gold mining [31]. Residents in the gold mining communities and downstream of the gold mining communities consume fish that may be heavily contaminated with methylmercury, and it impacts their kidney function [32]. Mercury is used in skin-lightening creams, Chinese traditional medicine (especially for rheumatoid arthritis), and hair-dyeing agents. In a study of 509 infants exposed to phenylmercury fungicide on cloth diapers [33] urinary excretion of gamma-glutamyl-transpeptidase increased in a dose-dependent manner when urinary mercury exceeded approximately 220 μg/L. This effect was completely reversible and was no longer detectable when the children were re-examined 2 years later.

Acute mercury poisoning presents with acute tubular necrosis, especially with involvement of the proximmal tubules. Chronic low-dose exposure to mercuric salts or elemental mercury vapor can present with edema, proteinuria and normal renal function. Chronic mercury exposure can induce an immunological form of glomerular disease. This form of mercury injury to the kidney is a common form of mercury-induced nephropathy [34–36]. Findings of this membranous nephropathy may include thickened glomerular basement membrane and mildly proliferative mesangial cells and deposits of IgG1 subclasses along the glomerular capillary loops [37].

Diagnosis

The exposure history is essential to making a diagnosis of mercury poisoning. When taking the history of exposure, it is important to document the occupation and hobbies of all home occupants, use of medicines, folk remedies and antiseptics. Essential questions in the environmental history are shown in Table 73.4. A 24 h urine specimen collected in an acid-washed plastic container is appropriate for patients who have been chronically exposed to elemental mercury or mercury salts. A first morning void can provide a close approximation of a 24-h collection, particularly if it is adjusted for the concentration of the urine (using the amount of creatinine present).

A urine mercury concentration of less than 8 μg/L in adults is considered background [37]. Urinary mercury concentrations from 12 to 100 μg/L are associated with subtle changes on some tests, even before overt symptoms occur. Background or toxic urinary mercury concentrations have not been determined for children [30].

Table 73.4 The Environmental History—focus on mercury

When obtaining the environmental history of a child with suspected mercury exposure, the following questions and actions should be included:
• What are the occupations and hobbies of adult household members?
• Has there been recent application of mercury-containing caulks, latex paints, and other materials in constructing or renovating homes and other buildings?
• Has there been recent use of folk medicines? (these may contain mercury compounds.)
• Has there been a recent move? (previous tenants may have spilled mercury.)
• Has there been recent use of cosmetics containing mercury? (mercury is contained in some mascaras and wave fixatives and some skin lighteners sold outside the US.)
• Has there been use of over-the-counter preparations such as nasal sprays, contact lens solutions, and topical antiseptics?
• Has there been use of elemental mercury in a school laboratory?
• Has the child been playing with mercury? (children are attracted to elemental mercury because of its unique properties.)

Management

Proper management includes finding and eliminating the source of the mercury. When a patient has ingested mercury salts, the goals of therapy are to remove mercury from the body and to prevent dehydration and shock. Inorganic mercury can be removed from the gastrointestinal tract by emesis, catharsis or lavage. It is imperative that adequate intravenous fluids be administered to prevent dehydration and to reduce the concentration of mercury in the kidneys. BAL or another appropriate chelating agent should be administered immediately; its usefulness depends on rapid administration [30].

Prognosis

If mercury exposure ceases, complete remission is expected [37].

Uranium

Exposure to uranium can damage the proximal tubules of the kidney [38]. A case series described a family in Connecticut, who discovered that their private well was contaminated with naturally occurring uranium. Twenty four hour measurements of urine uranium were obtained on all 7 family members, but only the youngest child (age 3) had an elevated β-2-microglobulin excretion rate [39]. They had lived in the house for the previous 5 years, and the 3-year-old had derived a major portion of her nutritional intake from infant formula that was prepared by mixing powdered formulas with contaminated well water. Soil and water can also become contaminated from depleted uranium in areas where armed conflict has occurred.

Diagnosis

The exposure history is essential to making a diagnosis of uranium poisoning. When taking the history of exposure, it is important to document the source of the family's water. If the child lives in an area where armed conflict has occurred, the physician should inquire if children play in areas where depleted uranium penetrators have impacted, if children have ingested heavily contaminated soil, and if a buried penetrator feeds uranium directly into a well. Environmental movement of depleted uranium from buried penetrators into local water supplies is likely to be very slow. Over decades levels of uranium could increase in local water supplies [40].

Urine analysis for uranium is the best test to determine whether a patient has been exposed to large amounts of uranium.

Management

Because well water used for drinking or to reconstitute infant formula may be contaminated with uranium, families who use well water should consider having the water tested. Most large municipal water supplies maintain uranium levels less than the U.S. EPA maximum contaminant level of 30 μg/L.

Table 73.5 Characteristic renal manifestations of exposure to selected pesticides

Manifestation	Characteristic of these poisonings	Occurs with these agents
Proteinuria	Inorganic arsenicals	Cadmium compounds
Hematuria	Copper compounds	Phosphorous
Sometimes leading	Sodium fluoride	Phosphides
To oliguria	Naphthalene	Phosphine
Acute renal failure	Borate	Chlorophenoxy compounds
With azotemia	Nitrophenols	Creosote
	Pentachlorophenol	Organotin compounds
	Sodium chlorate	
	Sulfuryl fluoride	
	Paraquat	
	Diquat	
	Arsine	
	Ethylene dibromide	
Dysuria, hematuria, pyuria	Chlordimeform	
Polyuria	Cholecalciferol	Fluoride
Hemoglobinuria	Naphthalene	
	Sodium chlorate	
	Arsine	
Wine-red urine (porphyrinuria)	Hexachlorobenzene	
Smoky urine	Creosote	
Glycosuria		Organotin compounds
Ketonuria		Borate

Source: Environmental Protection Agency. Recognition and Management of Pesticide Poisonings. 6th edition, 2013. Washington, DC: US Environmental Protection Agency, Office of Pesticide Programs

Pesticides

Many pesticides are toxic to the kidney; pesticides such as paraquat and diquat are particularly toxic [41]. Paraquat and diquat are bipyridyl herbicides that are widely used, primarily in agriculture and by government agencies and industries for control of weeds [42].

When humans are exposed to these herbicides the proximal convoluted tubules show vacuolation and cell necroses [43, 44]. Proteinuria, hematuria, glucosuria or all of the features of the Fanconi syndrome may occur. Severely poisoned patients develop acute oliguric renal failure. Characteristic manifestations of poisoning with other pesticides are shown in Table 73.5.

Diagnosis

Pesticide poisonings may go unrecognized because of the failure to take a proper exposure history. The exposure history is essential to making a diagnosis. When taking the history of exposure, it is important to document the location of the family's home and the occupation and hobbies of all home occupants. Essential questions in the environmental history are shown in Table 73.6.

Table 73.6 Environmental History—focus on pesticides

When obtaining the environmental history of a child with suspected exposure to pesticides, the following questions and actions should be included:
• Are pesticides (e.g., bug or weed killers, flea and tick sprays, collars, powders, or shampoos) used in your home or garden or on your pet?
• Has anyone in the family worked with pesticides that they might have brought home?
• Does parent or any household member have a hobby with exposure to pesticides?
• Has the patient ever lived near a facility which could have contaminated the surrounding area (plant, dump site)?
• Does the patient's drinking water come from a private well, city water supply and/or grocery store?
• If pesticides are used:
– Is a licensed pesticide applicator used?
– Are children allowed to play in areas recently treated with pesticides?
– Where are the pesticides stored?
– Is food handled properly (e.g., washing of raw fruits and vegetables)?

Management

If poisoning with paraquat or diquat is suspected based on the environmental history, a simple colorimetric test can be used to identify paraquat and diquat in the urine, and to give a rough indication of the magnitude of absorbed dose [42]. To one volume of urine add 0.5 volume of freshly prepared 1% sodium dithionate (sodium hydrosulfite) in 1-normal sodium hydroxide (1.0 N NaOH). Observe color at the end of 1 min. A blue color indicates the presence of paraquat in excess of 0.5 mg/L. Both positive and negative controls should be run to ensure that the dithionate has not undergone oxidation in storage [42]. Diquat in urine yields a green color with the dithionate test. Both paraquat and diquat can also be measured in blood and urine [42]. Treatment should be managed in conjunction with a pediatric toxicologist, and includes immediate gastrointestinal decontamination with an adsorbent such as activated charcoal, Bentonite, or Fuller's Earth (2 gm/kg for children under 12 years.)

Arsenic

Arsenic has been classified as a known human carcinogen [10]. Children's most common exposure to arsenic is from contaminated drinking water. Other major sources of children's exposure are foods including rice, organic rice syrup, other grains, fruits, and juices [45].

Chronic exposure to arsenic is associated, in a dose-related fashion, with an increased risk of bladder cancer [10, 46–53]. Arsenic also has been associated with an excess risk of cancers of the kidney [10, 54–56]. The chronic consumption of water contaminated with arsenic in a concentration of 500 parts per million is associated with an estimated risk of 1 in 10 people developing bladder cancer. At a concentration of 50 parts per billion (ppb), cancer mortality is estimated to be in the range of 0.6 to 1.5 per 100, or approximately 1 in 100. At 10 ppb, the US Environmental Protection Agency drinking water standard since 2006, the risk of bladder cancer is 1–3 per 1000 [57].

Infancy and childhood appear to be susceptible periods during which exposure to arsenic can have lasting effects [58]. Exposure to arsenic during pregnancy and childhood is associated with a greater risk of increased occurrence of kidney cancer than exposure during adulthood [59].

Diagnosis

The exposure history is essential to making a diagnosis of arsenic poisoning. When taking the history of exposure, it is important to document the source of the family's water.

Management

Because well water used for drinking or to reconstitute infant formula may be contaminated with arsenic, families who use well water should consider having the water tested. Most large municipal water supplies maintain arsenic levels less than the EPA standard of 0.010 mg/L.

Ochratoxin A

Exposures to certain ochratoxins can cause kidney damage. Ochratoxins are natural toxins produced by fungi including *Aspergillus ochraceus*, *Aspergillus ostianus*, and *Penicillium verrucosum* that grow on cereal grains (barley, oats, rye, corn and wheat) and contaminate foods and drinks such as milk powder, coffee, wine and beer [60]. Ochratoxin A is a potent nephrotoxin [61]. Outbreaks of **Balkan nephropathy**, a fatal, chronic renal disease occurring in limited areas of Bulgaria, the former Yugoslavia, and Romania, have been linked with exposure to ochratoxin A [62–65]. Levels of ochratoxin A are elevated in the blood of patients with Balkan nephropathy [66].

In the United States, ochratoxin exposure is highest in infants and young children who consume large amounts of oat-based cereals [67]. Infants may also be exposed through breast milk if the mother has consumed foods contaminated with ochratoxin [68, 69].

Although the primary route of children's exposure to ochratoxin has previously been assumed to be through ingestion, there is grow-

ing evidence that inhalation can be an important route of exposure [70]. A case report linked exposure to ochratoxin A to focal segmental glomerulosclerosis in a 5-year-old girl who was diagnosed after presenting with enuresis. The child had a significantly elevated urine concentration of ochratoxin A (9.1 parts per billion (limit of detection 2.0 parts per billion). She probably had been exposed to ochratoxin from a home environment that was water damaged and moldy [71].

Apart from its nephrotoxicity, chronic exposure to ochratoxin A is associated with tumors of the upper urinary tract in adults. In view of substantial evidence from animal experiments supporting the carcinogenicity of ochratoxin A, the substance is categorized as possibly carcinogenic to humans (Group 2B) [66].

Diagnosis

The exposure history is essential to making a diagnosis of kidney damage from ochratoxin. When taking the history of exposure, it is important to collect a dietary history and to carefully document the condition of the home environment, with special attention to a history of the child's exposure to water damaged and moldy indoor areas.

Management

Investigations are ongoing to study the use of aspartame, a structural analogue of ochratoxin A and phenylalanine, in preventing the toxic effects of ochratoxin A exposure on the kidneys [72]. Aspartame competitively prevents the binding of ochratoxin to serum albumin. Investigations are also being conducted to study ways to reduce the genotoxic effects of ochratoxin. The quantity of DNA adducts that are induced by ochratoxin A in animals can be reduced dramatically by pretreatment of the animals with aspirin and indomethacin, which inhibit prostaglandin H synthase [73].

Aristolochic Acid

Aristolochic acids belong to the *Aristolochia* genus of plants which are often used as herbal medicines [74]. The kidney injury caused by aristolochic acids was identified from a group of women who used the herbal weight-loss regimen *Guang Fang Ji*, which contains aristolochic acid [75]. The kidney disease caused by exposure to aristolochic acids is now formally termed **aristolochic acid nephropathy**. Cases of aristolochic acid nephropathy have been reported in Europe [76–81], the United States [82] Australia [83], Japan [84], Korea [85], China [86, 87], Taiwan [86], and Hong Kong [88]. In addition, some cases of Balkan endemic nephropathy have documented exposure to aristolochic acid [89]. In the area close to tributaries of the Danube River in Bosnia, Bulgaria, Croatia, Romania, and Serbia there is a weed, *A. Clematitis.* Seeds of this weed contain aristolochic acid. The contamination of wheat flour with the seed has been associated with some cases of Balkan endemic nephropathy. Many studies show an association between the exposure with high- dose and/or long-lasting aristolochic acid exposure and kidney disease as well as urothelial cancer. The mechanism for aristolochic acid nephropathy might be apoptosis in tubular cells, whereas the urothelial cancer might be caused by DNA adducts [90].

Diagnosis

There is no specific diagnostic feature for aristolochic acid nephropathy. However, a clinical inquiry for the possible use of herbal medicines containing aristolochic acid should be taken in patients with unexplained progressive decline in glomerular filtration rate.

Patients with aristolochic acid nephropathy usually have proximal tubular dysfunction, such as glycosuria and low molecular weight proteinuria. Urinalysis reveals few erythrocytes and leukocytes, and mild proteinuria. Anemia often is unusually prominent relative to the degree of GFR impairment.

Ultrasonography usually demonstrates contracted kidneys. Unfortunately, there are no defined biomarkers for diagnosing aristolochic acid nephropathy.

Kidney biopsy reveals extensive interstitial fibrosis associated with tubular atrophy and low numbers of chronic inflammatory cells. The most

specific pathologic feature is that injury decreases from the outer to the inner cortex [91–93].

Although no strict criteria for diagnosing aristolochic acid nephropathy are available, a set of criteria allowing the definite, probable and possible diagnosis of aristolochic acid nephropathy has been proposed [94]. A definite diagnosis can be made in the patient with impaired renal function and any 2 of 3 additional findings: renal pathology demonstrating hypocellular fibrosis decreasing from the outer to the inner renal cortex, phytochemical analysis proving the intake of products containing aristolochic acid, or the detection of AA–DNA adducts in renal or urinary tract tissue. If only one of the additional findings is present in patients with impaired renal function together with urothelial cancer at the time of presentation, a probable diagnosis can be made. A possible diagnosis can be made in patients with unexplained renal dysfunction and a history of taking herbal medicines likely containing aristolochic acid.

Management

There are no randomized clinical trials supporting an evidence-based therapeutic strategy. The discontinuation of ingestion of products containing aristolochic acid is certainly essential. A study on steroid treatment for aristolochic acid nephropathy showed significant slowing of progressive renal failure in 12 patients [95].

Melamine

The Chinese Epidemic

In 2008, an epidemic of melamine contamination from formula milk was associated with urinary tract stones in young children in China. Nearly 230,000 children were diagnosed with urinary stones revealed through a targeted screening program. Melamine contamination was detected in 22 commercial brands of formula. The level of melamine in contaminated formula was 2.5 mg/kg in many brands of milk powder, exceeding the estimated tolerable daily intake level (0.063 mg/kg body weight) multifold [96]. It appeared that melamine was added to milk powder to boost the protein content, because melamine is 66% nitrogen by mass.

Non-epidemic Exposures

Melamine is prevalent in the environment and the effects of low-level exposures to children have not been clarified. A study in the US found concentrations of melamine and cyanuric acid in children's urine to be higher than levels reported in children from other countries [97]. Cyanuric acid was associated with increased KIM-1 concentrations, suggesting kidney injury.

Pathogenesis of Renal Injury Induced By Melamine-Related Urinary Stones

Almost all the available data on the toxicology and toxicokinetics was obtained by animal experiments that included dogs, cats, rats and mice. Melamine and cyanuric acid are not metabolized and are eliminated from the kidneys unchanged [98–100]. Crystals form mostly in the distal tubules from melamine and cyanuric acid [101–103].

Kidney injury is thought to be induced by the crystals or via obstruction of urine flow [101–104]. In a study of rats administered a diet containing melamine, stone formation was associated with injury to renal tubular epithelial cells, apoptosis and inflammation [105]. Gut microbiota can convert melamine to cyanuric acid *in vitro*, implicating a role of intestinal microbiota in the pathogenesis of melamine-related renal injury [106].

Clinical Manifestations

Three quarters of the children with urinary stones caused by melamine-contaminated formula in the Chinese epidemic were asymptomatic [107]. A few patients presented with dysuria, hematuria, and unexplained crying when urinating; these patients usually had obstruction caused by urinary stones. A small portion of patients (about 5%) with acute obstructive renal failure caused by melamine-induced urinary stones presented with nausea, vomiting, edema and anuria [108].

Infants were the major victims because of their high exposure to milk powder formula. However, the age at diagnosis of melamine-

associated urinary stones ranged from 1.5 months to 10 years [108]. The incidence of melamine-associated urinary stones was 3.1 times higher in male than in female subjects; the excess risk of males was most marked in boys younger than 1 year of age [109, 110].

Diagnosis

The exposure history is essential to making a diagnosis of melamine-associated urinary stones. When taking the history of exposure, it is important to document the feeding mode, formula brand, duration and amount of daily feeding.

Ultrasonography of the kidneys along with the ureters and the bladder is the first choice to detect stones, hydronephrosis and obstruction caused by melamine-associated calculi [111–113]. The location and number of stones should be reported. Abdominal X-ray and CT urography can be performed if the stone or hydronephrosis cannot be confirmed by ultrasonography [114].

Management

The management of melamine-related urinary stones in the Chinese epidemic included a conservative treatment approach, extracorporeal shock wave lithotripsy and surgical intervention. The decision regarding the approach usually was made by taking into account symptoms, stone size, stone numbers and location, etc. In children with non-obstructive melamin-related stones smaller than 4 mm without serious clinical symptoms, only oral fluid prescription was increased [113]. In case of stones larger than 4 mm, management included infusion of fluids, forced diuresis and urinary alkalization aiming for urine pH between 6.0 and 6.5. A follow-up study after 4 years showed that among 45 children treated conservatively melamine-related urinary stones had disappeared in 34 children, partially discharged in six, were unchanged in four and had grown in size in one child [115]. Hence, the vast majority of children with melamine-related stones without serious clinical symptoms, recovered with time.

Many medical teams opted for extracorporeal shock wave lithotripsy (ESWL) for children with a single melamine-related urinary stone who failed conservative treatment. In a cohort of 189 young children ESWL was performed on stones 3.8–25 mm in size located in the renal pelvis (n = 141), proximal ureter (n = 17), mid ureter (n = 5), or distal ureter (n = 26). Most children (95%) required only one lithotripsy session. During 28 months of follow-up there was not a single case with a severe complication of lithotripsy [116].

Few children (5.6% according to a meta-analysis of 2164 cases) underwent surgical intervention because of failure of conservative treatment or obstructive kidney failure [108]. Surgery included minimally invasive percutaneous nephrolithotomy using ureteroscope and pneumatic intracorporeal lithotripsy, and lithotripsy with cystoscopy or ureteroscopy.

Per- and Polyfluoroalkylated Substances

Per- and polyfluoroalkylated substances (PFAS) are synthetic chemicals that repel both water and fat and are highly heat resistant. These substances are used in many consumer products including stain-repellant fabrics and carpets, food packaging, floor care and cleaning products and personal care products. Their half-life in humans is several years. PFAS are eliminated via the kidneys by tubular excretion. PFAS are suspected to cause cellular and tubular histological changes in the kidneys via oxidative stress, enhanced endothelial permeability and other molecular pathways [117].

Few studies addressed the relationship of PFAS exposure with children's kidney function. A study of children found an eGFR decrease of 0.75 mL/min/1.73 m^2 decrease per quartile increase in per-fluorooctanoic acid (PFOA) concentration [118]. Two pediatric studies linked serum PFA concentrations to high uric acid levels [119, 120]. A study among adolescents and young adults in Taiwan who had abnormal urinalysis found that children with CKD had higher serum PFUA concentrations [121]. Given their renal mode of elimina-

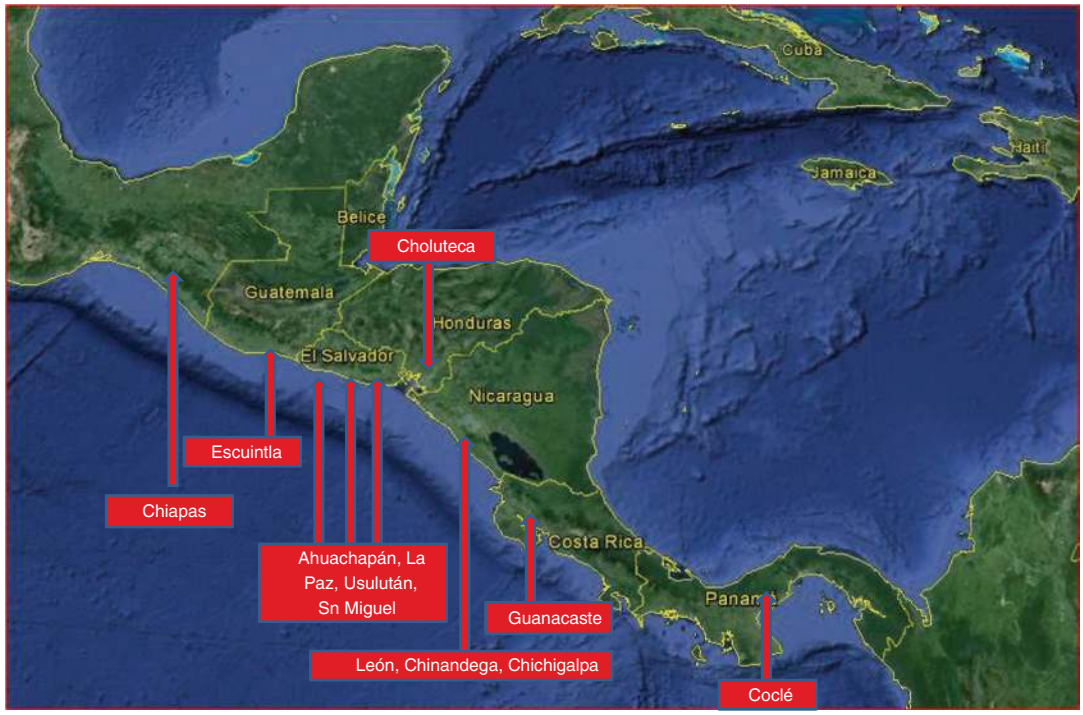

Fig. 73.1 Locations of studies of chronic kidney disease of unknown etiology in Central America. (Courtesy of Dr. Juan Jose Amador of Boston University)

tion, it is still controversial whether CKD may be the cause rather than the consequence of PFAS accumulation. However, in a recent long-term follow-up study in pre-diabetic adults, baseline PFAS concentrations were inversely correlated with long-term eGFR, with a reduction by 2.3 mL/min/1.73 m^2 per PFAS concentration quartile [122].

Chronic Kidney Disease of Unknown Etiology

Among young adults (primarily men) in Central American countries, an epidemic of non-proteinuric chronic kidney disease of unknown etiology has been occurring for at least the past 25 years on the Pacific coast (see Fig. 73.1). Young men working in the sugarcane fields appear to be most severely affected [123–126]. There is some evidence that the initial damage may be occurring at an early age [127]). A urine dipstick study conducted in the city of León among 423 pre-school children documented some level of proteinuria in 51% of the children in the study and hematuria in 20% [128].

Similar epidemics have been reported in Sri Lanka, India, and Egypt [129–131]. Investigators have evaluated the potential role of a variety of medicines, agrochemicals, arsenic, leptospirosis, and heat and strenuous labor combining to cause volume depletion, but a definitive etiology has not yet been identified [132–141].

Diagnosis

This is a diagnosis of exclusion. A thorough occupational and exposure history are essential if considering a diagnosis of chronic kidney disease of unknown etiology. When taking the history of exposure, the clinician should document the parents' occupation, and whether the child accompanies the parents to work. Adolescent work history should be obtained. Inquiries should be made about the source of the family's water.

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