Seth Kwabena Amponsah
Yashwant V. Pathak *Editors*

Recent Advances in Therapeutic Drug Monitoring and Clinical Toxicology

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I dedicate this book to my mother, Comfort Aboagye-Adu, who through her tireless efforts saw me through my education. Also to my wife, Twumwaa, and children, Ethan and Elsye, who continuously encourage me. I also dedicate this book to my sister, Adwoa, for all her support.

I cannot forget, Prof. George Obeng Adjei, Prof. Jorgen Kurtzhals, and Prof. Kwasi Agyei Bugyei, who have been my academic mentors.

—Seth Kwabena Amponsah

To the loving memories of my parents and Dr. Keshav Baliram Hedgewar, who gave proper direction to my life, to my beloved wife Seema who gave positive meaning, and my son Sarvadaman who gave a golden lining to my life.

I would like to dedicate this book to the loving memories of Ma Chamanlaljee, Ma Lakshmanraojee Bhide, and Ma Madhujee Limaye, who mentored me selflessly and helped me to become a good and socially useful human being.

—Yashwant V. Pathak

Foreword

Over the years, there has been growing interest in the fields of therapeutic drug monitoring and clinical toxicology. However, there appears to be few books that address recent trends. The current book, which I am very glad to write a foreword, has an excellent compilation of information on clinical toxicology and therapeutic drug monitoring.

Due to the fact that medicine is a fast-evolving field, it is important that information on current trends is documented. This book offers thorough, but succinct, details on drug monitoring and clinical toxicology. The book includes dedicated chapters for key topics, including analytical techniques in therapeutic drug monitoring and clinical toxicology, the role of artificial intelligence in therapeutic drug monitoring and clinical toxicity, and analysing data from therapeutic drug monitoring, pharmacokinetics and clinical toxicological studies. I am very confident that this book will be very beneficial to researchers, toxicologists, clinicians and students in the field of biomedicine and clinical sciences. The book contains 21 chapters with rich content, presenting fundamental facts, as well as practical and clinically related data, and ends with challenges and future directions of therapeutic drug monitoring and clinical toxicology. Each chapter is well written, clear, precise and easy to understand. The text integration is a bonus, making it appropriate for all in the sciences.

As a biomedical scientist, I strongly recommend this book to all researchers and students. I consider this book as essential for any reference library. My heartiest congratulations to Seth Kwabena Amponsah and Yashwant V. Pathak for such a laudable initiative. I would definitely have this book on my desk.

Gordon A. Awandare
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Preface

The correlation between drug concentration in body fluids and outcome is stronger than between drug dose and outcome. Hence, measuring systemic drug concentration is an essential part of therapeutic drug monitoring (TDM). Aside its role in therapy, TDM can also prevent unnecessary therapeutic interventions and subsequently reduce healthcare costs. There is no doubt that recent advances in TDM will shape clinical practice.

Toxicology is multidisciplinary, hence, contributions by diverse scientists. In the modern era, toxicologists share scientific knowledge to obtain accurate data about unwanted effects of different agents. Over the years, advanced tools used in toxicological and epidemiological research have been discovered.

This book gives an overview of TDM and its clinical application (analytical techniques, pharmacokinetic models, etc.). The book also highlights recent advances in toxicological studies.

Furthermore, this book focuses on major aspects of emerging and recent advances in TDM and clinical toxicology. The highlights include

(i) Analytical techniques in TDM and clinical toxicology
(ii) TDM and pharmacokinetic studies
(iii) TDM of drugs with narrow therapeutic indices
(iv) Artificial intelligence in TDM and clinical toxicology
(v) Future directions and challenges

The editors hope that this book will provide current information on TDM and clinical toxicology to healthcare professionals and academicians who work in the field of pharmacokinetics, toxicology, and pharmaceutical chemistry. Additionally, this book will affordably provide information on TDM and clinical toxicology to those interested in drug safety and the need for individualized therapy. The editors envisage that this book will be more of a reference and resource for all stakeholders in the health sciences.

The editors thank all contributing authors, who continue to play critical roles in the field of TDM and clinical toxicology. The editors also thank Springer Nature, for accepting to get this book published.

Accra, Ghana — Seth Kwabena Amponsah
Tampa, FL, USA — Yashwant V. Pathak

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About the Editors

Seth Kwabena Amponsah, PhD is a senior lecturer and head of the Department of Medical Pharmacology, University of Ghana Medical School. He has an MPhil and PhD in pharmacology. He has over 10 years' experience in teaching and research. His research focus includes clinical pharmacology (infectious disease and antimicrobial stewardship): prudent use of antimicrobials, antimicrobial level monitoring, and efficacy of antimicrobials in patients. He also has experience in therapeutic drug monitoring, population pharmacokinetic modeling, non-compartment pharmacokinetic and pharmacodynamic estimation, and pharmacokinetic evaluation of new drug formulations. He has published over 40 research articles, 3 book chapters, and several conference abstracts.

Yashwant V. Pathak has over 13 years of versatile administrative experience in an institution of higher education as dean (and over 30 years as faculty and as a researcher in higher education after his PhD). He now holds the position of associate dean for faculty affairs and tenured professor of pharmaceutical sciences at USF Health Taneja College of Pharmacy, University of South Florida and an adjunct professor at Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia. Dr. Pathak is an internationally recognized scholar, researcher, and educator in the areas of healthcare education, nanotechnology, drug delivery systems, and nutraceuticals. He has published extensively with over 50 edited volumes in the area of nanotechnology, drug delivery systems, artificial neural networks, conflict management, and cultural studies. Dr. Pathak has over 300 research papers, reviews, and chapters in books and has presented in many national and international conferences. He is also actively involved many nonprofit organizations, to mention a few, Hindu Swayamsevak Sangh (USA), Sewa International (USA), International Accreditation Council for Dharma Schools and Colleges, and the International Commission for Human Rights and Religious Freedom.

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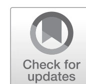

1 Therapeutic and Toxic Concentrations of Drugs in Biological Matrices

Seth Kwabena Amponsah and Yashwant V. Pathak

Abstract

Therapeutic drug monitoring (TDM) describes the measurement of chemical parameters of drugs during clinical laboratory testing. TDM aids estimation of the efficacy and safety of drugs, often a determinant of future dosing pattern. It combines knowledge of pharmaceutics, pharmacokinetics, and pharmacodynamics of drugs. TDM typically involves measuring of drug concentration in various biological fluids (matrices). Drug levels can be assayed in blood, urine, hair, tears, etc. The concentration of drugs measured in these matrices helps to estimate whether a drug is within its therapeutic range. Usually, when drug levels in these matrices attain toxic concentrations, it will lead to potential adverse effects, thus the need for documented data on therapeutic and toxic concentrations of drugs in the various biological matrices.

Keywords

Bioanalysis · Drug levels · Matrices · Pharmacokinetics · Toxic concentration

1.1 Introduction

For several decades, drug levels in biological samples have been estimated. Estimation of levels of drugs and other toxic substances in biological matrices has proven essential in the field of medicine, toxicology, pharmacology, forensic science, and environmental research. Bioanalysis of drugs and toxic substances aids decision making in pharmacotherapy and, under certain circumstances, legal decisions [1]. Furthermore, assay of drugs in biological matrices has seen tremendous technological advancement over the years [2, 3], and this has improved the practice of this science.

Detection of drugs and metabolites in tissues can aid clinical decision in pharmacotherapy, detection of illicit drugs and foreign substances (in toxicological and forensic contexts), estimation of trace elements and toxicants in biological matrices, and estimation of foreign compounds in biological materials for signs of poison and sometimes post-mortem [4]. The equilibrium between body fluids means that a drug present in the blood will also be present in oral fluid (saliva), but this concentration may be very low, sometimes below the analytical detection limit. In other instances, low levels of drugs may be

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deposited in growing hair. It is noteworthy that drug in biological matrices may be affected by several factors, including sample collection time, sample preparation, and stability of the drug [4–7].

1.2 Therapeutic Drug Monitoring

Many decades ago, it became clear that the administered dose of a drug alone does not predict drug exposure. This prompted determination of systemic concentrations of drugs and linking this to efficacy or adverse effects. This monitoring of drug in biological matrices has made it possible to tailor drug treatment in individual patients [5].

Therapeutic drug monitoring (TDM) describes the measurement of a chemical parameter of a drug during clinical laboratory testing. This parameter can, when paired with the right medical interpretation, have a direct impact on future drug dosing [6]. TDM combines knowledge of pharmaceutics, pharmacokinetics, and pharmacodynamics. It aids in the individualization of drug dosing by keeping concentrations of drug in plasma or blood within a therapeutic range or window [7]. Clinical pharmacists and pharmacologists use pharmacokinetic principles to interpret TDM data. TDM can be used to assess compliance to drug regimen and drug-drug interactions. TDM may also be necessary when there is suspicion of toxicity, when there is subtherapeutic effect, and when the manifestations of toxicity and disease state are similar [8].

1.3 Biological Matrices

The most common biological samples used to estimate drug levels are serum, plasma, and urine [9]. Drug level assays in whole blood, saliva, and cerebrospinal fluid can also be done, albeit less frequently. Drug concentrations in different biological matrices will not be the same since the drug is not uniformly distributed within the body [3]. Characteristics of the various biological matrices are summarized in Table 1.1.

1.3.1 Blood

Due to advancements in sample preparation, chromatography, and detection techniques, whole blood may be used as a screening matrix for drugs [10]. In one matrix, both identification and quantification can be done. Blood is a very uniform matrix because physiological factors vary within restricted boundaries. Serum, plasma, and whole blood are among the most important matrices in TDM [11]. Measurement of plasma or blood concentration may be a useful surrogate or indicator of the body's exposure to drugs [8].

Sampling of blood for TDM should be done steady state, which generally occurs at least five half-lives into dosage regimen [12]. If a loading dose is administered, steady state could be reached earlier. Before steady state is reached, however, patients with hepatic or renal impairment should be monitored to ensure that they do not experience drug toxicity [8].

1.3.2 Urine

Urine sample is more commonly used than blood to test for drugs of abuse [13]. The collection of urine and analysis of drug are relatively easy to undertake. A urine sample will test positive for a drug over a longer period than blood. Drug metabolites or the parent moiety can be found in urine for several days after a single dose [14]. There are countless applications of urinary drug determination in literature, even if a significant part of them may only be of toxicological importance. However, assaying drugs in urine can be used in several contexts [15–17].

Two of the main drawbacks of TDM using urine are inconvenience of collecting samples and the possibility of a loss of integrity. Unless urine voidance is observed, the authenticity of the sample can be questioned. It has been widely documented that urine adulterated with chemicals or diluted can lead to misleading results [18]. Witnessing the collection of samples is essential to prevent adulteration. However, this can be an extremely time-consuming and impractical sometimes [19].

Table 1.1 Characteristics of different biological matrices

Characteristic	Blood	Urine	Saliva	Hair
Maximum drug detection time	1–2 days	2–4 days	1–2 days	3–6 months
Intrusive sampling	Yes	Yes	No	Yes
Potential for adulteration	None	High	Low	Medium
Refusal rate	High	Medium	Low	High

1.3.3 Saliva

Water constitutes almost 99% of saliva, a viscous oral fluid. Additionally, saliva contains salts, enzymes, peptides, hormones, lipids, sugars, epithelial cells, food fragments, and microorganisms [20, 21]. In addition to maintaining the mucosa, saliva also aids in chewing, mineralization of teeth, regulating microorganisms, enhancing taste, and digestion [22].

Oral fluids are now being considered as viable candidates for TDM, despite their limited use in the past due to numerous restrictions [23]. Direct expectoration (spitting) is a good way to collect large amounts of saliva (more than 1 ml). Alternatively, saliva collectors in the form of absorbing pads, wipes, or sponges may also be used [22].

In pharmacokinetic studies, the use of saliva is advantageous because saliva contains fewer proteins than plasma [24]. Thus, a drug is less likely to bind to proteins in saliva. It is, therefore, possible to quantify the biologically active forms of unbound drugs (or their metabolites) [25]. In addition to providing noninvasive sampling and a great number of samples, saliva can be recovered from different types of patients (sometimes critically ill ones) [26]. In some cases, saliva is considered a substitute for urine samples in toxicology, since there is less chance that the patient will deliberately adulterate the sample [27]. Nonetheless, saliva samples are smaller than blood and urine samples; hence, drug concentrations can be substantially low [22]. The analytical method used in assaying saliva samples must be able to identify and quantify several analytes from a small sample volume at low concentrations, which places some constraints on the sample [28].

1.3.4 Cerebrospinal Fluid (CSF)

Using alternative matrices to conduct TDM can reduce pain, stress, and the general invasiveness of sampling. However, it is sometimes necessary to conduct highly invasive sampling to assess drug disposition over time [29]. The CSF is one of the most important sites for drug delivery because the blood-brain barrier (BBB) can vary the ratio of CSF to plasma concentration for many drugs [23]. The study of drug levels in the CSF and directly in the brain has helped characterize compartmental pharmacology of drugs used for diseases of the central nervous system [30].

Drugs injected at clinically safe doses must be able to penetrate the brain and CSF for effective treatment of brain or meningeal diseases. Knowing the concentration of a drug at the site of action (on-target or off-target) can assist in guiding pharmacokinetic and pharmacodynamic evaluations, which in turn guides dosing decisions. The brain and CSF are not readily or repeatedly accessible compartments, and given that plasma and CSF clear drugs differently, the dynamics of drug concentrations in CSF cannot be accurately predicted or extrapolated from plasma concentration data [31, 32].

1.3.5 Hair

Human hair consists of hair shaft and hair follicle. Unlike the hair shaft, which consists of dead keratinized epithelial cells, the hair follicle contains live epithelial cells. An intricate network of blood capillaries surrounds each hair follicle, supplying it with nutrients. Each hair follicle is directly connected to the sebaceous gland (oil gland). There are three axial layers in every hair

shaft: the medulla (inner layer), cortex (middle layer), and cuticle (outer layer). There are 65–95% proteins in the hair matrix, mainly keratins, water, lipids, and minerals [33].

Hair analysis may provide evidence of drug use over an extended period. Blood and urine concentrations can only reflect use of drugs over hours and days, respectively [34]. Although the specific process for drug integration into hair is unknown, it is thought that drugs enter through blood during hair development, sebum and sweat, and the external environment [35]. Blood sampling is more intrusive than hair sampling. Hair with a width of around a pencil and a weight of about 200 mg is typically taken from the back of the head [36]. The sample should be wrapped in aluminum foil and kept dry at room temperature. It is important to thoroughly decontaminate it by washing it with various solvents before drug testing.

1.4 Therapeutic Concentration of Drugs

The main goal of clinical pharmacokinetics is to improve efficacy of drugs, as well reduce toxicity. The discovery of robust correlations between systemic drug concentration and pharmacological effect has allowed clinicians to apply pharmacokinetic principles to real-life patient settings [37]. Drug concentration at the receptor site (and other tissues) can be affected by changes in the plasma drug concentration. Increasing the concentration of the drug in plasma will often lead to a corresponding increase in the concentration of the drug in most tissues [38].

The therapeutic range of a drug is the range of doses or plasma (serum) concentrations that typically leads to the desired therapeutic effect. While a patient may achieve benefit when drug concentrations are below the minimum threshold, he or she may also experience adverse effects when drug concentrations at that level continue for prolonged periods [39]. It is important to consider the benefit-to-risk ratio when determining the lower and upper limits of a treatment regimen. In the 1960s and 1970s, pharmacokinetic studies and expert opinions were used to assign therapeutic ranges to drugs [40, 41]. In general, drugs have a single therapeutic range for all indications, regardless of age, co-medication, or comorbidity.

1.5 Toxic Concentration of Drugs

Drug toxicity occurs when a drug's therapeutic effect is exceeded; nevertheless, toxic and therapeutic responses can occur at the same time [42]. The manifestation of drug toxicity could be behavioral and physiological. Drug toxicity can manifest itself behaviorally in a variety of ways, including decreased locomotor activity, loss of motor coordination, and cognitive impairment. Tissue damage, neuronal death, and hormone cycle disruptions are examples of physiological manifestations of toxicity [43]. Safety is one of the most significant challenges in drug development. Clinical trials are affected by unexpected toxicities, and post-market safety concerns can lead to the withdrawal of new drugs from the market [44]. For most drugs, there are therapeutic and toxic concentration ranges (Table 1.2).

Another principle that explains the toxic concentration of drugs is therapeutic index (TI), which compares the dose of a drug that causes therapeutic effect to the dose that causes death (in animals) or toxicity (in humans) [45]. TI can be computed in animal research by dividing the lethal dose of a drug for 50% of the population (LD_{50}) by the minimal effective dose for 50% of the population (ED_{50}), i.e., $TI = LD_{50}/ED_{50}$. Depending on the drug, the difference between the ED_{50} and the TD_{50} can be significant. The safer the drug, the larger the TI. A drug with a narrow TI, on the other hand, has a steep concentration-response relationship for efficacy, toxicity, or both, resulting in a relatively low risk-benefit range [42].

In clinical practice, TI can be the range of doses at which drugs are considered effective in clinical trials for a median of participants without causing unacceptable adverse reactions [47]. This range is sufficient for most drugs, so when the recommended doses of a drug are prescribed, the maximum plasma concentration and the area under the concentration-time curve are sufficiently above the minimum therapeutic concentration and below the toxic concentration [46]. It can therefore be

Table 1.2 Therapeutic and toxic concentrations of some drugs in blood

Drug	Therapeutic blood concentration (mg/L)	Toxic blood concentration (mg/L)	References
Acetylsalicylic acid (aspirin)	20–200	300–350	[48]
Alfuzosin	0.003–0.06	0.12	[49, 50]
Alprazolam	0.005–0.05 (–0.08)	0.1–0.4	[51]
Baclofen	0.08–0.4	1.1–3.8	[52]
Bisoprolol	0.01–0.1	0.2	[53]
Bromocriptine	0.1–0.3[a]	8[a]	[51]
Cabergoline	0.058–0.144[a]	0.39[a]	[51]
Candesartan	0.08–0.18 (–0.4)	0.54	[54]
Cetirizine	Appr. 0.1–0.6	2–5	[54]
Dapsone	0.5–2	10	[55]
Dexamethasone	Appr. 0.05–0.27	0.8	[56]
Ergotamine	0.36–0.42[a]	0.82[a]	[54]
Furosemide (Frusemide)	2–5 (–10)	25–30	[54]
Gentamicin	(2–) 4–10	12	[57]
Ibuprofen	15–30 (–50)	200	[54]
Levodopa (L-dopa)	0.3–2	5–20	[51]
Metformin	0.1–2	5–10	[49]
Naproxen	(20–) 50–100	200	[54]
Omeprazole	0.05–4	8	[50]
Paracetamol	(5–) 10–25	100–150	[58]
Quinine	1–7	10	[59]
Rabeprazole	0.2–1.8	3.6	[49]
Sulfasalazine	5–30	50	[49]
Tetracycline	1–5 (5–10)	30	[49]
Vancomycin	10–20	30–40	[57]
Warfarin	1–3	10–12	[60]
Zidovudine	0.1–0.3	2–3	[54]

[a]Units are in ng/mL

assumed that at recommended doses, drugs are clinically effective and are relatively safe.

1.6 Conclusion

The concentration of drugs measured in bodily fluids or tissues is important since it helps to estimate whether a drug is within therapeutic range. The most common biological samples used to determine drug levels are urine, serum, and plasma. When a decision has been made to monitor a drug's concentration, it is critical to obtain a biological sample that is clinically meaningful. Indeed, the relevance of literature on therapeutic and toxic concentration of drugs in biological matrices during drug monitoring cannot be overemphasized.

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2 Analytical Techniques for Therapeutic Drug Monitoring and Clinical Toxicology

Samuel O. Bekoe, Samuel Asare-Nkansah, and Kwabena F. M. Opuni

Abstract

Therapeutic drug monitoring (TDM) and clinical toxicology (CT) studies play significant roles in understanding and controlling the observed variabilities in therapeutic response of administered drug products, as well as proffering measures to improve the safety and efficacy of treatments that patients receive. However, the optimization of patient care through TDM continues to remain a challenge in many health jurisdictions despite the numerous advancements and progress in analytical techniques and technology. The practice of TDM and CT in the optimization of patient care is still evolving and requires a myriad of technical and material resources to achieve the needed optimal health outcomes. One of the critical elements in this endeavour is the availability of analytical techniques that are sensitive, cost effective, and high performing in terms of accuracy and precision and also possess seamless workflow. This chapter, thus, discusses the various high-throughput analytical techniques employed in TDM and CT, as well as the challenges associated with their respective applications as reported in the literature. It must be emphasized that consideration for a suitable analytical method for TDM and CT comes with careful planning and decision making. Factors to be considered include but are not limited to the availability of expertise (clinical and laboratory), equipment/instrument, the physicochemical nature of the target analyte (drug, metabolite, toxicant, or toxin), and the clinical situation presenting the need for TDM. Other important factors such as sample preparation and storage, analytical method development and validation, and interferences associated with matrix effect also require careful consideration in order to assure the reliability and quality of TDM or CT data needed for informed clinical decision.

Keywords

Analytical techniques · Bioanalysis · Clinical toxicology · Pharmacokinetics · Pharmacodynamics · Therapeutic drug monitoring

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2.1 Introduction

Therapeutic drug monitoring (TDM) involves the optimization of therapy through adjustment of dose at the individual level by monitoring concentrations of drug or drug metabolite in body fluids (e.g. blood, plasma, or serum) or a suitable physiological matrix [1, 2]. The scientific basis and practice of TDM date to 1946 with the establishment of a correlation between the pharmacological activity of a drug and its corresponding plasma concentration [3]. This scientific establishment resulted in the practice of dose adjustment for individuals with peculiar demographic and clinical characteristics, among others, to obtain optimized therapy. Such decisions, however, are premised on the fact that the pharmacological responses being observed by clinicians can be measured either clinically (e.g. in the use of analgesic or sedative drug) or with the use of an appropriate laboratory marker (e.g. in the use of lipid-lowering drug) [1, 2, 4, 5]. The situation, however, becomes a bit more complex and unpredictable when drug response cannot be easily evaluated clinically or when toxic side effects of the drug cannot be easily monitored or detected until irreparable damage has been caused. Thus, TDM can involve both the pharmacokinetic and pharmacodynamic aspects of drug action in applied pharmacotherapy. Therefore, analytical methods for the purposes of TDM should have the power to address relevant pharmacokinetic and pharmacodynamic parameters [2, 6–9].

To wit, different doses of a drug may be required by different patients/individuals in order to observe similar optimal therapeutic effects because of an individual patient's pharmacokinetic and pharmacodynamic variabilities. Different individuals absorb, distribute, metabolize, and excrete drugs after administration differently and as well are influenced by the action of a drug based on the unique characteristics of the patient [6, 7]. Beyond the pharmacokinetic and pharmacodynamic factors that influence drug action, other variables include:

(i) Suboptimal concentration of drug molecules in the plasma and at the receptor site or site of action in the patient because of potentially poor physicochemical quality of the drugs
(ii) An interruption in drug-receptor interactions and disruption of signal transduction pathways or processes [2, 6, 7, 9]

On the other hand, CT has been defined as a 'medical subspecialty focusing on the diagnosis, management, and prevention of poisoning and other adverse health effects due to medications, occupational and environmental toxins and biological agents' [10]. The strategy for CT includes among others the confirmation of the toxicant and/or poison using the appropriate analytical methods [11]. Since the dose of a drug makes it a poison, almost all drugs are studied under CT [12].

As earlier mentioned, the relevance of appropriate and sensitive analytical techniques in TDM and CT cannot be overemphasized. We propose in this book chapter that if sensitive high-throughput analytical techniques and detection technologies were more widely available, user friendly, and affordable, there would be better treatment outcomes, patient adherence, and reduced side effects for patients on certain drugs including antibiotics, anticancer agents, and immunosuppressants (cyclosporin), among others. In order to make available such simple, cost-effective, and efficient analytical methods, rigorous efforts that involve an investment of resources into analytical technology development and transfer and, more importantly, availability of resources to train and build capacity for TDM and CT implementation will be required in the global health delivery space [2, 6, 9].

Analytical techniques that have found successful use in TDM and CT can be very diverse, depending on the depth of the investigation being undertaken (i.e. the physicochemical properties of the drug, pKa values, ionized or unionized status at physiological pH, etc.), parameters

being monitored, the biological matrix (saliva, urine, plasma, etc.), and others. For instance, to establish a scientifically sound correlation between plasma drug concentration and pharmacological response, an analytical method must be developed and validated. Analytical techniques earlier employed for TDM studies included colorimetry, electrochemical techniques, spectrophotometry, and spectrofluorimetry [13–18]. Some of these methods currently find very little or no use at all in TDM studies due to their lack of specificity and inability to distinguish clear effects due to matrix from the drug of interest [13, 17]. Although such limitations exist for the majority of drugs, the application of colorimetry as well as flame atomic emission spectroscopy is still relevant for TDM of lithium [19]. Furthermore, bioassay techniques were previously used for TDM analysis of many antibiotics. However, this technique was found as laborious with poor specificity for polypharmacy patients and very slow in obtaining data required to make an informed clinical decision. The technique has also been reported as imprecise for present-day applications [2].

Thin-layer chromatography has also experienced a significant shift in its use for TDM analysis currently, as its limitations for total quantitative estimates are a challenge for such purposes [2]. Current high-throughput analytical techniques, which mostly find wide applications for routine monitoring and measurement of plasma drug concentrations, are electrophoresis, immunoassays, gas chromatography (GC), conventional high-performance liquid chromatography (HPLC), and recently, ultra-performance liquid chromatography (UPLC). The availability and compatibility of hyphenated modes such as LC-UV, LC-MS/MS, LC-MS, and GC-MS have positively impacted on sensitivities of such techniques in the successful quantitation of drug molecules in various biological matrices [2, 6, 20, 21].

This chapter focuses on a description of modern analytical techniques employed in TDM and CT, appraisal of the strengths and limitations of the various techniques described, as well as provision of some examples of relevant studies reported in the literature.

2.2 Immunoassays

These analytical techniques which involve the development and application of antibodies have found significant use in various disciplines including TDM and CT. The key aspect of the development and application of immunoassays borders mainly on antibodies. Antibodies, usually generated by beta-lymphocytes following exposure to substances such as foreign cells or proteins, required to trigger the immune system of mammals, are proteins. The main purpose of antibodies is to help fight infection in mammals. Various subtypes including IgA, IgD, IgE, IgG, and IgM have been reported and each has specific roles or functions within various compartments of the human body acting as sentinel sites in cases of re-infection or attack of new infections.

Although this technique comes with its own unique limitations (including the probability of obtaining false results, the effect of matrix on antibodies, poor specificity resulting in cross-reactivity with compounds that share similar physicochemical properties amongst others), immunoassays, with its cost effective, rapid detection, and robust nature, continue to play highly significant roles in the detection and quantification of minute amounts of target analytes in various complex matrices such as hair, plasma, and others. Immunoassays are usually designed for specific analytical purposes. For example, it could be designed and developed for solely qualitative purposes as well as for both qualitative and quantitative uses. Furthermore, it finds application for both low and large molecular weight molecules (compounds with molecular weights ≥250 daltons). The major common elements of interest in the design and development of an immunoassay technique include (i) target analyte (antibodies), (ii) a drug derivative needed to link to a reporter (hapten), (iii) the target for assay (analyte), (iv) required buffers or conditions such as pH of the sample, (v) a location to immobilize antibody (solid phase), (vi) the sample being analysed (matrix), and (vii) a recorder that amplifies the result (usually in the form of enzymes, fluorescence, chemiluminescence, and radioimmunoassay). Although various forms exist for

various purposes, the basic elements enumerated above remain basically the same for all immunoassay methods.

The development of such analytical techniques first involves the creation of the required antibody, followed by its production, and finally the immunoassay design. The evaluation of an antibody available for the design of the assay is a critical stage that informs the assay format that will be employed for the assay. Assay formats are generally grouped into two categories namely, (i) heterogenous and (ii) homogenous immunoassays. Heterogenous immunoassays require a separation step to remove materials that did not bind immunologically (separately bound from free materials). As an analytical method, certain key parameters required to evaluate the performance of the analytical technique under consideration include specificity/cross-reactivity, precision, limit of detection, interferences/adulteration, and stability. The optimization of such parameters assures the quality of data obtained from such determinations [2, 22–27].

Examples of compounds that have undergone TDM and reported in the literature employing various immunoassay techniques include immunosuppressants [28, 29], antiepileptic drugs [30–32], antibiotics [30, 33–36], bronchodilators [30], antimalarial agents [37], psychotropic drugs [38, 39], cardiovascular agents [40], anticancer agents, [35, 41, 42], and monoclonal antibodies [43–45] (Table 2.1). Also, immunoassays have been used in CT studies of diverse drugs [46].

Table 2.1 Analytical methods for TDM and/or CT of drugs

Analytical method	Analyte	Reference
Immunoassay	Immunosuppressants	[28, 29]
	Antiepileptic drugs	[30–32]
	Antibiotics	[30, 33–36]
	Bronchodilators	[30]
	Antimalarial agents	[37]
	Psychotropic drugs	[38, 39]
	Cardiovascular agents	[40]
	Anticancer agents	[35, 41, 42]
	Monoclonal antibodies	[43–45]
Electrophoresis	Antiepileptic drugs	[31, 32, 47, 48]
	Inflammatory bowel disease drugs	[49]
	Nonsteroidal anti-inflammatory drugs	[50, 51]
	Cardiovascular drugs	[47]
	Psychotropic agents	[47, 52]
	Diuretics	[47]
	Vasodilators	[47]
	Antibiotics	[53]
	Anthelmintics	[54]
	Anaesthetic agents	[55]
Biosensors	Anticancer agents	[56–59]
	Anticoagulants	[60]
	Monoclonal antibodies	[61–63]
	Antibiotics	[64–72]
	Bronchodilators	[73]
	Anticonvulsants	[74]
	Substance of abuse	[75]
HPLC/UPLC	Anti-infective agents	[76]
	Immunosuppressants	[28, 77–83]
	Antifungal agents	[81, 83, 84]
	Anti-arrhythmic drugs	[85]
	Monoclonal antibodies	[86]
	Antibiotics	[21, 83, 87–89]
	Antiepileptic drugs	[31, 32, 83, 90, 91]
	Anticancer drugs	[42, 83, 92, 93]
	Antiviral agents	[83, 94]
	Cardiovascular drugs	[83]
	Psychotropic agents	[38, 83, 95–105]
	Anticoagulants	[83]
	Antidiabetic agents	[83]
	Substance of abuse	[97]

2.3 Electrophoresis

Electrophoresis is a separation technique involving the use of a high-voltage electric field (electro-driven) to migrate and separate charged particles in a capillary-shaped separation compartment. Efficient separations resulting from capillary electrophoresis (CE) are usually employed in the analysis of charged compounds or drug molecules under the influence of an electric field generated uniformly across the separation compartment. The application of this technique especially in TDM is influenced by certain unique features that CE provides, and these include robustness of the instrument with

its high separation efficiency and sensitivity and minimal application of samples (sample size) and solvents, coupled with the versatile nature of its applications. The above-mentioned applications together with highly improved resolution, decreased separation time, and automation of the instrument provide the required real-time detection needed for such sensitive tasks. The principle of electrophoresis involves the high-voltage influenced migration of charged species between oppositely charged electrodes, dependent on electrostatic and electroosmotic forces. Three key parameters (i.e. size, charge, and shape of the analyte of interest) significantly influence the efficiency of separation. The commonly encountered CE modes are capillary gel electrophoresis (CGE), capillary isoelectric focusing (CIEF), capillary electrochromatography (CEC), capillary zone electrophoresis (CZE), and micellar electrokinetic chromatography (MEKC). Quite a significant number of drug molecules have had their levels in biological matrices measured using these named modes or techniques in TDM studies. Though CZE finds extensive use and application in TDM, all the other modes of separation are also utilized as appropriate for some drug molecules. It is also worth mentioning that hyphenation of CZE mode with mass spectrometry detectors is made possible through efficient compatibility and this further enhances structural elucidation for metabolite profiling [2, 106–108]. It must also be noted that of all the advantages listed for CE in TDM, some limitations do exist for its application, and these include the tendency of target analytes to undergo adsorption (which can be reversible or irreversible) onto the negatively charged surface of silica-based capillaries. It is as well difficult in handling the very small sample sizes and volumes with precision.

Electrophoretic methods have been used for the TDM of antiepileptic drugs [31, 32, 47, 48], inflammatory bowel disease drugs [49], nonsteroidal anti-inflammatory drugs [50, 51], cardiovascular drugs [47], psychotropic agents [47, 52], diuretics [47], vasodilators [47], antibiotics [53], anthelmintics [54], and anaesthetic agents [55] (Table 2.1). CT studies have been reported for different drugs using electrophoretic methods [109].

2.4 Biosensors

Biosensor-based techniques use antibodies, enzymes, membranes, molecularly imprinted polymers, and aptamers for the recognition of analytes of interest based on binding affinity [110]. Biosensors may be classified as either electrochemical, optical, piezoelectric, or nanomechanical [110]. This technique is advantageous due to low sample consumption, nearly non-invasive sample collection procedure, reduced reagent consumption, reduced analysis time, multiple analyte detection, and portability [111]. These advantages notwithstanding, there are challenges associated with sensitivity, qualitative, or semi-quantitative results obtained with the use of biosensors for TDM [112].

Biosensors have been used for the TDM of anticancer agents [56–59], anticoagulants [60], monoclonal antibodies [61–63], antibiotics [64–72], bronchodilators [73], anticonvulsants [74], and opioids [75] (Table 2.1). Biosensors have also been applied in CT studies [113].

2.5 Conventional HPLC and Emerging UHPLC Techniques

For decades, HPLC and recently (from the first decade of the twenty-first century) UHPLC have seen significant applications in various disciplines for the efficient separation and quantification of various analytes in various matrices. The ability of this unique and versatile technique to separate and analyse complex samples, both small and large molecules, continues to gain prominence in almost all basic and applied sciences of which TDM is no exception [114–119].

These liquid chromatography techniques have found versatile application and use in diverse settings as a result of its ability to separate a wide range of sample types, exceptional resolution power, speed of separation, and compatibility with a wide scope of highly sensitive detectors including mass spectrometric detectors. Such advantages that conventional HPLC as well as emerging UHPLC techniques provide have influ-

enced their high use and applications in forensic, biological, and pharmaceutical research including TDM and CT [2].

Both conventional HPLC and UHPLC have similar mechanisms of separation and resolution. However, when analysts and scientists in various fields wish to have faster separation without compromising data quality, then UHPLC becomes the obvious choice for such tasks. UHPLC also provides the requisite technological improvements in stationary support material (column) chemistry, detectors, and overall hardware required for ultra-fast separation without compromising the quality of data obtained.

The efficient separation of components of different samples could be due to the availability of diverse modes of separation including adsorption, partition, ion exchange, and size exclusion. The selection of the mode of separation usually depends on the type of task to be performed and the physicochemical properties (polar or nonpolar nature, etc.) of the analyte. Thus, a wide range of options with respect to the mode of separation such as normal phase, reversed phase, ion exchange, or size exclusion chromatography are available for well-defined tasks. The sensitivity of such analytical methods would depend on the type of detectors employed to monitor the column eluates, and these could be optical detectors such as ultraviolet (UV) absorption, fluorescence, diode array, and photodiode array detectors. It must be noted that the diode array (DAD) and photodiode array (PDA) detectors are advanced forms of UV detectors. Other equally well-known detectors include refractive index and electrochemical detectors (two types, namely, the coulometric detector and amperometric detector).

The development and introduction of hyphenated techniques especially with mass spectrometric detectors some decades ago have also seen significant improvement in the ability of conventional HPLC and UHPLC to separate and identify samples in highly complex matrices such as observed in TDM, and these include HPLC-MS, LC-MS/MS, and UPLC-MS/MS [2, 117].

Further to all of the above advancements, the availability of using a solvent system with constant composition for an entire analysis (isocratic mode) or being able to change/modify the composition of the solvent system for analysis with time (gradient mode) provides the required elution modes for efficient separation of compounds in even highly complex matrices.

However, there are several other factors that may influence the reliability of HPLC/UHPLC data for TDM and CT. These include the nature of the matrix being studied (urine, blood, liver, saliva, etc.), probable interference from endogenous substances/compounds from the matrix, the levels of the analyte available for detection and quantification (sensitivity of the detector being employed), and nature of sample preparation procedures used prior to pre-concentration of sample and analysis [2, 115].

Examples of drug compounds for which TDM has been performed and reported in literature with the use of the liquid chromatography technique include anti-infective agents [76], immunosuppressants [28, 77–83], antifungal agents [81, 83, 84], anti-arrhythmic drugs [85], monoclonal antibodies [86], antibiotics [21, 83, 87–89], antiepileptic drugs [31, 32, 83, 90, 91], anticancer agents [42, 83, 92, 93], antiviral agents [83, 94], cardiovascular drugs [83], psychotropic agents [38, 83, 95–105], anticoagulants [83], antidiabetic agents [83], and substance of abuse [97] (Table 2.1). CT studies of drugs using liquid chromatography have also been reported in the literature [11, 120–122].

2.6 Gas Chromatography (GC)

This analytical technique, which also borders on partition chromatography, has similarities to the HPLC technique already described. However, there are some significant differences with respect to the instrumentation, stationary phase support material (column), the mobile phase, and the nature of analytes. In GC, compounds suitable for analysis must be volatile in nature or if not volatile can be derivatized to provide samples that can be volatilized during analysis without thermal decomposition because of the high temperatures utilized in

such analysis. Unlike HPLC, the GC separation is ensured and performed on a stationary phase which is usually a steel capillary tube that is supplied with a continuous flow of inert gases or supercritical fluid (SCF) as a mobile phase in a temperature-regulated oven (usually around 400 °C). The mode of separation could be gas-solid or gas-liquid in nature, depending on whether the column contains either a solid (polymers) or liquid (polysiloxanes) stationary phase [2, 117].

The detection of compounds is successfully achieved using any of the following detectors:

(i) Nitrogen-phosphorus detector
(ii) Alkali flame ionization detector
(iii) Electron-capture detector
(iv) Atomic emission detector
(v) Flame ionization detector

Hyphenated modes with MS detectors are also available for GC in TDM analysis. Limitations such as lack of regular preventive maintenance and the presence of even trace levels of contaminants in the mobile phase (carrier gas), among others, could result in significant variabilities in data acquisition. Due to this challenge, few drug compounds have had their TDM studies reported in literature with the use of GC due to the huge tasks associated with the monitoring and control of variabilities in data acquisition.

Examples of drug compounds for which TDM data has been determined and reported with the use of GC include antiepileptic drugs [31, 32], psychotropic drugs [123–125], antihistamines [126], and narcotic analgesics [127] (Table 2.1). GC has also been applied in CT studies [128].

2.7 Conclusion and Outlook

In actualizing the cost benefits of TDM in clinical settings, it is imperative to re-emphasize the versatility of TDM in the establishment of benchmarks for individualization of therapy, dose optimization, screening for drug interactions, and prevention of drug toxicity. The objectives of any TDM project should be clearly defined in order to make room for appropriate decisions on the optimal selection of techniques and instrumentation to generate the required data for clinical interpretation and application. TDM should have comprehensive sample collection and handling protocols in addition to overarching quality control measures to govern sample analysis, data acquisition, data interpretation, and data management. The continuous introduction of novel and more efficacious drugs for the treatment of diseases, which can also be potentially toxic, further strengthens the need to have clinical and analytical mechanisms to improve drug therapy. For this purpose, advances in the various analytical techniques and related instrumentations for TDM and CT have led to the availability of many analytical tools with diverse costs and complexity as already described. Among the several analytical techniques, the immunoassay technique appears to be the most commonly used, with a lot of immunoassay kits commercially available. However, this technique might not be suitable in multicomponent analyses, especially in paediatrics where small sample volumes are used to monitor multiple analytes. Under such circumstances, the HPLC/UHPLC technique and its hyphenated forms are considered more appropriate because of the capacity to analyse and detect multicomponent analytes in a sample with a highly acceptable level of accuracy and precision, even when certain analytes co-elute (when mass spectrometric detection is applied). As a result of this, the HPLC/UHPLC technique has been shown to be suitable for a wide scope of drugs and matrices. Though the initial capital investment into the liquid chromatographs is high, they are known to have low running costs, making them a prudent choice for resource constraint environments that may need TDM to enhance therapeutic and clinical outcomes. Despite its limited role in TDM as a result of potential variabilities in data acquisition, GC has been very useful in CT screening, especially in its hyphenated modes (GC-mass spectrometric detection).

With the assessment of the advantages and disadvantages of the other analytical techniques for TDM and CT studies, an appropriate decision can be taken on optimal technique and instru-

mentation for any clearly defined TDM or CT programmes. It is still unclear when TDM will become routine in the health systems of emerging economies.

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3 Plasma Therapeutic Drug Monitoring and Clinical Toxicology

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Abstract

Therapeutic drug monitoring (TDM) is a branch of clinical pharmacology that seeks to optimize and maintain the concentration of medications within the bloodstream. The ability to predict a drug concentration within the serum or plasma following a predetermined dose is essential in treating disease; however, a given dose of a drug may not produce identical serum concentrations between patients due to variations in drug formulation, drug interactions, environmental factors, genetic variation, and renal and hepatic function. Therein lies the risk of undertreatment or overtreatment, particularly in drugs with narrow therapeutics indices. Identifying drug concentrations at steady state using proper measurement techniques specific to the drug of interest is both practical and maximizes safety and efficacy. Of note, pharmacogenetic variation in cytochrome P-450 enzymes between individuals, as well as age-related changes in drug metabolism, further complicates medication dosing. Therapeutic drug monitoring in the context of each patient serves to address these factors and requires collaboration between pharmacy, nursing, and medical teams.

Keywords

Therapeutic monitoring · Pharmacokinetic · Pharmacodynamic · Drug metabolism · Optimal dosing

3.1 Overview of Therapeutic Drug Monitoring (TDM)

Therapeutic drug monitoring (TDM) is a branch of clinical pharmacology in which serum or plasma medication concentrations are regularly measured to maintain a constant concentration in the patient's bloodstream. The practice of therapeutic drug monitoring stems from the observation that a given dose of a drug does not necessarily produce the same plasma or serum concentrations in every patient; instead, a multitude of patient factors and behaviors can influence drug absorption, distribution, metabolism, and elimination, ultimately impacting concentrations in the bloodstream [1]. By measuring drug concentrations in the serum or plasma at designated intervals, physicians can tailor treatment to

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maximize therapeutic benefit and minimize the risk of toxicity [2].

3.1.1 Assumptions

The concept of therapeutic drug monitoring to maintain efficacious and safe plasma or serum concentrations relies on two primary assumptions. First, TDM requires a definable relationship between dose and plasma or serum drug concentration [3]. There may be individual patient factors or behaviors that influence this relationship, including drug formulation, drug interactions, environmental factors, genetic variation, and renal and hepatic function [2]; however, the dose must somewhat predict plasma concentration. If a medication does not adhere to this assumption, then physicians would be less equipped to predict how a change in a dosing regimen would affect patient plasma concentrations, undermining the process of therapeutic drug monitoring.

In addition to a defined dose-blood concentration relationship, the concept of therapeutic drug monitoring relies on the assumption that a serum or plasma concentrations can predict therapeutic or toxic effects [3]. In other words, a predetermined therapeutic range, describing the blood plasma or serum concentration that is expected to achieve a therapeutic response while minimizing toxicity [4], is only valuable if therapeutic or toxic effects can be somewhat predicted based on concentrations. It remains true that individual patient factors may influence the concentration-effect relationship, such as drug interactions, electrolyte balance, acid-base balance, age, bacterial resistance, and protein binding [2]; however, a defined relationship must exist to ensure therapeutic ranges are not arbitrary and to maximize the utility of therapeutic drug monitoring.

Although these assumptions apply to several drugs, Aronson and Hardman (1992) describe specific criteria that decrease the utility of therapeutic drug monitoring: drug metabolism to active metabolites and a low toxic to therapeutic ratio [2]. Drugs that produce active metabolites require plasma measurement of both the drug and metabolites for proper interpretation of results, which is usually not possible in routine monitoring. In addition, there is reduced utility of TDM for drugs with a large therapeutic range such that high doses can be prescribed with little risk for toxicity, such as penicillin [5]. Instead, drugs with *narrow* therapeutic ranges and thus higher risk of toxicity or undertreatment, such as lithium, cyclosporine, and aminoglycoside antibiotics, are often indicated for TDM to ensure patient serum concentrations are maintained within a predetermined window and to inform changes to dose regimens if necessary. Although TDM assumes a clear dose-concentration and concentration-effect relationship, specific drug characteristics further impact its usefulness.

3.1.2 Process of Therapeutic Drug Monitoring

Therapeutic drug monitoring can begin immediately following diagnosis and selection of a drug or when an alteration in drug regimen is required at a later stage of treatment. First, the physician must determine if the drug is an appropriate candidate for TDM. Next, the normal range for the drug's concentration must be determined. Buclin et al. (2020) describe two types of percentiles that can inform the appropriate range: population percentiles, describing the range of concentrations expected for a given dose across the whole target population, and a priori percentiles, which take into consideration a given set of individual covariate values that better match the characteristics of the patient [6]. Given this information, the medical team then designs a dosing regimen to reach the predetermined window of plasma concentrations deemed appropriate for the specific patient. After administration of the drug, plasma or serum concentrations are measured and clinical assessments are performed to inform whether adjustments are necessary [3]. Clinical judgment and proper interpretation are necessary to determine how to adjust the dose regimen to bring concentrations closer to the target.

3.2 Indications for Measuring Plasma Concentrations

Although the primary purpose of TDM is to maximize efficacy and safety, other indications include individualizing therapy, diagnosing toxicity or undertreatment, monitoring adherence and drug interactions, prophylaxis, and guiding withdrawal of therapy [7].

3.2.1 Avoiding or Diagnosing Toxicity

Therapeutic drug monitoring is not only utilized to prevent toxicity, but to aid the interpretation of instances in which toxic effects are difficult to distinguish from the effect of the disease itself. For example, aminoglycoside nephrotoxicity clinically mimics that of generalized infection. In such cases, therapeutic drug monitoring of serum or plasma concentrations may better inform the cause of clinical symptoms, whether it be toxicity or infection [3]. This insight is necessary to appropriately determine how to proceed with treatment.

3.2.2 Diagnosing Undertreatment and Monitoring Patient Adherence

Just as high plasma drug concentrations are avoided to minimize the risk of toxicity, low plasma drug concentrations must be corrected as the patient would otherwise experience subtherapeutic effects. Low plasma concentrations can be indicative of undertreatment or low patient adherence, as the therapeutic benefit of many medications is strongly dependent on patient adherence to dosage regimens [7]. As such, it is incredibly important that physicians use clinical judgment to determine how to correct for low plasma or serum concentration. If the dosage is increased without any consideration of patient adherence, the patient could potentially be at risk for future toxicity.

Poor adherence may be indicated by a plasma concentration that is unlikely to be associated with the prescribed dose [3]. In addition, physicians can use previous measurements to guide the interpretation of plasma concentrations. If poor patient adherence is suspected, as opposed to undertreatment, then physicians must implement strategies to improve medication adherence, such as educating and empowering patients, reducing the barriers to obtaining and taking medication, improving communication during office visits, and understanding the underlying causes of non-adherence [8].

3.2.3 Prophylaxis

Whereas some drugs, such as antimicrobials, are prescribed for the therapeutic resolution of disease or illness, prophylactic drugs are administered to prevent the onset of disease or illness. For example, lithium is often prescribed to patients with affective disorders to prevent manic-depressive attacks [9]. Furthermore, cyclosporine can be prescribed to transplant patients to suppress the immune system and prevent transplant rejection. As these specific indications of lithium and cyclosporine use are prophylactic, physicians are unable to monitor clinical response. Instead, patients can undergo therapeutic drug monitoring to ensure plasma or serum concentrations indicate therapeutic benefit with minimized risk for toxicity. However, in such cases, physicians must continue to “treat the patient, not the plasma drug concentration” [7]; whereas the plasma drug concentration can suggest therapeutic benefits or risk for toxicity, individual patient factors must continue to be implemented into prophylactic and treatment strategies.

3.2.4 Drug Interactions and Termination of Treatment

One aspect of individualizing therapy is the management of drug interactions. When an interacting drug is prescribed, such as for an unrelated

condition or by a different provider, measurement of plasma concentrations can inform drug regimen alterations to avoid unanticipated toxicity or undertreatment. This is especially relevant for interacting drugs that impact hepatic or renal metabolism. For example, rifampin, an enzyme inducer, increases the clearance of cyclosporine, which may put patients receiving cyclosporine and rifampin at risk for undertreatment [10]. In this case, therapeutic drug monitoring can better ensure that the patient is receiving an adequate dosage of cyclosporine for therapeutic benefit, despite increased clearance. Lastly, TDM can be used to guide the withdrawal of treatment, specifically when the plasma concentration is below therapeutic range in a patient with an acceptable clinical condition and when the plasma concentration is high with little therapeutic benefit [7]. In the latter case, the increased risk for toxicity at high concentrations would likely outweigh the marginal therapeutic benefit, suggesting treatment should be terminated and other options explored. These two indications for withdrawal of treatment depict the risk of analyzing plasma or serum drug concentrations in isolation; to appropriately perform therapeutic drug monitoring, one must utilize both plasma concentrations and clinical assessment to inform adjustments in treatment.

3.3 Proper Use of Measurements

The timing of blood samples in therapeutic drug monitoring (TDM) is critical, as inaccurate readings can dramatically affect the interpretation of results and potentially diminish the value of the measured plasma concentrations. Four factors are needed to ensure the accuracy and success of TDM: timing of blood samples, types of samples used, measurement technique, and individualization of results [7].

3.3.1 Timing of Blood Samples

The adequate timing of blood sample collection is important in providing clinically useful measurements regarding a patient's plasma drug concentration. Following the onset of treatment, plasma concentration values should be measured once the drug has reached a steady state concentration [11]. Steady state occurs once equilibrium has been established between administration and elimination of the drug, which takes place after at least five half-lives, when the drug is eliminated by 94–97% [12]. This means that when patients are placed on a continuous dosing schedule, the steady state occurs after approximately five respective cycles of elimination and administration. Errors associated with improper timing of sample collection can negatively affect the interpretation of plasma drug concentrations. For example, when blood samples are collected prematurely, plasma concentrations may be higher than expected due to the reduced time available for tissue absorption. Most drug concentrations peak after 1 to 2 hours; however, in cases of delayed absorption or other distribution impairments, the time at which the drug levels peak may be prolonged [3]. In most cases of therapeutic drug monitoring, samples are collected prior to the administration of the following dose, otherwise known as the trough period, when plasma drug concentrations are at their lowest [11]. Samples acquired during the trough period are reflective of the drug's elimination phase and more accurately assess the steady state concentration. Table 3.1 highlights the timing necessary for measuring steady state concentrations for both individual drugs and drug classes [7].

3.3.2 Type of Sample Used

In addition to the proper timing of sample collection, the type of sample used has an important effect on the validity and reliability of plasma drug concentrations. For most drugs, the sample should be allowed to clot in its tube, a process called heparinization [3]. Prior to measurement, there are no important storage restrictions for most drug classes. Lithium and aminoglycosides differ from most drugs, as they require heparinization followed by separation within 1 hour of collection [3]. Cyclosporine also differs as it

Table 3.1 Variability in timing of serum drug measurement by drug class and route of administration

Drug/Drug Class	Route of Administration	Timing of Blood Sample
Aminoglycosides	Intravascular (IV)	Peak – 15 mins after infusion. Trough – before next dose.
	Intramuscular (IM)	Peak – 1 hour after injection. Trough –before next dose.
Cyclosporine	PO, IV	Before next dose, measure at same time of day.
Digoxin	PO	Approximately 6 hours after the last dose.
Lithium	PO	12 hours after the last dose.
Phenytoin	PO, IV	Timing not applicable.
Theophylline	During an infusion	4–6 hours after infusion starts; stop infusion for 15 min before sample.
	Oral	Before next dose, measure at the same time of day.

requires the consultation of local laboratory facilities for the necessary sampling and post-dosage collection techniques [3]. Ensuring that the proper sample types and collection techniques are utilized in TDM is critical, as errors in these stages may adversely affect the laboratory assay results and greatly reduce the value of plasma drug concentrations when providing patient care.

3.3.3 Measurement Technique

Following sample collection and processing, the laboratory performs a specific drug assay to determine the plasma drug concentration. The current standard practice involves the use of immunobinding assay procedures, most commonly fluorescence polarization immunoassay (FPIA), enzyme immunoassay (EMIT), and enzyme-linked immunosorbent assay (ELISA) [3]. These assays should be performed within 24 hours, especially when determining dosage adjustments or possible toxicity, as changes need to be made quickly in both scenarios to avoid complications [7]. The specificity of these assay methods contributes to their high accuracy; however, there may be potential interferences associated with cross-reactivity between the assay and the drug's metabolites, or the assay and structurally similar compounds [3]. These cross reactions may falsely increase or decrease the drug's plasma concentration, ultimately affecting the reliability of the immunoassay's results. For example, digoxin toxicity has been discovered when patients were co-administered spironolactone or canrenone, as these two drugs led to falsely low values of digoxin upon immunoassay [13]. This demonstrates the importance of ensuring adequate immunoassay techniques, which includes the evaluation of possible interferences and mitigation of any associated adverse effects.

3.3.4 Individualization of Results

Following sample collection and laboratory analysis, the results of the immunoassays must be interpreted in a way that is tailored to the patient's status. Phenytoin is an example of a drug that exhibits variation between individuals, especially those with comorbid conditions, and requires close monitoring of plasma concentrations as a result. The recommended dosage of phenytoin is 4–9 μmol/L, which is calculated based on the ideal plasma concentration of 40–80 μmol/L and the high affinity for binding albumin, as only 10% is unbound in the blood [14]. However, comorbid conditions that increase drug metabolism, alter albumin binding, or decrease albumin concentrations can lead to larger unbound concentrations of phenytoin, which alters the ideal plasma concentration range. One patient with chronic renal failure was treated with 300 mg of phenytoin per day for tonic-clonic seizures and was found to be asymptomatic 6 weeks later with a plasma concentration of 30 μmol/L, which is below the target range for phenytoin [14]. The clinician increased the dosage to 400 mg per day to fall within the recommended therapeutic range; however, 2 weeks later the patient exhibited mental dullness and difficulty walking, both being adverse effects associated with high

phenytoin plasma concentrations [14]. The patient's plasma concentration was found to be 60 μmol/L, and the clinician subsequently reduced the dose back to 300 mg per day, after which the patient returned to an asymptomatic state [14]. This patient displayed drug toxicity at a plasma concentration within the reference range, ultimately due to the concurrent renal failure altering the characteristics of phenytoin's absorption and metabolism. The patient's positive response at a lower therapeutic index indicates the need for repeated plasma monitoring, especially when altering the dosage regimen in the face of comorbid conditions.

Although phenytoin is just one example of a drug with varying effects between patients on the same dosage regimen, this case demonstrates the importance of combining the immunoassay results with the patient's full clinical picture to provide a more precise therapeutic range, regardless of the drug prescribed. In addition, plasma drug concentrations should not be used in isolation, as other therapeutic indices, such as protein binding ability in this scenario, are equally important when determining the dosage regimen necessary to achieve a desired effect.

Overall, therapeutic drug monitoring is a useful tool when evaluating a patient's response to a drug and determining plasma concentrations in comparison to a desired therapeutic range. Through the proper collection techniques, sample types, laboratory evaluation, and individualization of results, TDM conveys a considerable benefit for clinicians in providing patient care, especially when used among other therapeutic monitoring techniques.

3.4 Individualizing Therapy

It's well known that for a given dose of a drug, the physiologic impact varies greatly between individuals of both the same and different ethnic and geographical backgrounds. Variation exists in terms of the pharmacokinetics of a given drug, in that an individual may absorb the drug more readily, distribute it more rapidly, or eliminate it more quickly [3]. Further, the pharmacodynamic properties of a given drug affect individuals by treating disease states, by producing adverse side effects, and by producing tolerance [15]. On a broader scale, pharmacogenomics, defined as the effect of an individual's genetic composition on response to medications, has become increasingly recognized as a critical part of drug dosing [16]. Essentially, it is the pharmacodynamic, pharmacokinetic, and pharmacogenomic aspects of drug metabolism that impact drug therapeutic range and efficacy.

3.4.1 Formulation and Diet

Drug absorption is described is the process by which drugs enter the systemic circulation, which requires the passage through cell membranes. Passage through cell membranes is affected by molecular shape and size, as well as the solubility of the drug in water defined by the lipid-water partition coefficient [17]. Highly lipid-soluble drugs rapidly cross cell membranes without the need for molecular transport proteins, whereas drugs with a low lipid-water partition coefficient require a protein carrier [18]. Thiopental is an example of highly lipid-soluble barbiturate used for rapid intravenous general anesthesia. The drug rapidly crosses the blood-brain barrier due to its high lipid solubility [19]. When a drug administered orally is absorbed through the gastrointestinal tract through the hepatic portal system and undergoes biotransformation in the liver, the drug is converted to a more water-soluble metabolite [20]. For drugs, such as thiopental, for which their high lipid solubility is critical in distribution and mechanism of action, parenteral administration would be preferred.

With lipophilic drugs, a patient's body mass index (BMI) and obesity status is highly relevant. Individuals with a greater degree of adipose tissue tend to retain lipid-soluble drugs, thereby reducing drug elimination. Halothane is an inhaled general anesthetic that deposits in adipose tissue and undergoes reduced hepatic metabolism in obese individuals and increases the risk of hepatitis [21]. Halothane additionally has a high blood-gas partition coefficient, which

indicates that it binds to plasma proteins (such as albumin) in blood quite readily [22].

3.4.2 Pharmacokinetics: Ion Trapping

The unionized form of a drug readily crosses cell membranes; however, the ionized form becomes trapped and is unable to cross membranes. For example, a weakly acidic drug in the plasma will become trapped on the interstitial side if that side has a higher pH due to deprotonation of the drug [23]. Acetazolamide is a weak diuretic that is used to treat glaucoma, epilepsy, altitude sickness, and fluid retention [24]. The drug also decreases the plasma pH producing a mild metabolic acidosis. Doxorubicin is a chemotherapeutic drug that is a weak base and is prone to ion trapping in the presence of low pH environments. Consequently, concomitant use of acetazolamide and doxorubicin would lead to elevated concentrations of doxorubicin and increase the risk of doxorubicin toxicity [25].

3.4.3 Pathologic Disease States

Drugs typically undergo renal and/or hepatic clearance. Therefore, the physiologic status of these organs may have a direct impact on a drug's systemic concentration and biological effects. Metformin is frequently prescribed for type 2 diabetes mellitus and is a hydrophilic drug that is excreted unchanged by the kidneys [26]. At high doses of metformin, there is a risk of life-threatening lactic acidosis, which may result from drug accumulation in instances of severe renal impairment [27]. Therefore, in individuals with chronic kidney disease (CKD), metformin dosing must be carefully monitored and is contraindicated in late-stage CKD when the estimated glomerular filtration rate is <30 mL/min/1.73m^2 [28]. In the context of an individual with hepatic cirrhosis when liver function is severely reduced, the dosage of drugs undergoing hepatic modification or clearance may require adjustment. Hepatic biotransformation of drugs is of high clinical relevance as we begin to discuss cytochrome P450 enzymes and variations in functionality of these enzymes between individuals.

3.4.4 Cytochrome P450 Enzymes

Cytochrome P450 (CYP) oxidases consist of a family of more than 50 heme-dependent enzymes that play a key role in hormone synthesis but more importantly in xenobiotic and drug metabolism [29]. CYP enzymes are found in the smooth endoplasmic reticulum of cells and are most prominent in the liver, emphasizing the essential role of the liver in drug metabolism and clearance. CYP enzymes metabolize approximately 90% of drugs. Many drugs exist as prodrugs, such that the oral form of a given drug must be bioactivated by CYP enzyme(s) into its active functional form. For example, prasugrel is an antiplatelet drug that targets the P2Y12 receptor on the platelet and is useful in the treatment and management of acute coronary syndrome. Prasugrel is converted by esterases to an inactive form that is subsequently primarily converted by CYP3A enzymes and CYP2B6 to its final active form [30].

Individuals possess pharmacogenetic variation in CYP enzymes alleles such that for a given CYP enzyme, an individual may metabolize the drug at a more rapid or a slow rate. Clopidogrel has the same function as prasugrel but is metabolized differently. Clopidogrel is bioactivated mainly by CYP2C19 from a prodrug form into a primary metabolite, which is subsequently acted upon again by CYP2C19 to generate a second metabolite that is the true active form of the drug [31]. CYP2C19 has two key polymorphisms that influence drug serum concentrations and clopidogrel activity. CYP2C19*2 produces a loss-of-function polymorphism such that the less of the active form of clopidogrel is produced. In a complete homozygous case of CYP2C19*2, individuals should not be given clopidogrel; however, in the heterozygous case, a higher dosage would need to be administered to achieve the desired drug effect. This polymorphism is present in up

to 70% of Asian individuals. Conversely, CYP2C19*17 results in increased transcription of CYP2C19, thus producing more active clopidogrel and elevated drug potency for a given dose. Administration of clopidogrel to individuals with the CYP2C19*17 polymorphism would require reduced amounts of the drug to achieve the desired drug effect.

Certain drugs can induce or inhibit the activity of CYP enzymes that metabolize a given drug resulting in reduced or elevated serum concentrations of the latter drug, respectively. Warfarin is a popular anticoagulant used in the prophylaxis and treatment of deep vein thrombosis, pulmonary embolism, and atrial fibrillation [32]. The drug is inactivated by CYP2C9. Therefore, inhibition of CYP2C9 increases serum concentrations of warfarin and consequently increases the risk of severe bleeding [29]. Given that the antimicrobial metronidazole has been shown to inhibit CYP2C9, it would be necessary to reduce the dosage of warfarin and carefully monitor the patient for both external and internal bleeding if concomitant use of the drugs is necessary.

3.4.5 Age

The guidelines, contraindications, and dosage for medications vary depending on an individual's age. For infants and young children, drugs may be more potent than desired due to incomplete development of the physiological systems that metabolize medications [33]. However, in older children, other drugs may be less active for a drug dosed by weight because the liver and CYP enzymes develop more quickly than the relative increase in body mass [34]. In older and elderly patients, the activity of drug-metabolizing enzymes as well as kidney function declines with age [35]. Beers Criteria for potentially inappropriate medication use in older adults was first developed in 1991 to provide recommendations on prescription drug practices for adults 65 years and older. The goal of the guidelines is to reduce the risk to benefit ratio and reduce drug interactions that result from polypharmacy in older adults [36]. Drugs to be avoided include those that worsen a syndrome or disease, drugs with cautioned used, significant drug-drug interactions to be avoided in older adults, drugs with strong anticholinergic effects, and drugs that are contraindicated or require reduced dosage for patients with reduced kidney function.

3.5 Serum Versus Plasma Monitoring

Pioneer scientist and clinicians of drug monitoring demonstrated that incidence of toxicity could be reduced to drugs such as digoxin, phenytoin, lithium, and theophylline when patients are monitored within a therapeutic range [37]. This is a practice mainly utilized for drugs that have narrow therapeutic indexes, variable pharmacokinetic properties, and adverse side effects [3]. Drug monitoring consists of extracting various blood components to measure the drug concentrations within them and then comparing the concentration to clinical parameters [3]. Several key factors are important to pay attention to such as the timing of the blood sample and the type of blood sample used.

Components within blood are distinct as well as the processing after the blood draw. Whole blood should be used if the compounds are concentrated in the erythrocytes such as lead, cyanide, mercury, carbon monoxide, and chlorthalidone [41] or if the binding protein is on the erythrocyte such as with many immunosuppressants (e.g., cyclosporin, tacrolimus, azathioprine) [38, 39]. Whole blood that has been centrifuged becomes plasma and mainly consists of fibrinogen, clotting factors, plasma proteins (e.g., albumin), electrolytes (e.g., sodium, potassium, bicarbonate, chloride, calcium), and immunoglobulins. The chief difference in processing between plasma and serum is that anticoagulants are added to plasma, whereas coagulation enhancers are added to serum [40]. These coagulation enhancers facilitate clotting and separation of erythrocytes via centrifuge. Therefore, serum contains no fibrinogen or clotting factors. Frequently, serum and plasma are used

interchangeably, and reasons are not stated as to why one is used over the other.

One of the advantages to using plasma is that plasma has a larger volume. Because the yield for plasma is higher with about 50% of the original sample versus 30–50% for serum, a greater drug amount may generate a more accurate drug analysis. Another advantage is that there is no delay from the long duration of the clotting process. Clotting can occur even after centrifugation if enough time is not allotted. There will also be a decreased risk of hemolysis. When erythrocytes lyse, they increase the plasma concentration. The effect will be more pronounced in serum [41].

Some of the disadvantages to using plasma are related to the effect of anticoagulants on the sample. Anticoagulants can have a variable effect on the free concentration of the drug. For example, anticoagulants can increase lipolytic activity, resulting in an increase in non-esterified fatty acid that often displaces phenytoin. Phenytoin will then concentrate in erythrocytes and decrease the plasma concentration of phenytoin compared to the original. On the other hand, ibuprofen concentration can increase in the plasma with heparin usage because anticoagulants can disrupt protein binding. Some other potential disadvantages are associated with the additives or impurities found within the anticoagulants such as zinc, lead, aluminum, copper, and fluoride. They can falsely elevate the concentrations of these elements in the plasma [41]. In conclusion, while there are some advantages and disadvantages to utilizing plasma and serum, the concentrations overall are similar. The differences in whether one is used over the other may depend more on the hospital system.

3.5.1 Drugs Requiring Monitoring: Digoxin

Digoxin is one of the oldest cardiovascular medications, having been used for over 200 years to treat atrial fibrillation with fast ventricular rate and congestive heart failure with sinus rhythm [42, 43]. They are used in patients with later stage systolic heart failure that have recurrent symptoms despite therapeutic doses of ACE inhibitors and diuretics. Digoxin can be used chronically as a palliative medication or until a higher interventional step can be implemented [44]. However, utility of the drug is associated with adverse effects and a narrow therapeutic window. In addition, there are variable associations between clinical symptoms, plasma concentration, and therapeutic/toxic responses that make digoxin unpredictable and therefore further necessitate plasma monitoring. There is a higher correlation between clinical signs and therapeutic/toxic effects of digoxin in treating atrial fibrillation with a fast ventricular rate. Typically, the slowing of the ventricular rate is a good indicator that digoxin is having corrective effects. On the other hand, it's more difficult to measure the therapeutic response for patients with congestive heart failure due to overlapping symptoms between congestive heart failure and digoxin toxicity such as anorexia, nausea and vomiting, confusion, and cardiac arrhythmias. While evidence shows increasing the dosage of digoxin within the therapeutic window produces an increasing therapeutic effect when treating atrial fibrillation, there is not an established one for treating heart failure. The therapeutic effect is generally seen between 1 and 3.8 nmol/l with the risk of digoxin toxicity starting to increase above 2.6 nmol/l [45]. The recommended range for plasma digoxin concentration has decreased over time due to evidence of better outcomes with lower dosages [46, 47]. Demographics of patients who are more prone to digoxin toxicity are the elderly, especially patients who have poor kidney perfusion [42].

3.5.2 Drugs Requiring Monitoring: Phenytoin

Phenytoin is an anticonvulsant medication used in the management of epilepsy, generalized tonic-clonic seizures, complex partial seizures, and status epilepticus [48]. Phenytoin is another drug that has a narrow therapeutic window between 40 and 80 μmol/L with mainly neurological toxicities occurring above 100 μmol/L. Binding sites become saturated at relatively low doses and

therefore any increase in dosage beyond the saturation point rapidly increases the plasma concentration [14]. Furthermore, there is also a large variation in the dosage taken by patients and the resulting plasma concentration. Individual differences in hepatic enzyme system cytochrome P450 (CYP), predominantly CYP2C9 and CYP2C19 along with other drug interferences with this system, can contribute to the discrepancy between the dosage given to patients and the resulting plasma concentration. For example, drugs that inhibit CYP are cimetidine, amiodarone, allopurinol, azapropazone, chlorpromazine, imipramine, isoniazid, metronidazole, fluconazole, omeprazole, sulfonamides, thioridazine [48], and valproic acid [49], leading to an increase in plasma phenytoin. Conversely, medications that induce CYP are alcohol, rifampin, carbamazepine, barbiturates, theophylline, etc., leading to an increase in phenytoin breakdown and therefore a decrease in its plasma concentration [50]. Medical conditions that cause hypoalbuminemia, such as chronic liver disease, nephrotic syndrome, and pregnancy, will additionally increase plasma concentration due to a decrease in albumin binding sites. Approximately 90% of the time, phenytoin is bound to albumin and is inactive as a result [51]. Therefore, monitoring the plasma concentration is important to ensure the availability of phenytoin stays within the therapeutic range for sufficient anti-seizure effects and avoidance of neurological toxicities with higher concentrations.

Phenytoin therapeutic effects can be well demarcated by clinical evidence for patients with more severe seizure conditions but may not be as straightforward with patients that have milder symptoms or are taking phenytoin prophylactically for surgery. On the other hand, phenytoin's toxic effects are also insidious and difficult to distinguish from symptoms of other neurological conditions. In many of these cases, the measurement of plasma concentration within the therapeutic range can serve as an adequate marker for its efficacy [2].

3.6 Renal Clearance and Metabolism in Therapeutic Drug Monitoring

Another consideration when it comes to therapeutic drug monitoring is renal clearance and metabolism. Many disease states, medications, and individual variations contribute to differences in renal clearance among patients. It is imperative to take these variations into account when monitoring drug concentrations for different patient populations to ensure proper therapeutic effects and avoid toxicity. This section will discuss therapeutic drug monitoring regarding renally eliminated drugs in multiple patient populations with altered renal clearance including critically ill individuals, the geriatric population, the pediatric population, and individuals with acute or chronic renal failure.

3.6.1 Critically Ill Patients

Critically ill patients may experience a phenomenon called augmented renal clearance in which they have elevated creatinine clearance and thus increased renal elimination of renally cleared drugs. This phenomenon is especially prevalent in individuals with neurologic injury, sepsis, trauma, and burns [52]. While the normal range of creatinine clearance is 110 to 150 mL/min in males and 100 to 130 mL/min in females, creatinine clearance in patients with these conditions averages from 170 mL/min to more than 300 mL/min [53]. Specifically, neurologic injury, sepsis, trauma, burns, and interventions such as vasopressor use and fluid resuscitation in critically ill individuals increase renal blood flow, resulting in elevated renal clearance [54]. Augmented renal clearance may contribute to subtherapeutic drug concentrations in critically ill patients, especially regarding most antimicrobials, e.g., beta-lactams, vancomycin, and carbapenems due to their renal mechanism of clearance and standard use in septic patients [52].

Though augmented renal clearance is a well-documented phenomenon in many critically ill patients, it is also important to remember that many critically ill patients may also suffer from acute kidney injury (AKI) which may reduce GFR and subsequently renal drug clearance. Therapeutic drug monitoring in patients with AKI will be further discussed later in this section.

In terms of therapeutic drug monitoring, it has been deemed to be most useful in monitoring renally cleared antimicrobials, namely, aminoglycosides and beta-lactams, in critically ill patients as opposed to other types of medications in this population [55]. The bactericidal effects of aminoglycosides, specifically, are dependent on its concentration in the plasma. It has been recommended that therapeutic drug monitoring be performed in critically ill patients on aminoglycosides utilizing a concentration-time profile (Fig. 3.1) and the area under the concentration-time curve (AUC) in (mmol/L) x min divided by the minimum inhibitory concentration (MIC) in mmol [56]. The maximum concentration of aminoglycoside in the plasma and the renal elimination half-life can be used to approximate the AUC with mathematical modeling [57]. For critically ill patients, the target AUC/MIC was determined to be approximately 80–100 as opposed to 30–50 for non-critically ill patients [58]. Multiple days of prolonged elevated AUC values have correlated with increased ototoxicity and nephrotoxicity in critically ill patients though this population may require a higher initial single dose as well as an extended interval of administration due to augmented renal clearance [55]. For beta-lactams, therapeutic drug monitoring for critically ill patients is also recommended to optimize dosing and reduce the chances of toxicity [59]. Currently, the minimum concentration at steady state in a dosing interval, or trough sample, is the main way in which beta-lactam concentrations are monitored in the intensive care unit, but more accurate methods and models are currently explored to improve the monitoring of beta-lactams. The goal trough concentration for beta-lactams is equivalent to each antimicrobial's respective minimum inhibitory concentration [60].

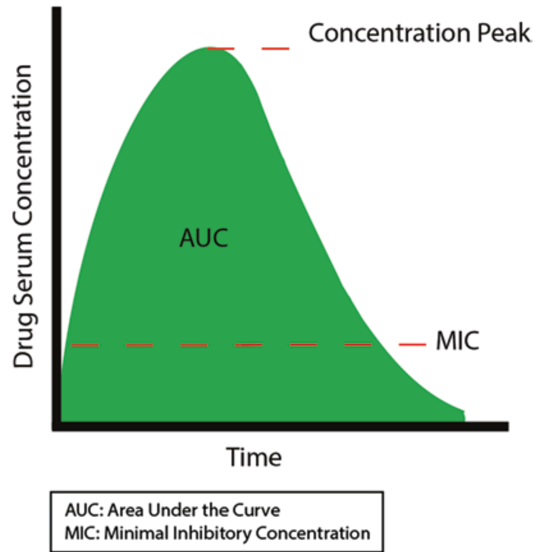

Fig. 3.1 Concentration-time profile commonly used in therapeutic drug monitoring

3.6.2 Geriatric Patients

Though glomerular filtration rate (GFR) and thus renal function and clearance are generally believed to decline with age, studies have shown that about one-third of elderly individuals do not experience a decline in renal function. Two-thirds of elderly individuals do experience an age-related decline in renal function and clearance, though these changes seem to correlate with other comorbidities such as cardiovascular disease [61]. Individuals affected by an age-related decline in renal function and clearance may be more susceptible to supratherapeutic drug concentrations and adverse drug events due to decreased renal elimination. Despite potential differences in renal clearance in comparison to the general population, therapeutic drug monitoring is not routinely used by default in this population unless there is a proven deficit in renal function [62].

3.6.3 Pediatric Patients

It is difficult to pinpoint renal function and clearance trends in the pediatric population due to the

numerous metabolic changes in infancy and early childhood. In general, metabolic clearance is very low in the newborn but increases rapidly to levels greater than metabolic clearance in adults in early childhood [63]. Later childhood and adolescent stages display similar renal function patterns to that of average adults [62].

Therapeutic drug monitoring is mainly utilized in the pediatric population for two specific types of medications: antiepileptic drugs and immunosuppressants. The main renally cleared antiepileptic drug utilized in pediatric populations is levetiracetam [63]. Renal clearance is very high in children particularly from the ages of 6 months to 6 years, requiring a dose per kilogram per body weight of levetiracetam about 30% greater at these ages relative to later childhood, adolescence, and adulthood [64]. Because of this variation in required dosage due to rapidly changing renal function during childhood, therapeutic drug monitoring may be useful in discovering the best therapeutic dose of levetiracetam for individual children [62]. Therapeutic drug monitoring for levetiracetam utilizes trough samples instantly before the next dose due to its short half-life of 6–8 hours, which may result in great variability in serum drug concentration if samples are taken at other time points [64]. The goal trough concentration for levetiracetam is currently 12–46 mg/L as established by the International League Against Epilepsy [65]. In terms of immunosuppressants in transplant patients, mycophenolate mofetil is a commonly used renally cleared immunosuppressant in the pediatric population. Therapeutic drug monitoring of this drug has the potential to decrease the likelihood of underexposure, which can improve outcomes for transplant and nephrotic syndrome patients. The current mechanism of monitoring for mycophenolate mofetil involves measuring AUC, which was discussed previously regarding aminoglycosides [66]. Simulations have shown an optimal AUC of 30 to 60 (mmol/L) x min for pediatric transplant patients to decrease the risk of acute rejection [67]. The AUC method, however, requires excessive sampling within a 12-hour period, which may not be feasible for children; thus, an equally reliable but more convenient method of monitoring mycophenolate mofetil concentrations in children may be necessary. In addition, data shows that total immunosuppressant monitoring in addition to mycophenolate mofetil monitoring may be more useful in promoting therapeutic effects in pediatric transplant and nephrotic syndrome patients than sole mycophenolate mofetil monitoring [66].

3.6.4 Acute or Chronic Renal Failure Patients

Regardless of an acute or chronic etiology, patients with renal failure have severely decreased renal function and clearance. This makes them more susceptible to supratherapeutic drug concentrations, putting them at higher risk for toxicity. Many of these patients are on dialysis, however, and this further changes their pharmacokinetic profile. Drug clearance in dialysis patients is affected by the membrane, dialysate and its flow rate, duration of dialysis, and the individual properties of the drug. High molecular weight, high protein affinity, and large volume of distribution are all properties that decrease the likelihood of clearance by renal replacement therapy [68].

Therapeutic drug monitoring plays an extensive role in the medical treatment of renal failure and dialysis patients due to their respective increased risks for toxicity and sometimes unpredictable drug reactions with dialysis [68]. The antibiotic vancomycin can best illustrate this concept in these patient populations. Patients with renal insufficiency have increased rates of nephrotoxicity with vancomycin, and therapeutic drug monitoring utilizing trough concentrations along with mathematical models forecasting the trough concentration based on dosing has been shown to decrease the incidence of vancomycin-associated nephrotoxicity in patients with poor renal function [69]. In the case of dialysis patients, vancomycin is typically administered in the last hour of dialysis because though low-flux dialyzers have no pronounced effect on vancomycin clearance, high-flux dialyzers may elimi-

nate up to 40% of the vancomycin starting dose during dialysis [70]. Because of this, therapeutic drug monitoring with trough concentrations is considered a necessity in dialysis patients to ensure proper drug concentrations are reached to treat the patient's infection [71]. Traditionally, the goal trough concentration for vancomycin has been 15–20 mg/L, though there has been increased nephrotoxicity associated with these concentrations with no proven improvement in therapeutic outcome. A goal trough concentration of 10–15 mg/L for renal insufficiency and dialysis patients may result in decreased incidence of vancomycin-associated nephrotoxicity [72].

3.7 Role of Cytochrome P450 Systems in Liver Clearance and Metabolism

The liver is the site of drug modification and clearance. To appreciate the properties of the drug and its downstream effects, understanding the mechanics of the liver is valuable. For drugs that are taken orally, it is the site of first-pass metabolism which can result in secondary activation of the drug or removal. The heterogeneity of the liver can be attributed to its unique portal venous system, the biliary system, the protein interactions occurring at the hepatocyte and sinusoidal surfaces, and the variety of transporters and enzymes located intracellularly [73].

There is functional diversity in the liver lobule given the zonal location. For instance, zone 1 is known as the periportal zone and is sensitive to changes in oxygen and nutrients. This region plays a role in carbohydrate, fat, and urea metabolism. This region is particularly vulnerable to oxidative stress [74]. In zone 3, which is closest to the central vein and furthest from the portal triad, there is increased expression of cytochrome P450 enzymes which has a wide range of biochemical activities especially drug metabolism, in addition to bile acid synthesis. This region is more susceptible to ischemia [74]. Although there is not much evidence highlighting the synthetic or metabolic properties of zone 2, there is new evidence suggesting it is a prominent source of liver regeneration in settings with or without liver injury likely due to hepcidin antimicrobial peptide 2 (Hamp2) gene expression [75].

The accepted model on liver clearance is the physiologically based pharmacokinetic (PBPK) model by Rowland et al. The PBPK model is a compartmental model but deviates from its classical predecessors in that the compartments are equivalent to the actual organs and their volumes, and it considers the sequential processing in the primary organ of metabolism, accounts for drug clearance in organs where secondary metabolites form, considers the differing kinetics between the various primary and downstream metabolites, and differentiates between the permeability and transport of the different metabolites [76]. Current PBPK models account for the role of futile cycling in phase I and II reactions which involve reduction/oxidation and conjugation/deconjugation reactions, respectively [77]. There is a combined PBPK model which accounts for first-pass metabolism occurring within the intestinal and hepatic systems [76]. The traditional model focuses on flow and volume of the intestinal compartment; meanwhile the segregated flow (SFM) model differentiates between serosal and absorptive or enterocyte layers of the intestine [76]. Majority of the blood flow is shunted to the serosa rather than the enterocyte layer [76]. This fact accounts for the variable intestinal and hepatic processing which occurs when drugs are administered orally or intravenously since there is minimal secondary metabolite formation associated with the latter [76]. Although there is less biochemical or phase I and II reactions involving the intestines compared to the liver, the dose-dependent exposure of metabolites and drugs to the liver varies largely when drugs are dosed IV or orally [77]. The first-pass effect by the liver changes when the intestines are summed into the equation [76]. Even if the intestines play a minute role in the overall metabolic processing, the organ is the gateway to downstream liver processing [76]. The PBPK intestinal and liver model is becoming the more commonly employed approach for drug development and understanding drug processing.

3.7.1 Acetaminophen

Acetaminophen (APAP) is metabolized by the cytochrome P450 (CYP) system which generates N-acetyl-p-benzoquinone imine (NAPQI), an electrophile, that reacts with glutathione (GSH) and other proteins (particularly in the mitochondria) [78]. The NAPQI-GSH conjugate is how APAP is detoxified from the liver [78]. Other metabolic reactions include sulfonation and glucuronidation which are also hepatoprotective [78]. To a lesser degree, there is oxidation of APAP to 3-OH-APAP [78]. The main CYP enzymes which metabolize APAP are CYP2E1, CYP1A2, CYP3A4, and CYP2A6 [78]. CYP3A4 more commonly bioactivates APAP and generates the toxic NAPQI metabolite [78]. In organisms where CYP3A4 completed the greatest bioactivation, there were fewer sulfonate and glucuronide conjugates [78]. CYP3A4-specific inhibitors like troleandomycin did not show a reduction in NAPQI production [78]. When adding GSH there was some enhancement of CYP3A4 activity with certain substrates [78]. These specific drug-drug interactions suggest that there is possible allosteric modulation involved in CYP3A4 activity. CYP2E1 was a significant activator at low doses of APAP; meanwhile CYP1A2 does so at high doses of APAP [78].

In addition to the cytochrome P450 system, there are other adjacent reactions occurring in the liver such as conjugation to proteins via the sulfhydryl group and some GSH depletion [79]. The original presumption was that APAP-CYS adducts would be detectable only when hepatocyte necrosis and cell death were occurring [80]. It is now suggested that these adducts can be present without alanine aminotransferase (ALT) elevations, suggesting that hepatic necrosis does not concomitantly occur when APAP is conjugating to proteins. Protein adducts can be generated without significant GSH depletion [80]. Originally, it was assumed that major reductions in GSH would permit for an environment where protein adducts could be created. APAP-CYS adducts form regardless of whether low or high concentrations of APAP are administered [80].

When APAP is dosed, the sulfation pathway followed by the glucuronidation pathways becomes saturated [79]. Normally these pathways are activated prior to NAPQI formation [79]. Toxic doses of APAP can cause hepatic damage which limit sulfation and glucuronidation [79]. Sulfotransferases (SULT) increase the propensity of APAP elimination by increasing its hydrophilicity [79]. The primary sulfotransferases are mediated by SULT2A1 and SULT1A1 [79]. Glucuronidation is mediated by UDP-glucuronosyltransferases (UGT), particularly by UGT1A9 and UGT1A1, at normal or toxic doses, and UGT1A6 at lower doses [79]. N-Acetylcysteine (NAC) treatment of APAP overdose increases flux to the SULTs to limit production of the NAPQI and promote elimination of APAP and its conjugates [79].

3.7.2 Rifampin

Rifampin is an antituberculosis medication that is a notable cytochrome P450, i.e., CYP3A4, inducer. Given this property, it enhances warfarin and benzodiazepine metabolism. In addition, there is induction of P-glycoprotein (P-gp) activity which can also reduce bioavailability and mitigate intestinal absorption and lower plasma concentrations [81]. Hence, drugs which are metabolized by the cytochrome P450 system may be initially excreted by MDR1 proteins [81]. Without considering this relationship, the role of cytochrome P450 metabolism in clearance or bioactivation of certain drugs may be inflated.

To monitor CYP3A4 activity and its expression is normally done by measuring the area under midazolam plasma concentration-time curve (AUC), but a more accurate measure would be to see increased CYP3A4 expression in the gastrointestinal and hepatic systems [82]. The administration of rifampin caused reductions of midazolam by 75% [82]. This is mediated by the activation of the nuclear pregnane X receptor (PXR). CYP3A4 enzymes were more commonly present in the intestines than in the liver, although it was found to be that the cytochrome P450 sys-

tem in the liver was rate-limiting in terms of CYP3A4 activation and deactivation [83].

It is important to consider the role of hepatotoxicity after oral rifampin administration given there are elevations in alanine aminotransferase (ALT) as seen in mice studies [84]. In addition, there were increases in bilirubin, LDL, and total cholesterol. Alkaline phosphatase (ALP) concentrations did not increase [84]. The elevations in lipid concentrations are associated with increased expression of peroxisome proliferator-activated receptor-γ (PPARγ) and upregulation can also be induced by rifampin [84]. Overall, this suggests that activation of the PPARγ and CYP3A4 activity can both contribute to hepatotoxicity following rifampin administration [84].

3.7.3 Erythromycin

Erythromycin is a macrolide antibiotic and a known cytochrome P450 inhibitor. To mediate its effects on the CYP system, it relies on both competitive inhibition and mechanism-based inactivation (MBI) where intermediates bind with the enzyme and impair reactions [85]. Given that the latter method occurs through covalent binding, overall drug clearance does not regenerate the enzyme due to the powerful bond created by the intermediate [85]. Hence, there is allosteric inhibition when these reactive metabolites are generated.

It's important to consider the CYP3A4 variants present in patients. In particular, the changes in hydrophobicity in the helical structure of these enzymes are heavily responsible for substrate interaction and changes in the K_m [85]. The variants also have changes in their allosteric sites, as well [85]. Certain substrates are required prior to activation of the enzyme at the allosteric site first [85]. These substrates can be rate-limiting factors in terms of accessibility for the main substrates required at the activation site.

Given that CYP3A4 enzymes are heavily affected by MBI, the rate of degradation and synthesis of these enzymes influence bioactivation and downstream reactions normally mediated by this system [85]. In vitro studies have not shown major differences in turnover which can likely be due to minimal allelic differences [85]. It is difficult to isolate the role of competitive inhibition given that MBI is still at play. The role of MBI was so significant between certain CYP3A4 phenotypes and variants that inhibitory potencies, K_1, had upwards of six-fold difference [85].

With respect to CYP3A4 inhibition, studies were completed to investigate the role of erythromycin with biopterin administration. When erythromycin was administered, the AUC of biopterin was doubled [86]. The inhibitory role of CYP3A4 increases the bioavailability of substrates but is unclear if it does so with CYP inhibitors such as erythromycin. Erythromycin is converted to N-desmethylerythromycin by the enzymes and was present in equal quantities when administered with biopterin, suggesting that biopterin has little to no role in CYP3A4 metabolism [86]. Erythromycin relies on P-gp for transport and biopterin is a known P-gp inhibitor [86]. Erythromycin increased after P-gp administration, suggesting that this mechanism is the likely explanation for elevated plasma concentrations and not due to CYP3A4 inhibition, although both mechanisms are pathways that play a role in clearance [86].

3.8 Antiretrovirals and Glucose in Therapeutic Drug Monitoring

3.8.1 The Role and Influence of Glucose in Therapeutic Drug Monitoring

Glucose monitoring in diabetic patients is one of the most common, routine forms of serum monitoring. Multiple variations of a "glucometer" exist, ranging from test strips to continuous glucose monitoring with feedback, also known as "closed-loop" systems. Common, at-home blood glucose tests require a whole capillary blood sample in which glucose reacts with either the glucose oxidase, glucose dehydrogenase, or hexokinase enzymes, leading to a chemical reaction [87]. A particular challenge with this mecha-

nism is that it must be done quickly, as glucose concentrations in a whole blood sample decline with time due to erythrocyte glycolysis and consumption of glucose.

Hemoglobin A1C (HbA1C) has long been shown to be a surrogate for blood glucose levels over a 3-month period and is routinely measured in diabetic patients. The two main methods for determining HbA1C are in-office point of care tests (POCT) and cation exchange chromatography [88]. Interestingly, studies have shown that hemoglobinopathies such as sickle cell (HbS) can influence the accuracy of POCT HbA1C [88, 89].

Many drugs are cleared within hepatocytes via the cytochrome P450 enzymatic system. Varied subtypes have been shown to be significantly upregulated or downregulated in response to either hypo- or hyperglycemia, in vitro [90]. Further, prolonged hyperglycemia in cultured hepatocytes was shown to significantly increase accumulation of lipids as well as exhibit insulin resistance. Current data suggest hepatic lipid accumulation, commonly known as hepatic steatosis or nonalcoholic fatty liver disease, can change the pharmacokinetics and pharmacodynamics of hepatic drug metabolism.

Elevated serum glucose concentrations can have significant effects on the pharmacokinetic properties of drugs cleared by the kidney. Diabetes mellitus (DM) is associated with increased glomerular filtration rate (GFR) and subsequent renal "hyperfiltration," with eventual progression to chronic kidney disease (CKD) [91]. Renal clearance of a drug is dependent on the urine flow rate and inversely related to the plasma concentration of the said drug. As a result, a patient with uncontrolled DM may develop significant changes in their GFR and thus varying concentrations of renally cleared substances in their serum as the disease progresses. This warrants potential changes to drug dose in patients with varying stages of CKD.

Glucose plays a very large and active role in drug metabolism via detrimental effects on the architecture of the liver and kidney. Maintaining blood glucose within the normal range is ideal, especially in diabetic patients, as it will limit the potential impact on drug clearance in the aforementioned systems.

3.8.2 Therapeutic Drug Monitoring of Antiretroviral Drugs

Human immunodeficiency virus, commonly known as HIV, is a retrovirus spread through bodily fluids, leading to significant immune system impairment and eventual acquired immunodeficiency syndrome (AIDS). As a result, many pharmaceuticals have been developed to combat HIV, colloquially known as "antiretrovirals." Many forms of antiretrovirals exist, ranging from integrase, entry, and protease inhibitors, as well as analogs including nucleoside and non-nucleoside reverse transcriptase inhibitors (NRTI and NNRTI, respectively).

Although very effective, many of these antiretroviral drugs have been associated with hepatotoxicity [92]. Further, many of these drugs are associated with resistance in HIV-infected individuals [93]. Cases have highlighted the beneficial effects of therapeutic drug monitoring (TDM) in HIV-infected individuals with comorbid hepatic and renal failure [94]. As a result, TDM of these compounds can be beneficial to determine both minimum effective concentration and potential dose-dependent toxicity. Many of these compounds are bound to plasma proteins in vivo and only contribute therapeutic and toxic effects when free and unbound [95]. Despite this, many of these compounds have notable toxicity profiles and thus highlight the benefit of routine TDM.

Protease inhibitors, including ritonavir, have long been associated with toxic side effects including hepatotoxicity [96]. Cases of dolutegravir-induced neurotoxicity following supratherapeutic dosing of the drug as well as severe thrombocytopenia have been noted [97, 98]. Interestingly, many studies exist regarding TDM and ritonavir, specifically in the pregnant population. Drug toxicity in pregnant patients is important due to the potential consequences of placental transfer of toxic compounds. Dolutegravir has been shown to cross the pla-

centa in an ex vivo placental model with a fetal-to-maternal concentration ratio of 0.6, which is considered relatively high compared to other antiretrovirals [99]. Further, mouse models of dolutegravir have noted increased rates of neural tube defects at therapeutic, but not supratherapeutic, concentrations [100]. The aforementioned studies highlight the benefits of TDM in HIV-infected individuals.

Currently, the most common published methods for determining serum concentrations of antiretrovirals are via high-performance liquid chromatography (HPLC), mass spectrometry, and ultrafiltration. Pharmacokinetics of common antiretroviral drugs can be seen in Table 3.2. Integrase inhibitors such as raltegravir, dolutegravir, and elvitegravir concentrations can be determined via HPLC as well as tandem mass spectrometry in both plasma and cerebrospinal fluid (CSF) samples [101]. Ultrafiltration has been noted to be a plausible technique for determining unbound, active plasma concentrations of dolutegravir, raltegravir, and darunavir [95]. These methods are ideal in resource-rich countries with widespread access to complex laboratory techniques. In resource-poor regions, the use of dried blood spots has been shown to be an effective method for TDM of antiretroviral drugs [102].

Despite the aforementioned routes of TDM, the most common and routine method of TDM in HIV-infected individuals is via surrogate laboratory markers. Patients may receive routine monitoring for hepatic, renal, or hematologic abnormalities. Common markers include alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyl transferase (GGT), as well as creatinine and complete blood count (CBC). Routine hematologic monitoring is recommended for zidovudine, hepatic monitoring for nevirapine, and renal monitoring for tenofovir [104]. Given that many antiretrovirals are administered together in a regimen known as highly active antiretroviral therapy (HAART), using routine laboratory markers is ideal and more cost effective.

3.9 Conclusion

The ability to accurately monitor drug concentrations remains a critical component of patient care. Knowledge of the pharmacokinetic properties of a given drug allows for members of the care team to determine the serum concentration of the drug and predict the effect the drug will have on the individual. Given that the absorption, biotransformation, and clearance of particular drugs may have interpatient variation, treating patients based on their known comorbidities, characteristics, and genetic profiles will maximize the therapeutic safety and efficacy of their medical treatment. Furthermore, knowledge of how to properly administer and follow the concentrations of higher risk drugs with narrow therapeutic windows requires special consideration and pharmacist involvement. Plasma provides insight into the often cryptic manner by which drugs are metabolized and function.

Table 3.2 Pharmacokinetic variations in antiretroviral drugs

Drug	Bioavailability	Hours to peak serum concentration	Half-life (hours)
Abacavir	50% protein bound	0.7–1.7	1.5
Darunavir	95% protein bound	2.5–4	15
Dolutegravir	>98% protein bound	2–3	14
Maraviroc	70% protein bound	0.5–4	14–18
Raltegravir	83% protein bound	3	9
Ritonavir	98–99% protein bound	2	3–5
Tenofovir	<7% protein bound	1–2	17

Adapted from Brody's Human Pharmacology: Mechanism Based Therapeutics [103]

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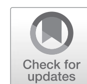

4 Dried Blood Spots in Therapeutic Drug Monitoring and Toxicology

Raphael N. Alolga, Qun Liu, and Qi Lian-Wen

Abstract

In the quest for suitable surrogates to venipuncture and conventional biological matrices (plasma and serum), dried blood spot (DBS) sampling has emerged as a very credible candidate with good analytical prospects. The use of DBS for qualitative and quantitative purposes has garnered much attention from the scientific community over the past 60 years. This chapter details the applicability of DBS in therapeutic drug monitoring (TDM) and toxicology. Specifically, this chapter highlights topical issues including but not limited to the application of DBS sampling in pharmacokinetics (PK), toxicokinetics (TK), forensic toxicology, and biomonitoring of environmental contaminants. The strengths and weaknesses associated with its use, the various presumptions that need to be considered in the use of same for TDM, and other pertinent issues are herein addressed. Due consideration has also been devoted to discussing the implications of blood hematocrit variations (known as the hematocrit factor) and ways to tackle or cope with this pitfall. Finally, this chapter delineates the procedural steps in DBS sampling, recent innovations in DBS sampling, and high-throughput application of same.

Keywords

Dried blood spots · Therapeutic drug monitoring · Toxicology · Hematocrit · Biological matrices

4.1 Introduction

The gold standard for TDM has been serum or plasma, but the emergence of DBS in the early 1960s makes it an alternative that is worth considering. Interest in DBS for TDM was ignited by the work of Guthrie and Susi, who reported the utility of DBS in the screening of phenylketonuria in newborns [1]. Their work paved way for numerous newborn screening programs using DBS [2–4]. The scope of applicability for DBS has increased considerably over the years, from

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newborns to adults, animals, and even for post-mortem purposes. DBS has also been applied for the analyses of proteins, DNA, trace elements, and small molecules [5].

DBS sampling is a relatively simple and convenient method compared to venipuncture. Generally, in DBS sampling, an individual disinfects his/her finger and pricks same with a sterile lancet. The first drop of blood is usually discarded since it contains mainly tissue fluid. Subsequent blood drop is then deposited on a filter paper with premarked circle, allowed to dry at room temperature, and then sent for laboratory analysis. Laboratory analysis is hence conducted using a punched circular disc (representing a defined volume) of the blood spot with sensitive analytical technique [6, 7]. The advantages of this technique over the conventionally established venous sampling include [8]:

- Ease of sampling and minimal invasiveness of technique. This is a technique (which basically involves a finger prick with a lancet) that can be performed by any adult without the need for a phlebotomist.
- A small amount of blood is required.
- Enhanced stability of analytes in the DBS than frozen samples.
- Storage and transportation convenience to the laboratory for analysis.
- Risk of infection from dried sample is reduced.
- Use of DBS leads to simplification in the processing of samples for analysis.

The weaknesses or challenges encountered in the use of DBS include:

- The small sample volumes which are characteristic of DBS call for sensitive analytical techniques.
- Only one assay per spot can be performed at a time.
- There are usually no spare DBS samples for confirmatory analysis.
- Independent sampling by non-health workers requires adequate training and even that does not automatically guarantee success by first-time users.
- Successful and meaningful application of this technique requires substantial validation. Factors such as the influence of hematocrit (Hct) variations on the size and homogeneity of the blood spot, impact of blood volume and spot homogeneity, and the type of filter paper used all require due consideration and validation.
- There is need to take into consideration the fact that capillary blood (which is what is used in DBS) concentration of an analyte is essentially different from venous blood, and appropriate translational corrections/calculations done.

Undoubtedly, the most notable challenge in the use of DBS remains the “hematocrit factor.” This is a double-sided problem that is both analytical and physiological in nature [8]. Analytically, the influence of hematocrit alterations affects the accuracy and precision of the analytical method used to determine the level(s) of the analyte(s). The physiological effect of the hematocrit factor relates to the plasma/analyte ratio of the analyte under investigation [8]. Details of these problems and how to possibly circumvent them are discussed in this chapter. This chapter also discusses the applicability of DBS in TDM, toxicology, and innovations made to improve upon the potential of DBS toward possible adoption by clinical biochemistry laboratories.

4.2 Therapeutic Drug Monitoring (TDM)

TDM basically entails measurement of the levels (concentrations) of drugs in the bloodstream of individuals (patients) at specified time intervals with the ultimate aim of establishing optimal dosage regimens. It is mainly employed for drugs with the following characteristics: (1) drugs with narrow therapeutic indices, (2) drugs that exhibit significant variabilities in their pharmacokinetic (PK) profiles, (3) drugs whose systemic concentrations are not easy to monitor, and (4) drugs with known or predicted to have serious adverse effects. TDM operates on the

premise that a reasonably established relationship exists between the dose and plasma or blood concentration of a drug and the concentration and therapeutic effect(s) of same. A plethora of variables influence the interpretation of drug concentration data, from dose and dosage form to sampling time, handling of blood samples, analytical method, health status of individual (healthy, sick, presence of comorbid conditions, etc.), and reliability of PK models used. TDM generally involves factors and variables that influence the PK and pharmacodynamic (PD) monitoring of drugs. These variables therefore need to be taken into consideration during TDM for the data to be clinically useful. Clinically, TDM aims at enhancing patient care by modifying the dosages of drugs to suit individual/specific patient needs [9].

4.2.1 DBS in PK Monitoring

In a much as venipuncture remains the mainstay blood sampling technique for PK studies, DBS sampling is gradually attaining recognition as a credible surrogate or complementary technique. The invasiveness of venipuncture constitutes a major limitation that accounts considerably for poor volunteer recruitments in such studies. Also multiple withdrawal of large volumes of blood samples from experimental animals constitutes another limitation for venipuncture. Under such circumstances, meeting the ethical requirements of the "3Rs" (replacement, reduction, and refinement) becomes a strenuous task. Blood sampling by DBS particularly meets the latter two of the "3Rs," thus reduction and refinement. DBS sampling allows for a reduction in the number of animals (rodents) needed. This comes with reduced cost of experiments particularly when knockout or transgenic animal models are used. The need for refinement is met in the way and manner blood is sampled from the animals. Aside from the general strengths and advantages of DBS as a sampling technique earlier outlined, the analytes are subjected to relatively fewer processing stages prior to analysis and do not need to be stored under strict cold-chain conditions. This makes DBS sampling a suitable technique for multicenter studies in low-resource settings where multiple sampling is required and cold-chain facilities are unavailable [10]. DBS has hence been variously used for the PK studies of different classes of drugs including but not limited to antimicrobials (antibiotics and antifungals), immunosuppressants, analgesics, antidepressants, antiretrovirals, anticonvulsants, etc. Table 4.1 summarizes the classes of drugs, names of drugs, and the analytical techniques applied for the PK studies by various research groups.

4.2.2 Factors to Consider in PK Studies Using DBS Sampling

In order to fully tap from the benefits of DBS sampling in PK studies as well as other strategies of TDM, certain inherent correlational establishments and assumptions need to be duly recognized [11]. Due consideration should be given to the relationships that exist between the total plasma concentration, total whole blood concentration, and the unbound concentration of the analyte as shown in Eqs. 4.1 and 4.2.

$$C_t = \frac{C_u}{f_u} \tag{4.1}$$

$$C_b = \left[\frac{1-}{f_u} + \quad \times \rho \right] C_u \tag{4.2}$$

C_t is the total plasma concentration; C_u is the unbound concentration; C_b is the total blood concentration; f_u is the unbound plasma fraction; H is the hematocrit and ρ is the blood cell-to-unbound plasma concentration ratio. It is evident from these equations that the plasma concentration of an analyte is proportional to its unbound concentration if f_u is constant. The blood concentration of the analyte is proportional to its unbound concentration when f_u, H, and ρ are constant. Under such ideal circumstances, accurate determinations of the analyte concentration can either be done using plasma or whole blood, since the result of using any of these accurately reflects the unbound concentration of same.

However, under non-ideal conditions these general assumptions do not hold validity. For instance, for drugs that do not have any binding affinity for plasma proteins (in this case $f_u = 1$) such as the aminoglycosides, total plasma concentration (C_t) is approximately equal to the concentration of the unbound drug (C_u). Plasma (or even whole blood) could therefore be appropriate matrix for the analysis of these drugs. In the instance where the drug binds to plasma proteins (as is the case for most drugs), f_u becomes a crucial parameter. The value of f_u is influenced by disease (e.g., kidney diseases), burns, pregnancy, age, species, etc. Under these circumstances, it advisable to directly measure the unbound drug concentration and calculate the f_u value therefrom. There are also instances where the hematocrit (H) and the blood cell-to-unbound plasma concentration ratio (ρ) are additional confounding parameters. Though relatively constant, the hematocrit of the blood can fall drastically below the average value of 0.45 to about 0.2 under anemic conditions and should therefore be taken into account in blood analysis and data interpretation. For polar, hydrophilic drugs that cannot permeate the blood cells, the blood cells tend to act as a diluent for such drugs. The blood concentration of the analytes (C_b) is thus represented by Eq. 4.3:

$$C_b = \left[\frac{1-}{f_u}\right] C_u \tag{4.3}$$

Such drugs also exhibit either poor or no binding affinity to plasma proteins ($f_u = 1$), thereby changing Eq. 4.3 to:

$$C_b = \left(1- \quad\right) C_u \tag{4.4}$$

For these drugs (e.g., the aminoglycosides), use of blood is a good matrix to monitor their unbound levels. For drugs such as caffeine or alcohol that freely permeate the blood cells and do not bind to the plasma proteins, their C_b is almost equal to C_u. In this instance, blood again is a good matrix to determine their levels.

For drugs that permeate the blood cells as well as bind to same, ρ becomes a critical factor to consider. Strong cellular binding of the drug (especially to the RBCs) than to plasma protein ($\rho > 1/f_u$) changes Eq. 4.4 to:

$$C_b = \quad \times \rho \times C_u \tag{4.5}$$

As noted from Eq. 4.5, the blood concentration of an analyte becomes directly proportional to its unbound concentration and is not prone to alterations in plasma protein binding, thereby making blood superior to plasma as an analytical matrix. This ideal situation does not play out all the time because the blood concentrations of most drugs are usually sensitive to changes in ρ. Also drugs that fall under this category possess high blood-to-plasma concentration ratios (C_b/C_t). Blood-to-plasma concentration ratio therefore provides a useful guide to the significance of either plasma binding or blood cell binding in the use of DBS for PK studies. For instance, blood cell binding becomes the predominant issue when the $C_b/C_t > 1.5$.

In brief, under ideal conditions, the blood concentration of a drug is the sum of its concentrations in the RBCs (predominantly) and plasma [10]. Preferential and differential binding of a drug to other components of the blood and other tissues result in variations in the concentrations of same in the plasma and RBCs and invariably the blood concentration. To this end, for a comparison between the amount of a drug in the plasma or serum and the DBS to hold any validity, the hematocrit (H), RBC to plasma partitioning ($K_{\mathrm{RBC/plasma}}$), and blood-plasma partitioning ($K_{\mathrm{Blood/plasma}}$) need to be determined. To calculate the hematocrit of blood, a packed RBC volume is divided by total blood volume. The packed RBC volume is simply determined by centrifuging heparinized blood in microhematocrit tubes at specified speed and time. The $K_{\mathrm{RBC/Plasma}}$ or $K_{\mathrm{Blood/plasma}}$ could be estimated by spiking a drug in plasma or plasma with suspended RBCs, equilibrating the mixture before centrifugation. The amount of the drug in the plasma and RBCs can then be quantified [12]. Determinations of these parameters make it possible for the plasma concentration of the drug to be estimated from the DBS results based on Eq. 4.6:

$$\text{Estimated plasma conc.} = \frac{\text{Drug Conc. in DBS}}{\left[(1-\text{H}) + \text{H} + \text{K}_{\text{RBC/plasma}}\right]} \quad (4.6)$$

For a drug with $K_{\text{RBC/plasma}}$ or $K_{\text{Blood/plasma}}$ values greater than 1, the amount of that drug will be higher in the DBS than plasma, hence making DBS a superior matrix for PK analysis. $K_{\text{RBC/plasma}}$ or $K_{\text{Blood/plasma}}$ values equal to 1 lead to comparable amounts of the drug in plasma or serum and the DBS. Use of DBS under such circumstance does not offer any obvious benefit over plasma or serum. However, when the $K_{\text{RBC/plasma}}$ or $K_{\text{Blood/plasma}}$ values are less than 1, the amount of the drug in the DBS will be lower than in plasma or serum, making DBS not the recommended method for PK studies [13]. DBS is best suited for the analysis of drugs with higher RBC binding affinities relative to plasma.

4.2.3 Toxicology

Aside from the relevance of DBS in TDM, its application in various aspects of toxicological assessments such as toxicokinetics, forensic, environmental, and clinical toxicology is gaining traction. In neonatal screening programs, DBS samples are used not only to determine the presence or otherwise of inborn errors of metabolism but serve as useful matrices to monitor the effects of certain prenatal exposure conditions, notably exposure to potentially harmful and toxic compounds. In this respect, the ability of these compounds to cross the fetoplacental barrier is assessed, albeit with caution since postpartum exposure via the mother's milk needs to be taken into consideration. The information obtained therefrom could retrospectively reveal the extent of exposure to these chemicals. Since early exposure to these harmful chemicals could have debilitating effects on the child later in life, the toxicological information obtained could be used for specific follow-up studies and possible interventions [14].

4.2.3.1 Toxicokinetics

DBS sampling has also been applied for toxicokinetic studies. Toxicokinetics, which is similar to PK, is mainly focused on toxicants – the absorption, distribution, and elimination of toxicants in a living organism. In this respect, the earlier stated advantages, weaknesses, assumptions, and correlational evaluations with the use of DBS for PK studies apply for toxicokinetics. Details of specific toxicokinetic studies are summarized in Table 4.1.

4.2.3.2 Forensic Toxicology

Due to the inherent strengths of DBS sampling, it has been suggested for use in forensic toxicological analysis as either an alternative or mainstay technique. Forensic toxicology generally deals with the application of the principles and knowledge of toxicology to investigate issues that are of interest to the law enforcement authorities [86]. In this regard, the testimonies of forensic toxicologists in the delivery of fair and just judgments by the law courts cannot be overstated. This branch of science mainly consists of three major disciplines: postmortem toxicology, forensic drug testing, and human performance toxicology. Broadly, forensic toxicology refers to the qualitative as well as the quantitative evaluation of biological samples for both legal and illegal drugs. Hence, drugs or substances of abuse including the benzodiazepines, opiates, cannabinoids, cocaine, amphetamines, gamma-hydroxybutyric acid, and alcohol (including its metabolites, ethyl glucuronide and ethyl sulfate, phosphatidyl ethanol) fall under this category. Table 4.2 summarizes some drugs or compounds of interest for which DBS sampling was performed. Despite the merits of DBS sampling, it is still used with caution. For instance, in the determination of a DUI case (driving under the influence of alcohol), the accepted convention is to determine the amount of alcohol in the exhaled breath of the person using a breathalyzer. Also, for substance abuse cases, urine usually serves as the main biological matrix.

Table 4.1 PK and TK studies of drugs for which sampling was done by DBS

Class and name of drug	Type of study	Analytical platform	References
Antivirals			
Amprenavir	PK	LC-MS	[15]
Atazanavir	PK and TDM	LC-MS	[15]
Darunavir	PK and TDM	LC-MS/MS	[15, 16]
Saquinavir	PK and TDM	LC-MS/MS	[15]
Amprenavir	PK and TDM	LC-MS/MS	[15]
Lopinavir	PK and TDM	LC-MS/MS	[15]
Ritonavir	PK and TDM	LC-MS/MS	[15]
Etravirine	PK and TDM	LC-MS/MS	[15]
Efavirenz	PK and TDM	LC-MS/MS	[15]
Nevirapine	PK and TDM	LC-MS/MS	[15]
Tenofovir and emtricitabine	PK	LC-MS/MS	[17]
Oseltamivir	PK	LC-MS/MS	[18]
Zidovudine	TDM	RIA	[19]
Raltegravir	TDM	LC-MS/MS	[20]
Nevirapine and efavirenz	TDM	LC-MS/MS	[21]
Etravirine	TDM	LC-MS/MS	[15]
Efavirenz	TDM	RP-HPLC-UV	[22]
Anticonvulsants/antidepressants			
Levetiracetam	TDM	HPLC	[23]
Lamotrigine	TDM	HPLC	[23–25]
Phenobarbital	TDM	HPLC	[23]
Carbamazepine	TDM	HPLC	[23, 25]
Gabapentin	PK	LC-MS/MS	[26]
Rufinamide	TDM	LC-MS/MS	[27]
Topiramate	TDM	FPIA and LC-MS/MS	[28]
Fluoxetine	TDM and PK	NICI-MS-MS	[29]
Norfluoxetine	TDM and PK	NICI-MS-MS	[29]
Reboxetine	TDM and PK	NICI-MS-MS	[29]
Paroxetine	TDM and PK	NICI-MS-MS	[29]
Clozapine	TDM	HPLC	[30, 31]
Diazepam	PK	LC-MS/MS	[32]
Donepezil	PK	LC-MS/MS	[33]
Midazolam	PK	LC-MS/MS	[34]
Valproic acid	TDM	LC-MS/MS	[25]
Phenytoin	PK	LC-MS/MS	[35]
Antimicrobials			
Actinomycin-D	PK	LC-MS/MS	[36]

(continued)

Table 4.1 (continued)

Class and name of drug	Type of study	Analytical platform	References
Gemifloxacin	PK	HILIC with fluorescence detection	[37]
Linezolid	TDM	LC-MS/MS	[38]
Moxifloxacin	TDM	LC-MS/MS	[39]
Posaconazole	PK and TDM	LC-MS/MS	[40, 41]
Fluconazole	TDM	LC-MS/MS	[41]
Voriconazole	TDM	LC-MS/MS	[41]
Rifampicin	TDM and PK	HPLC-UV; LC-MS/MS	[42, 43]
Pyrazinamide	PK	LC-MS/MS	[43]
Ethambutol	PK	LC-MS/MS	[43]
Rifaximin	TDM and TK	LC-MS	[44]
Sisomicin and netilmicin	TDM	Post-column HPLC with fluorescence detection	[45]
Mycophenolic acid	TDM	LC-MS/MS	[46]
Ertapenem	TDM and PK	LC-MS/MS	[47]
Metronidazole	PK	HPLC	[48, 49]
Ampicillin	PK	LC-MS/MS	[50]
Benzathine penicillin	PK	LC-MS/MS	[51]
Solithromycin	PK	LC-MS/MS	[52, 53]
Ceftriaxone	PK	LC-MS/MS	[54]
Piperacillin-tazobactam	PK	LC-MS/MS	[55]
Analgesics			
Acetaminophen	TK	LC-MS/MS	[56]
Flurbiprofen	PK	LC-MS/MS	[34]
Methadone	TDM	HPLC	[57]
Naproxen	PK	LC-MS/MS	[58]
Anticancers			
Busulfan	TDM	LC-MS/MS	[59]
Paclitaxel	PK	LC-MS/MS	[60, 61]
Vincristine	TDM and PK	LC-MS/MS	[62]
Larotrectinib	PK	LC-MS/MS	[63]
Abiraterone and delta(4)-abiraterone	TDM	LC-MS/MS	[64]
Radotinib	TDM	LC-MS/MS	[65]
Immunosuppressants			
Tacrolimus	TDM	LC-MS/MS	[66]
Sirolimus	TDM	LC-MS/MS	[66, 67]
Everolimus	TDM	LC-MS/MS	[66, 67]
Cyclosporin A	TDM	LC-MS/MS	[66]
Antihypertensives			
Clonidine	PK	LC -MS/MS	[68]
Atenolol	TDM and PK	LC-TOF-HRMS	[69]
Bisoprolol	TDM	LC-HRMS	[70]
Ramipril	TDM	LC-HRMS	[70]
Simvastatin	TDM	LC-HRMS	[70]
Bosentan	PK	LC-MS/MS	[71]

(continued)

Table 4.1 (continued)

Class and name of drug	Type of study	Analytical platform	References
Losartan	PK	LC-MS/MS	[72]
Metoprolol	PK	LC-MS/MS	[73]
Propranolol	PK	LC-MS/MS	[74]
Guanfacine	PK	LC-MS/MS	[75]
Antimalarials			
Tafenoquine	TDM	HILIC with fluorescence detection	[76]
Sulfadoxine and sulfamethoxazole	TDM	HPLC-UV	[77]
Sulfadoxine and pyrimethamine	TDM	HPLC-UV	[78]
Quinine and 3-hydroxyquinine	PK	HILIC with fluorescence detection	[79]
Monodesethylchloroquine, chloroquine, cycloguanil, and proguanil	PK	HPLC	[80]
Amodiaquine, chloroquine, and chlorthalidone	PK	LC-MS/MS	[81]
Chloroquine and desethylchloroquine	PK	HPLC-UV	[82]
Antidiabetics			
Metformin	TDM	HPLC-UV	[83]
Metformin and sitagliptin	PK	LDTD-MS/MS	[84]
Pioglitazone	TK	LC-MS/MS	[85]
Rosiglitazone	PK	LC-MS/MS	[34]

LC-MS liquid chromatography-mass spectrometry; *LC-MS/MS* liquid chromatography-tandem mass spectrometry; *LC-HRMS* liquid chromatography-high-resolution mass spectrometry; *LC-TOF-HRMS* liquid chromatography-time-of-flight high-resolution mass spectrometry; *NICI-MS/MS* negative-ion chemical ionization-tandem mass spectrometry; *LDTD-MS/MS* laser diode thermal desorption tandem mass spectrometry; *FPIA* fluorescence polarization immunoassay; *RIA* radioimmunoassay

4.2.3.3 Screening for Environmental Contaminants

DBS has served as the matrix of choice for the screening of various environmental contaminants in humans and animals. These contaminants range from heavy metals, benzene oxide (metabolite of benzene), perfluoroalkyl compounds, perchlorate, organochlorine pesticides, polychlorinated biphenyls, to polybrominated diphenyl esters. Exposure of newborns to toxic compounds such as dichlorodiphenyldichloroethylene (metabolite of DDT), perfluorooctane sulfonate, perfluorooctanoate, etc. has been assessed using DBS sampling [120–122]. Animals intoxicated with biohazard materials can be monitored using the DBS sampling technique. The levels of cholinesterase inhibitors such as the carbamate and organophosphate insecticides, in birds for instance, can be assessed by DBS sampling. As earlier indicated, the utility of this technique allows for samples to be collected in secluded areas where no special equipment are available. It is also possible to monitor the levels of biohazard compounds in the environment by targeting the animals at the top of the food chain. This strategy has been applied to monitor the level of exposure of the bottlenose dolphin to the biotoxins of *Pseudo-nitzschia* and *Karenia brevis*, thus domoic acid and brevetoxins, respectively [123]. These toxins have also been detected in other experimental animals (mice, rats) using DBS sampling. Analysis of these biotoxins in the DBS samples was accomplished using radioimmunoassay [124], receptor-binding assay [125], or competitive ELISA [126].

4.3 The Hematocrit Factor

The hematocrit factor remains by far the single most relevant and discussed issue in DBS sampling. Since the viscosity of whole blood is determined by its hematocrit, it invariably affects the spreadability of same on a filter paper. Hence, aside from the type of filter paper used, the content of an analyte in punched discs from the DBS card obtained using blood of different hematocrit values tends to differ and ulti-

Table 4.2 List of some drugs or markers of drugs of abuse for forensic purposes

Name of drug (marker compounds)	Analytical platform	References
Fentanyl and its metabolites (norfentanyl and despropionyl fentanyl) and analogs	LC-MS/MS	[87–89]
Ethylglucuronide – ethylsulfate	LC-MS/MS	[90]
Phosphatidylethanol	LC-MS/MS	[91–93]
Cotinine	LC-MS/MS	[94–96]
Tramadol and O-desmethyltramadol	LC-MS/MS; LC-HRMS	[97, 98]
Tetrahydrocannabinol and metabolites	LC-MS/MS	[99–101]
Methadone and metabolites	LC-MS/MS	[102, 103]
Buprenorphine and metabolites	LC-MS/MS	[104]
Ketamine and norketamine	LC-MS/MS	[104]
Opiates and metabolites	LC-MS/MS	[105, 106]
Gamma-hydroxybutyric acid	GC-MS	[107–109]
Benzodiazepines	LC-MS/MS	[110, 111]
Zolpidem	CE-MS	[112]
Cocaine, benzoylecgonine, and other metabolites	LC-MS/MS	[113]
Methamphetamine	LC-MS/MS	[105]
[a]MDEA, MDMA, MDA	LC-MS/MS	[114]
Amphetamine	LC-MS/MS	[115]
Anabolic steroids	LC-MS/MS; GC-MS	[116–119]

LC-MS liquid chromatography-mass spectrometry; *LC-MS/MS* liquid chromatography-tandem mass spectrometry; *LC-HRMS* liquid chromatography-high-resolution mass spectrometry; *GC-MS* gas chromatography-mass spectrometry
[a]*MDEA* 3,4-methylenedioxyethylamphetamine; *MDMA* 3,4-methylenedioxymethamphetamine; *MDA* 3,4-methylenedioxyamphetamine

mately introduces significant assay biases and errors. The hematocrit of blood is the volume ratio of red blood cells to the total blood volume. The typical hematocrit range for men is 0.41–0.50, while that for women is 0.36–0.44 [127]. Elevated values are generally observed for people living in areas of high altitude, newborns, and persons with primary polycythemia and chronic obstructive pulmonary disease, while anemic persons, immunocompromised individuals, and persons receiving chemotherapy have lower hematocrit values [8].

Several strategies have been advanced with the aim of determining how to either minimize the impact of hematocrit variations or tackle (avoid) it entirely. To avoid the hematocrit problem, researchers tend to use volumetrically generated DBS and analyze the DBS punches holistically. Others use dried plasma spots (DPS) in place of DBS. The impact of hematocrit can be minimized by spotting the blood on special filter substrates, using calibrators with hematocrit values within the range of the target population and estimating (measuring) the hematocrit of the DBS [8].

4.3.1 Holistic Analysis of Volumetrically Spotted DBS

A convenient way to tackle the hematocrit issue is to avoid it by analyzing whole-cut volumetrically applied DBS. It involves mainly two approaches. The first approach involves the use of the entire DBS punched out of their respective filter papers after specific volumes of blood have been applied. In the other approach, volumetrically generated blood is spotted on pre-punched discs, dried, and the whole discs analyzed. This approach at tackling the hematocrit problem requires that the volume of blood used for spotting be accurately and precisely delivered. Accurate and precise volumes of blood are obtained using specialized microcapillary sampling devices that are best used by trained persons. This obviously tends to be a limitation since this approach cannot be readily used outside a specialized environment (e.g., at home) by non-trained individuals, thus defeating one of the main strengths of DBS sampling.

4.3.2 Use of DPS in Place of DBS

An alternative strategy for circumventing the hematocrit problem is the use of dried plasma spots (DPS) instead of DBS. By virtue of the fact that plasma is used instead of whole blood as the matrix, RBCs are excluded – ultimately avoiding the hematocrit problem. Many a researcher have

reported an almost excellent association between the concentrations of various drugs (such as paroxetine, nevirapine, triazole antibiotics) in DPS and plasma [128–131]. Most researchers obtained plasma from blood using various methods that eventually involved centrifugation, a process that cannot be readily carried out at home. Other researchers have however devised various preparatory steps that are independent of centrifugation. A study worth mentioning is that of Li et al. who, in the biomonitoring of guanfacine, used a device that was capable of separating plasma from whole blood [13]. The DPS was obtained from blood drops applied onto a filter membrane (multilayered and polymeric) with collection and separation membranes, and a top layer that is removable. The plasma seeped through the separation membrane to the collection membrane at the bottom, while the solid residue (RBCs) remained on top and could then be peeled off after about 5 min [13].

4.3.3 Use of Special Filter Substrates

As a means of minimizing the hematocrit effect, special types of filter material or paper have been manufactured by several companies. Examples of these special filter materials include Bond Elut Dried Matrix (a noncellulose filter paper developed by Agilent Technologies), HemaForm™, HemaSpot™ (developed by Spot on Sciences Inc.), glass paper filters, chitosan and alginate forms, etc. These special materials exhibit unique strengths and weaknesses compared to the conventional cellulose filter paper and therefore require validation before use [8].

4.3.4 Use of Hematocrit Calibrators

To better minimize the impact of hematocrit on any DBS-based analytical assay, it is advisable to consider the target population (e.g., healthy persons, newborns, the elderly, anemic individuals, immunocompromised persons, etc.) and establish an acceptable hematocrit range prior to analysis and validation of the analytical method. Since hematocrit could vary based on specific target groups, its impact could hence be reduced by using blood samples from such groups as calibrators. In line with this, when quantitatively monitoring the level of an analyte in newborns (healthy) who usually have hematocrit values above 0.50, the same calibration line cannot be used for immunocompromised patients, typically with lower than the 0.30 average hematocrit value [132]. In the situation whereby the target population is heterogeneous, the calibrators can be prepared using blood with approximate average hematocrit value of 0.4 [133]. On the other hand, one could also use two calibration graphs, one constructed within the upper limit and the other within the lower limit of acceptable hematocrit range.

4.3.5 Estimation or Prediction of Hematocrit

Different analytical strategies have been propounded or applied to cope with the hematocrit factor [8]. These strategies essentially allow for confirmation of the hematocrit of DBS samples and acquisition of a correction factor to compensate for the impact of the hematocrit. It has been suggested that extra venous blood sample be taken for hematocrit estimation in addition to the capillary blood used for the DBS. This however is feasible under controlled conditions with the aid of trained personnel. Also with this approach, one wonders the essence of using capillary blood for the DBS if venous blood is available except for issues of stability. Differences in the hematocrit of capillary blood relative to venous blood make this approach quite problematic. Ideally, the hematocrit of the capillary blood used for the DBS should be determined. To achieve this, a calibrated microcapillary coated with an anticoagulant can used to deliver a minute volume of capillary blood and the hematocrit read after the sample is centrifuged. Here again, the ease of sampling which is central to DBS is eventually

lost. Though costly to the patient, a more convenient and practical option is to rely on point-of-care tests (e.g., those developed by HemoCue®) that are comparatively fast, easy to handle, and require minimal amount of blood (10 µl) to determine its hematocrit [134–136].

Some researchers have also suggested that the hematocrit be estimated on the basis of certain physical characteristics of the DBS including the surface area, diameter, frustum volume, and color [39, 127, 137]. Needless to mention, but this approach also has its shortfalls and consequently introduces certain errors and biases in the determination of hematocrit. Notably its reliance on the exactitude of the volume of blood spotted ranks topmost since the characteristics of the DBS that depend on its dimensions such as area (diameter) of spread, color of spot, and frustum volume are in turn dependent on the amount of blood spotted. For instance, using the photographs of DBS, Denniff and Spooner sought to measure the spot area of blood on different kinds of filter paper [127]. This proved difficult to accomplish since the differences in the type of filter paper affected the homogeneous spread of the spot, making it difficult to determine the exact edges of the spot for the frustum volume to be calculated. They however found that the frustum volume was linearly correlated with hematocrit. Other authors established a correction factor in a bid to eliminate biases resulting from the punch diameter, blood volume, and DBS area. That notwithstanding, the biases were not totally eliminated.

A more reliable and resilient alternative to measure or estimate the hematocrit is to rather measure the level of an endogenous compound that has a strong correlation with it (hematocrit). According to De Kesel et al., for a compound to be considered as a hematocrit marker, it needs to satisfy a five-point criterion [8]: (1) the compound should have a strong correlation with hematocrit; (2) the compound should be universally applicable, in the sense that, it should be easily measurable in any population regardless of factors such as age, sex, or race; (3) the compound should exhibit minimal interindividual variation, irrespective of even diseased conditions; (4) the compound should be stable in both new and old DBS, for its measurement to be possible; and (5) determination of the level of such compound should be relatively easy to accomplish. On the basis of this criterion, potassium totally fulfilled all requirements and has therefore been variously recommended by researchers [138, 139].

Hemoglobin seems an obvious candidate to accurately estimate the hematocrit of blood. Hemoglobin is an iron-binding protein exclusively found in the red blood cells (RBCs) and a transporter of oxygen through the body. As earlier outlined, the calculation of hematocrit of fresh blood is already done using hemoglobin. However, the use of hemoglobin to calculate the hematocrit of DBS is met with some challenges that consequently compromise the accuracy and precision of the method. It is worth noting that hemoglobin changes with time as evidenced by the change in color of DBS. This implies that for routine measurement of hematocrit using analyzers that only measure absorption at specific wavelengths, there is a tendency to obtain false readings (lower than fresh samples) especially for older DBS samples. This could be the result of conversion of hemoglobin to another form that does not absorb at that wavelength but not necessarily due to lower amount of hemoglobin *per se. Calibration curves should therefore be constructed using the appropriate samples. Hence, quantification of hemoglobin in fresh* DBS samples should be based on calibration curves obtained with fresh samples and not using calibration curves of aged samples.

4.4 General Steps in DBS Sampling

As summarized in Fig. 4.1, the main procedural steps in the analysis of various compounds (analytes) in the matrix of blood via DBS sampling include selection of filter material, collection of specimen, drying of spotted cards, packaging of DBS for storage and transport, and extraction and analysis of analyte.

4.4.1 Selection of Filter Material

DBS cards consist of mainly cellulose (paper) or noncellulose matrix with varied pore sizes and thickness. Commercially available DBS cards include the Whatman fast transient analysis (FTA) elute cards, the FTA-drug metabolism and pharmacokinetic, FTA-DMPK cards (types A, B, C), and Whatman 903® cards. The quality specifications of these cards define their respective applications. For routine screening of newborns, the Whatman 903® cards are used. The FTA-DMPK cards are suited for PK/TK studies, while the FTA elute cards are designed to accurately collect and purify DNA for subsequent analysis [10].

The DMPK cards come in two main forms, regular and indicating. When colorless samples such as plasma, urine, synovial fluid, etc. are to be analyzed, the indicating cards are the most appropriate type. Types A and B of the DMPK cards are pretreated chemically such that they cause cells to lyse and proteins to denature, as well as inactivate enzymes and inhibit bacteria growth on contact with the biosample (blood, plasma, urine, etc.). These precoated cards also allow for cellular and nuclear membranes to lyse so that nucleic acids that are very stable remain for analysis and/or storage. Moreover, the DMPK cards denature and activate enzymes such as the esterases in the blood, thereby preventing enzymatic breakdown of drugs such as aspirin (acetylsalicylic acid) and procaine, leading to their increased stability. The DMPK-C type is not pretreated with any special chemical and therefore does not denature proteins or produce any chemical that could interfere with sample analysis. Another type of card that exhibits the same properties as the DMPK-C type is the Ahlstrom 226 card (ID Biological Systems, Greenville, SC). These two are therefore recommended for protein-based biomolecular analysis. There are also commercially available noncellulose-based DBS cards for drug monitoring and PK studies such as the Bond Elut DMS card by Agilent Technologies. They are deemed to be superior in quality, require less punching effort, enhance mass spectrometry signals of the analyte, and are not affect by hematocrit variations [10].

4.4.2 Collection of Specimen

In humans, whole blood is sampled from a finger, toe, or heel after a prick with a sterile and disposable lancet. Blood can be collected from rats and

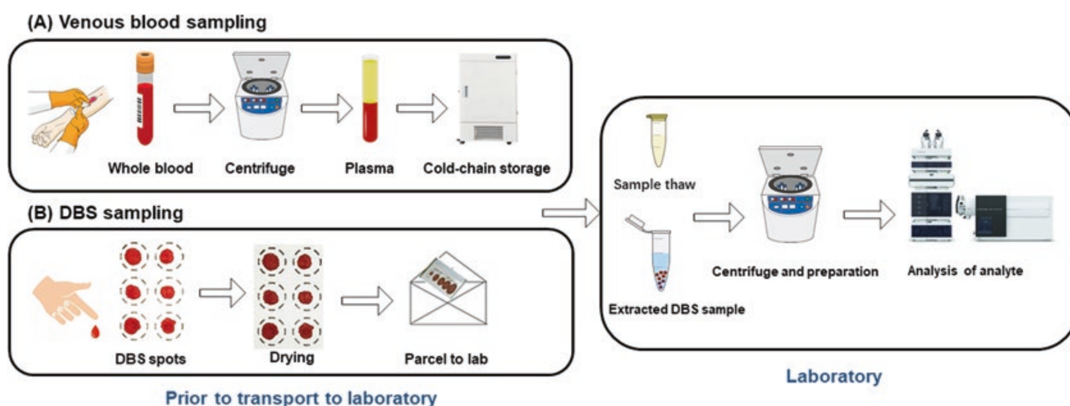

Fig. 4.1 Schematic representation of the main steps in venous blood sampling (**a**) and DBS sampling (**b**). In venous blood sampling (**a**), blood is collected via venipuncture into special sample tubes (for plasma or serum) and centrifuged and the supernatant (plasma or serum) collected and stored under strict cold-chain conditions till analysis or for transport to the laboratory for analysis. For DBS sampling (**b**), capillary blood from a finger prick is spotted on the DBS card, dried under room temperature, and transported as a parcel by normal courier to the laboratory for analysis. In the laboratory, the frozen sample is thawed, centrifuged, and prepared for analysis, while the analyte in the DBS sample is extracted, centrifuged, and prepared for analysis

mice for the purposes of PK and TK studies, from the caudal vein. For the purposes of qualitative determination, drops of blood from the finger, toe, or heel are cautiously spotted within defined areas on the DBS card. For quantitative analyses, accurate volumes of blood are spotted on the DBS cards using a micropipette or capillary tube. It is common in such instances to use anticoagulants such as heparin or ethylenediaminetetraacetic acid (EDTA), though they need to be used with caution in quantitative polymerase chain (qPCR)-based analysis since they affect telomere length measurements [140]. When the samples are analyzed by MS (i.e., LC-MS), EDTA interferes with the spectrum of the analyte of interest. That notwithstanding, it is preferred to heparin for its drying effects and inhibition of ester hydrolases and phospholipases (calcium-dependent) [141–143].

Since the usability of the DBS is dependent on the volume of blood spotted and the accuracy and sensitivity of the analytical platform, it is advisable that the sampling process be done correctly. Improperly spotted cards and DBS cards that are discolored, supersaturated, dark in color, clotted, or contaminated cannot be used for any purpose, be it qualitative or quantitative. As earlier indicated, the differential hematocrit values of blood also need to be duly considered and corrected in order to accurately determine the content of the analyte.

4.4.3 Drying of Spotted Cards

Drying of the spotted cards could be a crucial step particularly for unstable, heat-sensitive analytes or metabolically unstable analytes. The cards need to be completely dried prior to transportation, storage, or analysis so as to avoid microbial growth and other quality defects. Drying time is mainly dependent on the type of filter material used and the volume of blood spotted. Generally, the cards are dried at room temperature for 2–3 h or under controlled humidity using a gentle stream of nitrogen [144, 145]. Exposure to direct sunlight, dust, and insects should be avoided in order not to hamper the integrity of the DBS specimen [145].

4.4.4 Packaging of DBS for Storage and Transport

DBS sampling offers a very convenient avenue for the storage and transportation of the DBS cards. Compared to traditional biological matrices (plasma, serum, urine, etc.), the need for strict adherence to cold-chain during storage or transport is almost totally eliminated with DBS. One does not require to use bulky equipment such as freezers or even dry ice for the storage or transportation of DBS. Once humidity is controlled, DBS cards can be stored or transported at ambient temperature. To guard against the influence of humidity from the environment, the cards can be packed in sealable plastic bags with a desiccant and humidity indicators to monitor when to replace the desiccant. For compounds that are light-sensitive, it is advisable to use an aluminum-coated bag [145]. The DBS cards can be transported safely using normal postal mail or courier without threat of exposure of handlers to infection. It is however recommended that the packaged cards be labeled to clearly reflect the biohazardous nature of sealed content before transportation to the analytical laboratory for analysis.

4.4.5 Extraction and Analysis of Analyte

In the analytical laboratory, the spotted portions of the DBS cards are punched out and used directly or analyzed after extraction using appropriate solvents. A crucial step in the extraction process is the addition of internal standard (IS). The IS could be directly added in the extracting solvent or spotted on the DBS card subsequent to the spotting of the blood. For the sake of homogeneity and reproducibility, the IS can be sprayed on the card [145]. Regardless of the method used to introduce the IS, the extraction efficiencies of various solvents and their combinations for the analyte need to be assessed and the best solvents used and extraction conditions optimized. After optimized extraction, the analyte(s) can be subjected to either qualitative or quantitative analy-

sis under various analytical platforms such as high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), liquid chromatography-tandem mass spectrometry (LC-MS/MS), matrix-assisted laser desorption mass spectrometry (MALDI-MS), inductively coupled plasma mass spectrometry (ICP-MS), enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), etc. With the advent of automated online high-throughput equipment, the sampling process and analysis of DBS cards have become easier, faster, and more accurate and precise [145].

4.4.6 Validation of DBS-Based Analytical Processes

As is required of every quantitative analytical process, DBS-based quantitative methods need to be validated. The guidelines by various established authorities such as the US FDA, the EMA, and the ICH for the validation of bioanalytical methods could be referenced. However, since these guidelines pertain particularly to quantitative analyses in liquid matrices like plasma, serum, urine, etc., the solid matrix of DBS presents certain challenges that require additional validation. In addition to the known validation parameters, certain parameters applicable to only DBS-based samples need to be considered. The following parameters need special attention in DBS-based quantitative analysis: spot volume, hematocrit (already discussed), spot homogeneity, spot-to-spot carry-over, recovery, matrix effects, stability under transport and storage conditions, and dilution integrity. Acceptance criteria for these parameters similar to those of the recognized regulatory authorities need to be met (e.g., ±15 for accuracy and precision) [146].

Spot volume is usually controlled when calibration standards or quality control samples are prepared by spotting exact volumes of whole blood on DBS cards. In the case of clinical samples however, this cannot be controlled since the blood from a pricked finger or heel is applied directly on the DBS cards and could differ. The DBS samples need to be visually checked to make sure that the blood is evenly spread on the card, and larger than the defined punch area. Depending on the type of DBS card and the physicochemical properties of the analyte, differences in spot volume could be critical to the attainment of reproducible and accurate results from quantitative analyses. A series of spot volumes ranging from 15 to 40 μL is usually recommended for the validation process. Triplicate analyses of at least two concentrations (low and high) are recommended for all spot volumes used. Finally, the spot volumes used for the calibration curves and quality control samples should be within the range of the clinical samples under study, so that the effect of differential spot volumes of the samples on the analyte can be controlled [147].

Sample homogeneity using the liquid matrices could be achieved by thorough mixing (vortexing) and centrifugation. This is not possible with the DBS samples since the analytes within the blood are spotted and spread on the card. The need for spot homogeneity becomes paramount when a specific part within the spotted area is used for analysis rather than the whole spot. In such cases, the blood spots need to be evenly distributed on the card, so that the spot size could be equated to a fixed volume. The influence of spot homogeneity on the outcome of quantitative analysis is also dependent on the DBS card type and analyte under investigation. Differences in analyte content in punches gotten from the center and periphery of the DBS card need to be evaluated at least three times at two concentration levels (high and low) during validation. Marked differences should guide in the choice of punches to use (i.e., bigger or smaller) and the need to conduct whole-spot analysis.

Spot-to-spot carry-over basically concerns the device used to punch out the spotted DBS. This is a source of preanalytical error that could be reduced to the barest minimum by performing "between-punches" using blank DBS cards [147]. That is, after punching out the defined diameter on the DBS sample, the same device should be used to perform two punches on blank DBS cards, so that the carry-over of the analyte from one punch to another could be minimized or avoided totally. According to the EMA guidelines, the

spot-to-spot carry-over should not be more than 20% of the lower limit of quantification.

Per the guidelines of the US FDA, EMA, and ICH, the recovery of an analytical method determined at different concentration levels needs to be precise, reproducible, and consistent. These requirements hold true for DBS-based analyses. The addition of IS to the spot prior to extraction could commensurate somewhat for variations in the extraction efficiencies of the solvent(s) as earlier indicated. Aging and hematocrit are known to affect the extraction recovery of some analytes in DBS. It is recommended that a method that results in at least 85% extraction recovery of the analyte be used [146]. Similar to the requirements for matrix effect evaluation during validation, it is recommended that the recovery be assessed using a minimum of six batches of DBS cards, with different hematocrit values and at least two concentrations. It is also advisable to use an accurate volume of whole blood that produces a spot size within the punch size of the punching instrument used so as to ensure whole-spot extraction. Analyses can then be performed on three different types of samples, thus the DBS samples at low and high concentrations (here referred to as "A"), solutions that represent 100% recovery (i.e., neat solutions of the analyte at low and high concentrations, "B"), and solutions of blank DBS sample prepared with neat solutions ("C"). The recovery can then be calculated using Eq. 4.7:

$$\text{Recovery}(\%) = \frac{\text{Content of analyte in A}}{\text{Content of analyte in C}} \times 100\% \tag{4.7}$$

Matrix effect is an essential validation parameter in mass spectrometric analysis of biosamples. Results of matrix effects also need to be consistent and reproducible. Following the laid down protocols by recognized authorities, matrix effects can be investigated and validated in DBS-based samples. Particular attention needs to be paid to the DBS samples spotted on precoated cards since these could introduce additional components that could adversely affect the detection (ion suppression) of the analyte. It is a normal practice to assess the matrix effects using at least six different batches of whole blood at two concentrations (high and low). Hence, the following solutions can used: (1) neat solutions at high and low analyte concentrations (100% analyte recovery) and (2) blank extracts of DBS sample prepared with neat solutions [146]. The influence of the matrix could then be calculated as follows:

$$\text{Matrix factor} = \frac{\begin{array}{c}\text{Content of analyte}\\ \text{in neat solution}\end{array}}{\begin{array}{c}\text{Content of analyte in blanck}\\ \text{BDS extract in neat solutions}\end{array}} \tag{4.8}$$

Stability of the analyte at every stage of handling from sampling, storage, transport to analytical laboratories is a major quality requirement. With respect to storage and transport, the DBS samples need to be stored under controlled temperature and humidity. The time and route of transport of the samples should also be considered. If the samples are to be transported in mailboxes via regular postal service, potential temperature fluctuations would therefore be a significant factor to consider. This is particularly crucial when the storage and transport of the samples are done during seasons of very high (summer) and very low (winter) temperatures [146, 147].

Another essential validation parameter worthy of attention is dilution integrity. It should be assessed at concentrations that foreseeably fall within that of the clinical sample (DBS sample). This can be achieved in one of two main ways: (1) by diluting the final DBS extract with the final blank extract that contains the IS or (2) the analyte can be processed using a high concentration of the IS to obtain a final extract solution. This final extract is then diluted with the final extract of the blank solution (obtained with the same dilution factor as the final analyte extract) that does not contain the IS. This method is known as the IS track dilution method. A minimum of five determinations for every dilution factor is recommended regardless of the methodological approach [146].

4.5 Recent Innovative DBS Sampling Alternatives

In recent times, various innovative approaches have been made in respect of improving the extraction efficiency of DBS as well as addressing its shortfalls. An example of such was introduced by Damon and company called, the three-dimensional (3D) dried blood spheroids [148]. Instead of the conventional hydrophilic cellulose-based DBS cards, they used a functionalized hydrophobic paper substrate that was obtained via gas phase silanization of trichloro(3,3,3-trifluoropropyl)silane on a triangular-shaped filter paper. The silanization process of the filter paper led to a reduction in its surface energy, resulting in the maintenance of the shape of the blood droplets, consequently forming the 3D dried blood spheroids. Using this analytical strategy, they were able to tackle known systemic challenges in traditional DBS sampling such as the volcanic and chromatographic effects and the hematocrit factor. Due to minimal interactions of the analytes with the surface of the paper, its extraction efficiency was enhanced as evidenced by extreme low LOD values. Also, the spheroidal shape of the blood droplets tends to protect the analyte(s) from the effects of ambient air such as oxidative degradation – enhancing the collective stability of the analytes.

In a bid to determine the content of a drug with a narrow therapeutic index (carbamazepine) by capillary electrophoresis, Nuchtavorn et al. coated a Whatman® Grade 1 filter paper with a hybrid of homogenous polystyrene and silica gel polymer to form what they termed molecularly imprinted-interpenetrating networks (MI-IPN) [149]. Using the MI-IPN for extraction of the analyte, they were able to eliminate proteins and other matrices that could affect the results of the capillary electrophoresis instrument. The MI-IPN provided better on-spot extraction efficiencies than conventional DBS cards (Whatman® 903 protein saver card and GenCollect™ 2.0 card).

As early on indicated, the analyte variability in punched spots of DBS due to hematocrit differences poses a major analytical challenge during its quantification. Numerous attempts have been made by different researchers to nib this problem in the bud. For instance, Genk and coresearchers recently devised a microfluidic sampling device consisting of a combination of DBS paper, plastic foils, and a thin dissolvable film [150]. This device operates on capillary forces, while the dissolvable film valves function as a timer that allows for automation of volume metering. The metering chip of this device was able to collect and store microliter quantities of whole blood on the DBS paper substrate in 20 s. Neto et al. established an accurate and precise blood collection and metering system capable of preparing DBS with as low as 3 μL of whole blood [151]. Their proposed methodological approach proved superior to traditional microvolume collection systems such as using a micropipette or an analytical syringe and also almost completely solved the hematocrit problem. A group of researchers from Japan, Nakahara and colleagues in 2018 developed the volumetric absorptive paper disc (VAPD) and its scaled-down version (VAPDmini) for accurate and simple volumetric collection of blood for DBS [152]. The VAPD is made up of a filter paper disc and a filter paper sheet with holes slightly larger than the disc. With the addition of whole blood to the disc, saturation of same allows for the surrounding filter sheet to absorb the excess blood. The accuracies and precisions of their devices were tested using clozapine and its metabolites and found to be within acceptable standards. Also, the analytes' recoveries were found to be hematocrit-independent (not influenced by blood with different hematocrit values).

4.6 High-Throughput Application of DBS Sampling

A very notable application of DBS sampling is in various metabolomics studies. The advantages of this microsampling technique have been variously applied to characterize and aid in the diagnosis or identification of potential biomarkers for different diseases. Metabolomics, which is generally defined as the holistic qualitative and quantitative analyses of as much as practicable

all metabolites present within a biological system under defined conditions, is an ever-evolving field in systems biology. It is an analytical technique that can provide a sneak peek into metabolic processes that underlie various pathophysiological conditions. Also with metabolomics, unique chemical fingerprints of specific metabolic processes can be obtained and provide useful clues on diagnosis and treatment options [153–155]. The metabolomes of diverse diseased conditions including but not limited to the following have been explored using DBS sampling; Diamond-Blackfan anemia [156], cystic fibrosis [157, 158], acute lymphoblastic leukemia [159], biliary atresia [160], pediatric acute myeloid leukemia [161], colorectal cancer [162], ischemic and hemorrhagic stroke [163], breast cancer [164], pyruvate kinase deficiency [165], autism [166, 167], small cell lung cancer [168], etc.

DBS sampling has also been applied for the detection and quantification of proteins and peptides, particularly in immunoassays. The use of DBS sampling for enzyme-linked immunosorbent assay (ELISA) is characterized by high selectivity, specificity, and reproducibility of data [169]. Of note, this sampling technique has been successfully used for the detection of antibodies against various viruses such as rubella virus [170], Epstein-Barr virus [171], hepatitis C virus [172], and dengue virus [173]. The surge in the use of high-throughput analytical platforms such as LC-MS, LC-Q-TOF-MS, MALDI-TOF-MS, etc. has made combinations of these platforms with DBS sampling for both targeted and untargeted analyses of proteins and peptides possible. Using such combinations, it has been possible to determine the level of ceruloplasmin during neonatal screening for the Wilson's disease [174, 175], quantify C-peptide as a measure of normal beta cell function [176], quantify amino acids and acylcarnitines as part of a newborn screening strategy [177, 178], predict major depressive disorder based on proteomics data [179], quantify metanephrines to aid in the diagnosis of pheochromocytoma and paragangliomas [180], screen for primary immunodeficiency disorders in newborns [181], and screen for sickle cell disease in neonates [182], to mention a few.

4.7 Conclusion and Future Perspectives

The increased interest in DBS sampling by the scientific community over the past 60 years not only points to its gradual acceptance but also its potential applicability in research areas that hitherto received little to no attention. Its application in various fields under TDM with the potential for large-scale clinical adoption is worth appreciating. Among the numerous advantages associated with the use of DBS sampling, the ease of sample (whole blood) collection and the stabilizing effect DBS stand out. This assertion holds true for both animal and human studies. For instance, DBS sampling in animal studies allows for adherence to the principles of the 3Rs, such that the number of animals required is reduced and there is marked refinement in respect of the collection of blood without the need to warm the animals (rodents) to ease blood flow. The stabilizing effect conferred on the analyte by the DBS largely prevents its degradation *ex vivo and/or de novo formation of other analytes. These and other advantages notwithstanding, a few points are worth noting in the use of* DBS for both qualitative and quantitative purposes. Due to the possibility of sample contamination and the quality implications therefrom, much attention needs to be devoted to the process of blood collection, spotting, drying of the DBS cards, and transportation of the dried cards. Second, the influence of factors such as hematocrit variation and its attendant issues as well as volume of blood spotted and site of punching need to be addressed (as earlier outlined). Third, for the findings of any DBS-based study to possess any potential clinical significance and be reproducible, it is essential that the entire analytical process be validated in accordance with the guidelines of recognized standard authorities.

As researchers work at providing innovative ways to improve on this microsampling technique and tackle inherent challenges with its application, the aim ultimately is to establish a robust analytical system that could form part of routine tests in the clinic. As most of the processes are being automated, in the foreseeable

future, it might be possible to perform all the analytical steps, from DBS sampling through to analysis using a simple but fully automated equipment.

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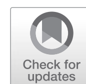

5 The Role of Artificial Intelligence in Therapeutic Drug Monitoring and Clinical Toxicity

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Abstract

The core concept of drug design is data analysis and the methods used in such analysis. The angle of artificial intelligence (AI) in drug discovery is mainly concentrated towards the methodological aspect of computation such as deep learning. With the growth in computing power and advancement in AI methods, this field including machine learning/deep learning (ML/DL) has moved away from theoretical to real-world application. The generation of AI and its subset learning models are based on properties of the training data sets such as physiochemical properties, quantum mechanical, 2D properties, 3D descriptors, molecular patterns, molecular finger prints, etc. Methods such as PCR plus support vector machines (SVMs), naïve Bayes, random forest, neural networks and recursive partitioning are quite often used for the generation of ML models by correlating descriptors with experimental activity. Therapeutic drug monitoring and clinical toxicity are faced by new challenges every day as the problem-solving approaches are fetched with more complex causes in regulatory and health technology to assist the healthcare system. Drug monitoring for the patient is enhancing its functioning by various technical tools supported with numerous treatment models. There exist many platforms to improve disease management by digitally leveraging the best patient care. In this chapter, we have discussed the various key points of AI along with its role in monitoring drug toxicity. Additionally, implementing AI in drug discovery and obtaining medical big data is also discussed. The pipeline which is critical in monitoring diseases and the implementation of AI during pharmacovigilance are described. Other topics such as systematic collection of adverse drug reactions (ADR) through spontaneous logical methods and the impact of AI during pharmacovigilance and toxicity are also discussed. Finally, recent advances in AI for drug safety and post-market surveillance along with the illusions, reality and future of AI use in drug design and discovery were also mentioned.

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Keywords

Artificial intelligence · Machine learning · Deep learning · Drug design · Toxicity · Pharmacovigilance · Adverse drug reaction

5.1 Introduction

The interdisciplinary nature of the drug discovery field has always been on wheels fuelled by new ideas and development in sciences (biological or physical) along with the application of computers and algorithms such as computer-aided drug design (CADD), chemoinformatics, machine learning and artificial intelligence. The core concept in all the aforementioned is data analysis and the methods utilized for such analysis. The artificial intelligence angle in drug discovery is mainly concentrated towards the methodological aspect of computation such as deep learning [1]. The Food and Drug Administration (FDA) recently has been promoting the term real-world data (RWD) to be used in drug discovery which means data collected from other conventional research settings like billing data, administrative claims, electronic health records, etc. [2]. The status of treatment, disease, adherence to treatment, comorbidities, outcomes, concurrent treatments, etc. which can be tracked are present in such RWD. This RWD information can provide critical evidence (real world) to develop good patient care, safety surveillance, therapeutic development, and comparative studies of effectiveness. [3].

The field of artificial intelligence (AI) including machine learning/deep learning (ML/DL) has moved away largely from theoretical to real-world application due to the growth in computer systems and advancement in AI methods [3]. AI is used in target identification, understanding disease mechanism, biomarker development and stages of drug discovery [4]. Digitization of the pharmaceutical sector has given birth to various challenges such as acquiring these data and scrutinizing and applying them for solving clinical problems [5]. These challenges have brought about the use of AI as it can handle large volume of data with increased automation [6]. The advanced tools and methods used in AI can mimic human intelligence without replacing the physical presence of humans [7]. In this chapter, we have discussed the various key points of AI along with its role in monitoring drug toxicity.

5.2 AI Terms

5.2.1 Machine Learning (ML)

Machine learning (ML) is a subset of AI which enables a software, system or application to function more accurately to predict outcomes. Machine learning algorithms use trained data sets as input to predict new rules considering the underlying understanding and reasoning. Data classification is a common process but when the data set becomes complicated with n-numbers of attributes, machine learning comes into the picture. Broadly machine learning is supervised learning, unsupervised learning, semi-supervised learning and reinforcement learning. Each type has its own structure to predict different results.

Supervised learning: In this type of machine learning labelled training data are used. It defines the variables to the different labels to associate correlations with the algorithm.

Unsupervised learning: In this type of machine learning function with unlabelled data are used. The possible useful connection is scanned in the data sets to process further.

Semi-supervised learning: Semi-supervised learning is the combination of supervised learning and unsupervised learning. The algorithm is mostly fed with labelled and training data. There is the freedom to model and explore the data on its own understanding of the data set.

5.2.2 Deep Learning (DL)

Deep learning is a subset of machine learning techniques which enables systems, software or

application to operate naturally. It learns from various examples based on labelled data only. The labelled data can be classified as images, text, sound or other relevant sources. The computer model associated with deep learning performs classification tasks directly from labelled data. Associated models are using a large set of labelled data in multi-layer neural network architectures.

Deep learning applications are popular in aerospace, defence, automated car driving, electronics, healthcare, etc. To detect the particular disease cell automatically, high-dimensional data set of concerned diseases are classified as per need. The trained data is modelled to identify the concerned disease cell.

Reinforcement Learning Here the machine is given the complete multi-step process based on earlier defined rules which is reward-based learning that performs on the principle of feedback. Tasks are completed with positive or negative cues in the flow but majority of the algorithms decide the next steps. In disease cell identification processes, the feedback from various sources will be fitted into the desired reinforcement model. The model will then predict cell legacy belonging to a particular disease or not based on rewards and feedback fitted in the data set.

5.2.3 Artificial Neural Network (ANN)

ANN is similar to the human brain that consists of connected neurons which communicate via signals. It works with three layers which are input layer, hidden layer and output layer. Data are fed to the input layers which forward them to the hidden layer. The main operations are performed at hidden layers, and after completion, results are forwarded to output layer. Generally, ANN works with all nodes interconnected to each other with output node value and the associated weight with each link. The ANNs are based on making the right connections with silicon and wires as live neurons and dendrites to provide the best output.

5.3 Implementing AI in Drug Design: *Early Stages*

Artificial intelligence (AI) may work as humans and involves artificially incorporated human-like intelligence built using complex algorithms and mathematical functions [8]. AI is a technology-based system with advanced tools and networks, models and algorithms to function as human intelligence without replacing the physical presence of humans completely. AI provides such system which learns from the input data to make independent decisions for completing specific objectives [9].

Drug designing involves huge expenditure, time and high cost along with many existing factors that affect the success and failure of drug discovery, development and marketing. It is a slow process with high investment and is quite challenging due to rapid changes in drug demand and technological developments [10]. Pharmaceutical companies and academic institutions spend a lot for commercial potential and societal benefit. A huge amount of experimental data has been accumulated from the past decades, including the biochemical backups and clinical trials which may become a valuable source for learning and understanding the success patterns and failures of compounds in the entire drug discovery process. Data collection, analysis and digitalization for drug design are rapidly increasing in the pharmaceutical sector. Digitalization of data has many challenges such as appropriate data collection, scrutinizing relevant attributes and then applying that knowledge to selected data frames to solve complex clinical problems [11].

Many subsets exist in AI such as ML-DL, neural network, expert system, robotics, machine vision, speech recognition, etc. Such technology provides a machine with the capability to learn from data and experience through algorithms [8]. The integration of various tools enables AI to serve as an end-to-end point in drug design and development with millions of experimental data available in the public domain. The experimental data includes multidimensional attributes with a wide range of elements. Properly curated data should be considered for the generation of pre-

cise AI learning models. Different types of AI methods are widely used in small molecule drug designing using either supervised or unsupervised learning methods. Unsupervised methods cluster the molecules based on chemical similarity. Clustering methods are useful to identify the nearest neighbours and have a greater application in repurposing the off-target prediction. Clustering methods with shifting, Gaussian mixture can be applied to yield great results. The models for data sets, having the experimental activity, are predictive methods, with either quantitative continue data or qualitative categorical based data on the experimental activity of the training data. Generating models for each protein or type of disease when applied may classify the unexplored data more precisely to identify new hits. However, the quality of input data mainly dictates the precision with many models and this can accelerate the drug design process. Supervised learning methods will have a major role to identify the druggability of compounds based on ADMET data. The most druggable compounds for biological studies can be obtained by building a highly predictive model for absorption, distribution, metabolism, excretion and toxicity; using adequate samples and filtering the compounds during the screening process [12]. Physiochemical properties, quantum mechanics, 2D properties, 3D descriptors, molecular patterns and molecular finger prints of the training data sets are used to generate the AI and its subset learning models. Methods such as PCR plus support vector machines (SVMs), naïve Bayes, random forest, neural networks and recursive partitioning are often used for the generation of ML models by correlating descriptors with experimental activity. AI associates along with their role in drug discovery, design or toxicity monitoring are listed below in Table 5.1.

Therapeutic drug monitoring and clinical toxicity face new challenges every day. Problem-solving approaches are required in regulatory and health technology to assist the healthcare system [13]. Drug monitoring for the patient can be enhanced with various technical tools supported with numerous treatment models [11]. There are many platforms to improve disease management, leveraging on the best patient care. Such systems also supplements the physician's information processing. AI is amongst one of the most appropriate ways to provide solutions for personalized drug monitoring [2]. Figure 5.1 represents the functioning of AI in drug monitoring and toxicity.

5.4 Essentials for Implementing AI in Drug Discovery

The current computational drug discovery process has certain weakness which exhibits complex challenges when AI is applied. These challenges include the basic chemical and biological differences which are both crucial for the application of AI in drug designing and monitoring. Chemistry has been well described from data available from drug assays which can be used for modelling. However, biology has its own difficulty in setting its own finite parameters for which uncertainty exists. Some of the chemical and biological differences are discussed under the following subheadings [5].

5.4.1 Data Describing the System

In the chemical information field, small molecule in its entirety (crystal packing) can be described by chemical structures; however, dynamic chemical states such as tautomeric ones remain unclear. For biological information, the signal type may be unclear and thus the information is unclear [14].

5.4.2 Integration of Data

Once the chemical structures are determined, their representation remains static across experiments. On the other hand, due to the differences in bioactivity across various assays and laboratories, performances of models differ [15]. Biological data are often not reproducible as the results are highly dependent on assay or system used for the experiment.

Table 5.1 Artificial intelligence in drug discovery, design and development

AI associates	AI involvement	URL of AI associates
BenevolentAI, AstraZeneca	To identify new drug candidates for chronic kidney disease and idiopathic pulmonary fibrosis	https://www.benevolent.com/news/astrazeneca-starts-artificial-intelligence-collaboration-to-accelerate-drug-discovery
Insitro, Gilead	To identify new drug targets for non-alcoholic steatohepatitis	https://www.gilead.com/news-and-press/press-room/press-releases/2019/4/gilead-and-insitro-announce-strategic-collaboration-to-discover-and-develop-novel-therapies-for-nonalcoholic-steatohepatitis
Exscientia, Rallybio	To discover small-molecule drugs for rare diseases	https://www.biospace.com/article/exscientia-enters-broad-rare-disease-drug-discovery-collaboration-with-rallybio/
Microsoft, Novartis	To identify and develop therapeutics	https://news.microsoft.com/transform/novartis-empowers-scientists-ai-speed-discovery-development-breakthrough-medicines/
ZebiAI Therapeutics, Google Accelerated Science	To discover small-molecule drug candidates	https://www.globenewswire.com/news-release/2021/04/16/2211478/0/en/Relay-Therapeutics-Extends-Leadershipin-Integrating-Computational-and-Experimental-Approaches-to-Create-Precision-Medicines-by-Acquiring-ZebiAI.html
Exscientia, Bayer	To discover cardiovascular and oncology drug candidates	https://media.bayer.com/baynews/baynews.nsf/id/Bayer-Exscientia-collaborate-leverage-potential-artificial-intelligence-cardiovascular-oncology
BioSymetrics, Sema4, Johnson & Johnson	To predict onset and severity of COVID-19 among different populations, with a goal of developing new treatments and vaccines	https://www.prnewswire.com/news-releases/biosymetrics-collaborates-with-janssen-and-sema4-to-predict-the-onset-of-covid-19-301114823.html
Recursion Pharmaceuticals, Bayer	In drug development, to deal with new small-molecule therapies to treat fibrotic diseases	https://media.bayer.com/baynews/baynews.nsf/ID/Bayer-collaborates-Recursion-strengthen-digital-discovery-advance-therapies-fibrotic-diseases
Insitro, Bristol Myers Squibb	To identify potential drug targets by developing predictive models of amyotrophic lateral sclerosis and frontotemporal dementia	https://www.businesswire.com/news/home/20201028005276/en/insitro-Announces-Five-Year-Discovery-Collaboration-with-Bristol-Myers-Squibb-to-Discover-and-Develop-Novel-Treatments-for-Amyotrophic-Lateral-Sclerosis-and-Frontotemporal-Dementia
Genesis Therapeutics, Genentech	To identify drug candidates for a range of disorders	https://www.businesswire.com/news/home/20201019005182/en/Genesis-Therapeutics-Enters-AI-driven-Multi-Target-Drug-Discovery-Partnership-with-Genentech
Roivant, Silicon Therapeutics	In silico small-molecule drug design	https://silicontx.com/news/press-releases/roivant-grows-computational-drug-discovery-engine-with-acquisition-of-silicon-therapeutics/
Exscientia, University of Oxford	Collaborate to develop treatments for Alzheimer disease	https://www.ndm.ox.ac.uk/about/news/exscientia-and-the-university-of-oxford-announce-partnership-to-develop-treatments-for-alzheimer2019s-disease
Iktos, Pfizer	De novo design software to a number of Pfizer's small-molecule programmes	https://www.businesswire.com/news/home/20210302005501/en/Iktos-Announces-Collaboration-With-Pfizer-in-AI-for-Drug-Design

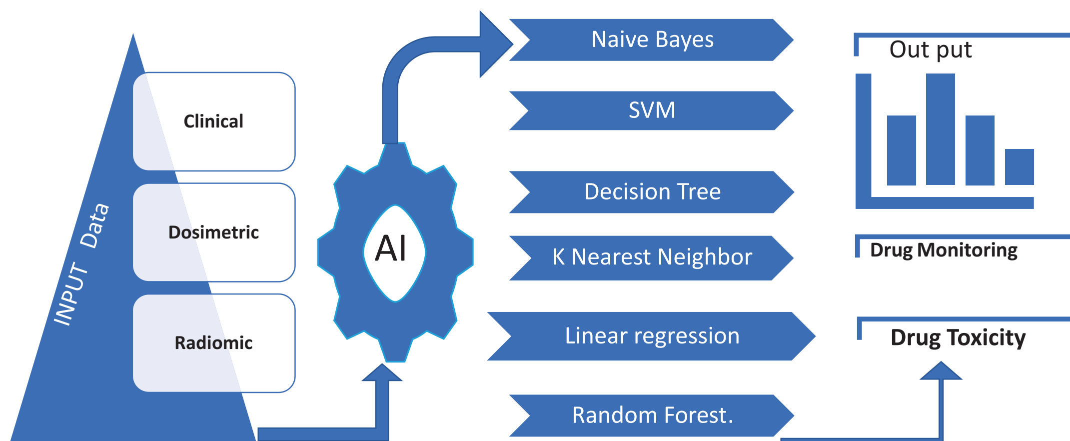

Fig. 5.1 Functioning of AI in drug monitoring and toxicity

Corrections required for batch effects, normalization and integration of data measured in different scales, time points and resolutions are needed, viz. histopathology images, and single cell RNA-seq data integration. [16].

5.4.3 System Stability

A chemical structure needs to be highly stable when used as a drug so that it can be easily synthesized. Significant compound degradation does not occur when stored for a long time because degradation may lead to a problem of compound libraries [17]. Cell plasticity and heterogeneity present difficulty to biological systems because this leads to different drug responses within subpopulation of cell lines and tumours [18].

5.4.4 Dimension and Interdependency

Chemical structures can be represented with high dimension, while no dependency is observed among the dimensions [19]. Biological information has medium to high dimension and sometimes even higher interdependency among data types is observed. This can be observed among protein interactions, gene expressions, etc. [20].

5.4.5 Field Knowledge

Proper understanding of the chemical field such as physical, chemical and thermodynamics is relevant for the display of the chemical information. The biological field is still not well understood and with limited knowledge. A relevant signal for certain types of biological endpoints remains unclear; this includes the pathways/molecule for a given disease [21, 22].

5.5 AI or Ligand Discovery: *Which One Is Critical in Drug Discovery*?

Significant attention has been given to de novo drug design wherein computation devises and novel structures are desired [23]. Fields of retrosynthesis prediction and forward prediction [24] which screens the chemical worth of experimental investigation are also relevant. The area of ligand binding to target has been revived recently with large-scale comparisons [25], applying methods such as matrix factorization [26] and deep learning [27]. AI usage in the chemical have been of research interest in recent times. As sufficiently well-labelled data prevail in these areas, data mining and computational analyses have positive impact in protein-ligand interactions. Deep learning has however some impact in

increasing the performance measure [28] but recent large-scale studies have not corroborated this data [29]. When special emphasis is paid on the metrics of model performances, practical and significant tweaks in model quality are identified, which are were practical and relevant [30]. Even though the efforts for identifying disease driver genes are of great interest, this alone does not offer an easy path for target identification [31].

An integrated approach is currently required for AI in drug discovery including factors such as protein-ligand activity and PK properties of the compound. The steps involved in quantitative systems pharmacology (QSP) include interaction modelling of small molecules with the interacting partners, understanding the expression of target in diseased tissue and its role in disease modulation, the PK behaviour in the molecule in relation to the in vivo system and considerations for safety and efficacy [32].

The application of AI in drug discovery is rather more fragmented in the current situation. AI is capable of designing ligands as per the interested protein making the synthesis much easier, thus helping in the discovery of ligands. Technical facilities as those offered by IBM (https://www.ibm.com/in) and other robotic platforms [33] to achieve multi-objective optimization endpoints have been around for some time [34]. These technical facilities however do not enable us to design an efficacious drug in vivo. Thus, we need better decisions on considering the type of molecule for further studies but very little of such data is provided by the proxy data we possess in practice. The question remains on how these decisions can be made and how AI can help in decision making and to what length. When these fundamental questions are answered, AI can be of great help in the drug discovery sector.

5.6 AI in Drug Monitoring: Gathering Medical Big Data

The physiology and pathophysiology of a disease can now be characterized at an unprecedented level along with the environmental risk associated with the emerging medical technologies. Examples of such big data integration from large population for helping drug discovery process are Alzheimer's Disease Neuroimaging Initiative (ADNI), UK Biobank, Osteoarthritis Initiative (OAI), The Cancer Genome Atlas (TCGA), etc. In the near future, millions of such patient data will be integrated across multiple diseases and with exponential growth of data compiled within biomedical databases such as those maintained by the US National Center for Biotechnology Information (NCBI) and European Bioinformatics Institute (EBI).

When such huge medical data are captured, one demerit remains to be the proper availability and selection of standard data sets in machine-readable forms. Data complexity, scarcity and heterogeneity further add to the problem, making data capturing a huge challenging task. In the data life-cycle management step, the integration of multimodal massive data produced by different technologies poses a significant difficulty with respect to reliability and consistency of using attributes. Availability to curated and accurate data is a critical aspect when it comes to improving machine learning (ML) repeatability. However, good computer hardware architecture settings in relation to the requirements of life sciences can solve these issues as most of the information is deported to cloud. For example, FAIR guiding principles [35] and Clinical Data Interchange Standards Consortium (CDISC) [36] are such efforts made in this line. The implementation of coherent and functional data control is imposed by agencies such as the US Health Information Technology for Economic and Clinical Act and European General Data Protection Regulation (GDPR) for regulatory demands for storage, sharing and access to sensitive and personal data of patients [37].

Collaboration between academic labs and pharmaceutical companies such as Drug Target Commons [38] or MELLODY provides novel initiatives to gather, curate and share huge data for ML-based algorithm development. Several drug companies were brought under the same umbrella to share their individual chemical libraries for training predictive algorithms (multitask

ones) by MELLODY. The challenge is to standardize the proposed reference data set for benchmarking and testing new algorithms by multiple crowd-source challenges such as PrecisionFDA [39], Kaggle [40] and Dream [41] in order to address complex medical problems.

5.7 Pipeline for Disease Monitoring

Disease monitoring has been in existence for about a decade and remains to be a critical aspect in detecting outbreaks and epidemics. Traditional disease monitoring relied on time-consuming laboratory diagnosis, while the computer-based system is significantly fast to alert hospitals, state and medical officers. The latter relies on databases, intelligent systems, advanced informatics and advanced analytical techniques such as text mining, social-network analysis, time-series analysis, monitoring, visualization and analysis. Thus, with these advances real-time or near to that identification of serious diseases and future exposure to bioterrorism agents can be detected [42].

The merging of biotechnologies and AI has provided us with the opportunity to create disease models which can help to position therapies to targeted subpopulations. Extensive molecular profiling of patients in comparison with normal individuals is required for such models. Multi-omics technologies are used to represent subtypes of a disease based on the underlying pathophysiological conditions [43]. This type of information is generated by the follow-up of large groups of people via public-private partnership through patient arrangement using supervised and unsupervised learning methods. Such clustering is based on clinical phenotypes which aid precision medicine [44]. Conventional bioinformatics cannot integrate massive and multimodal data for which comprehensive modelling by AI is required [45].

5.7.1 Target Selection, Validation

Currently, AI has changed the way pathway or targets are identified for a particular disease due to the incorporation of genomic data, biochemical data and target manageability [46]. “Open Targets” is one such platform for target prediction which consists of gene-disease association. This has shown that animal models exhibiting a disease-related phenotype with a neural network classifier greater than 71% makes the most accurate prediction [47]. Five new RNA-binding proteins (RBP) were identified by IBM’s Watson AI drug discovery platform related to the pathogenesis of amyotrophic lateral sclerosis (ALS) [42].

Computational methods are being developed to identify disease-related proteins or genes of which deregulated molecular pathways of a certain disease can be constructed from protein-protein interaction [48] or reconstructed from correlation or Bayesian network [49], also known as knowledge graphs. Further computational analysis of the congenital topology in such graphs leads to identification of nodes [50, 51]. Nodes exhibiting little or no evidence of disease link are amplified using network propagation algorithm [52], with the challenge of multi-layer network integration [53] and their large-scale representation [54].

5.7.2 Design, Optimization of Drug Design

The use of chemical space is required in the utilization of AI for the discovery of small drug-like molecules. Novel and high-quality molecules are discovered from this stage of chemical space due to computational cataloging of probable organic compounds [43]. Also, the identification of virtual molecules specifically designed for targets is made possible by the predictive software model and ML along with the optimization of safety and efficacy data.

Repurposing drugs is possible using the proximity analysis of network-based methods used for drug-target interactions [55] such as deepDTnet algorithm [55]. Deep learning and neural networks together with better versions of algorithms and higher computational power have addressed the gradient problem and overfitting of quantitative structure-activity relationships (QSAR) anal-

ysis of the last decade [56]. Drug interactions with targets in large ligand-based virtual screens are monitored using trained ML-based neural networks which are also used to predict absorption, distribution, metabolism, excretion and toxicity (ADMET) or repurpose [57]. Bioactivity and ADME prediction can also be performed using deep learning as it allows multitask prediction. Besides accuracy, multitask prediction enhances drug monitoring as compared to classical ML methods such as support vector machine and random forest [58].

5.7.3 Clinical Studies (Virtual)

Recognition of disease in patients, gene target identification and prediction of molecular efficacy along with the on-and off-target effects are the assistances provided by AI in clinical trials. It was observed in AiCure, an AI-based mobile application, that it increased adherence by 25% when compared to the traditional way of medication adherence [59]. In Phase II and III clinical trials, AI approaches are developed for identification and prediction of human-relevant biomarkers, thus helping in the recruitment of certain specific patient pool which has the possibility to increase the success rate of clinical trials [60]. The efficacy and success rate of a drug can be improved by using AI during the design, execution and monitoring [61]. Disease understanding and patient heterogeneity helps in the recruitment of patients for trials. Real-world evidence or health records are mined using natural language processing to examine the eligibility of patients for clinical trials [62]. The selection and identification of patients is done by automated text mining which fulfils the inclusion criteria such as specific target organs, disease severity and background therapies. The design of innovative precision medicine trials is possible by incorporating huge imaging (medical), biological and clinical data that define patient's specificity. Trial monitoring is done by AI in remote fashion for patients using wearable devices or sensors. Therapeutic or symptomatic decisions are easy to make for the physician when critical information such as cognitive function, sleep patterns, motricity, symptoms, pain, etc. can be mined using AI [63]. AI and ML are used to analyse data from both successful and failed studies to generate models capable of predicting multiple clinical parameters of multimodal nature [64]. All these analyses are essential for biomarker prediction for severity, progression, and response to treatment [65]. The acceptance of such virtual trials by regulatory agencies remains a major challenge in the application of AI in clinical studies for synthetic patients, virtual trials, validation of algorithms and digital endpoints. Few real-world clinical trials will still be needed which may be simple and better guided by AI.

5.8 AI in Pharmacovigilance

Monitoring the safety of drug applications is a common issue in today's era [10]. The systematic collection of adverse drug reactions (ADR) through spontaneous logical methods and analysis of adverse events associated with the use of drugs are very essential to solve emerging problems, record signals and communicate to minimize or prevent harm. Safety is always a major concern in any drug design process. During certain clinical trials, the use of the drug may be a major source of erosion is unpredictable toxicities which may cause morbidity [9]. To manage such unpredictable toxicities, the etiquette data will play a vital role with AI.

AI has a wide scope of covering heterogeneous sectors such as medical diagnosis, clinical situations, trial treatment, and toxicity detection and prediction. It is used in various healthcare sectors to monitor health problems. AI provides different subsets as machine learning and deep learning with many algorithms to extract feature attributes from a large data [66].

There are different stages in license holder ADR data collector to authenticate pharmaceutical companies and local drug regulators. The same data set will be recreated with feature abstraction, feature alignment, noise removal, splitting and labelling desired attributes to implement relevant AI-ML algorithms. AI can shorten

the process of detection, reporting and labelling technical terms of ADRs associated with individual reports and their relationship with the suspected drug. AI has introduced the minimum human interface during the collection of ADRs. Big data cannot be analysed manually, however, AI an be very helpful.

AI techniques play a significant role in pharmacovigilance by predicting the related ADRs from the well-collected data set. The authenticated data set can be fitted into various working models to predict the desired effect. The repository of ADRs is analysed with numerical and categorical data. The data set is classified as per the selected model. Various algorithms like linear regression, logistic regression, decision tree, SVM, naïve Bayes algorithm, kNN, K-means, random forest, dimensionality reduction algorithms, etc. are used on the pre-processed data set to the derived prediction of ADRs and their effects. Figure 5.2 depicts AI impact in pharmacovigilance/ADR repositories.

AI and machines are utilized in enhancing the understanding of the science of drug safety. The impact of pharmacovigilance starts with the development process employing in vitro and in vivo studies and proceeds into clinical trials and post-marketing surveillance [67]. Mockute et al. proposed an idea about the pharmacovigilance chain value and this was to amplify it by involving the contextual analysis and cognitive load theory. The investigators recognized 51 contending decision points and validated the process to develop clarity about the information of pharmacovigilance. Further, the SMEs were integrated and acceptability was validated by employing quality inspections. The results of validation of such 126 cognitive services were interesting; however, the acceptability of the proposed use of AI needs more confirmation [68].

Danysz et al. discussed the present status of pharmacovigilance and drug safety professionals, along with an investigation carried out at Celgene's Global Drug Safety and Risk Management (GDSRM). The outcomes of the aforementioned investigation indicated that pharmacovigilance professionals are in the favour of utilizing their knowledge and expertise at the full extreme for imparting value to their activities. It was suggested that machine learning algorithms may facilitate the improvement in decision making with regard to drug safety and better control over the processing of cases [69]. The in-depth knowledge of artificial intelligence in pharmacovigilance will prove to be beneficial for setting up the methods, action plans and data sets [70].

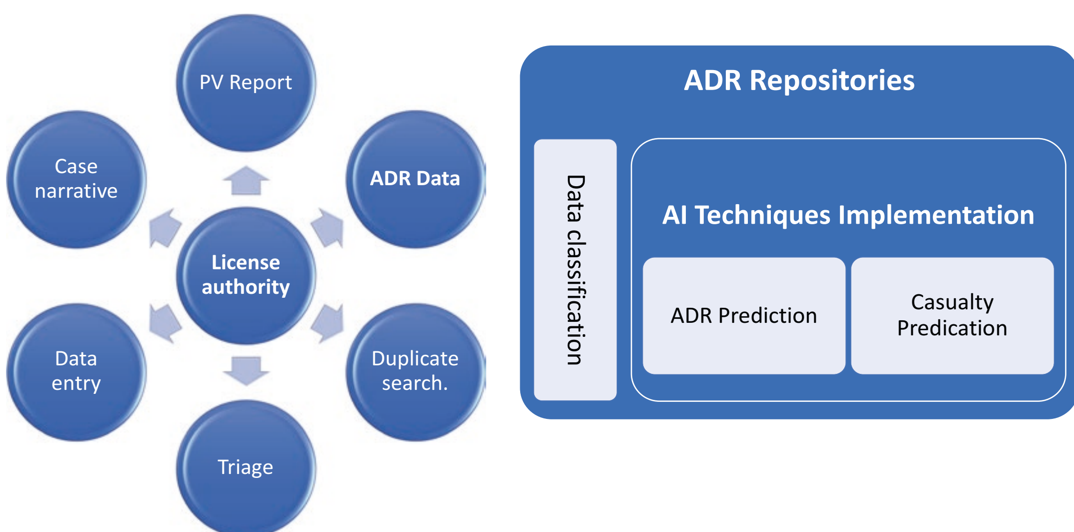

Fig. 5.2 Artificial intelligence and its impact in pharmacovigilance

Although the technological advancements related to artificial intelligence and machine learning have shown a lot of potential in the pharmacovigilance and drug safety field, its implementation in everyday pharmacovigilance has been problematic. The implementation should take place readily. Enhancing patient safety and synchronization of technologies with current pharmacovigilance processes is also a challenge [71]. Routray et al. designed a method based on augmented intelligence for recognizing the adverse drug reactions from the extemporaneous, supplicated and medical reports. The study involved the development of three neural networks for approaching recognition of the severity of adverse reactions, like classifiers for a level of adverse events, classifier for severity categorization of adverse events and annotator for recognizing the severity criteria. The outcomes of the study showed that the strategy of using neural networks has sufficient accuracy and scalability [72].

The assessment of textual information from various sources may be evident for pharmacovigilance professionals to get a better understanding of the use and effects of medicinal compounds in individuals. Text mining strategy can be improvised to fit in various fields by using machine learning approach which is characterized by experts who employ guidelines of annotation. Thompson et al. put forward a connoted body of work from about 597 abstracts from MEDLINE, and PHAEDRA, which gave evidence of drug effects and interactions. The literature has furnished a preliminary illustration of mining textual information by training baseline classifiers for the named entities and events. This occurs by employing available tools which can also be implemented to other information sources as well [73]. Segura-Bedmar et al. highlighted the implementation of the natural language processing and text mining techniques to pharmacovigilance for resolving the impenetrable problems and as a prospect for future research [74]. Ward et al. designed the models for envisaging the probability that an individual will develop an adverse outcome of acute coronary syndrome by using their drug history and comorbidities as inputs. The explainable artificial intelligence (XAI) was employed in quantifying the role of each drug and a method for incorporating the principles of XAI. The research comprised of using multiple models to check whether the individuals aged over 65 (administered with musculo-skeletal system or cardiovascular system drugs) between 1993 and 2009 were recognized and the drug history, comorbidities and other characteristics were taken out from Western Australian data sets. It was found out that adverse reactions related to acute coronary syndrome had some linkages with dispensing attributes for rofecoxib and celecoxib and prediction of the adverse results was 72% accurate. Moreover, the local interpretable model-agnostic explanations (LIME) and shapely additive explanations (SHAP) were able to recognize essential and non-essential characteristics, although SHAP performed slightly better than LIME [12].

5.9 AI in Toxicity

The study of drug toxicity is one of the key parameters for regulatory approvals. Almost 90% of new drug moieties failed, of which around 40% of failure was due to safety [12]. In the last decade, AI is extensively studied for drug discovery, diagnosis, toxicity studies and clinical research which reduce time, and increase productivity and quality [75]. AI in toxicity studies also improves accuracy and multilevel correlations [76]. In the developing world, the demand to reduce animal studies at laboratories attracts the focus of regulatory agency to replace the traditional animal study with AI and ML-based in silico models [77]. Figure 5.3 indicates an analysis of the different types of toxicities using AI.

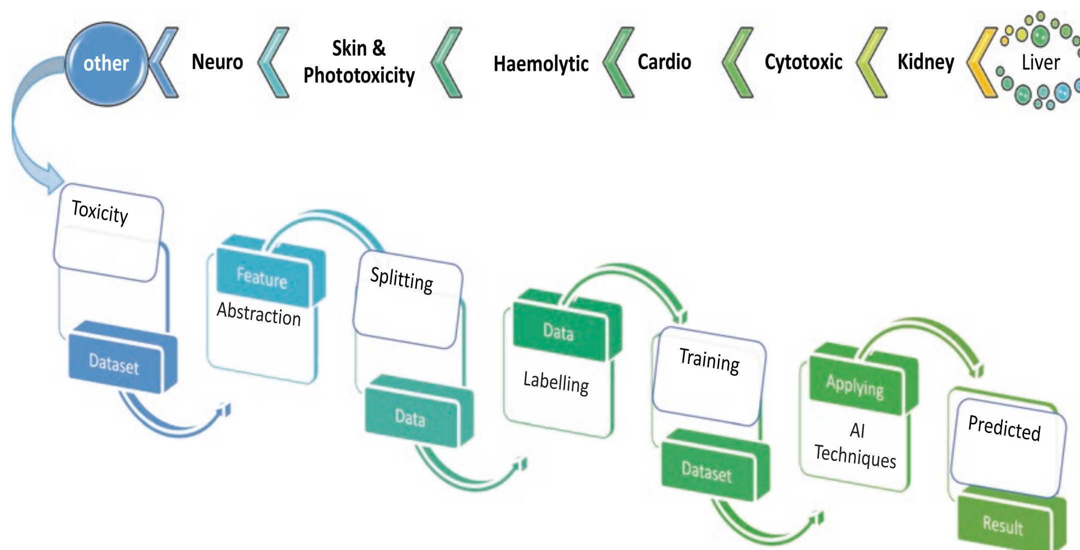

Fig. 5.3 Analysis of different types of toxicity using AI

5.10 Structural Toxicity for Endpoints

Various kinds of toxicity studies are typically used to investigate specific adverse reactions or endpoints of the drug. The common classes of toxicities are liver toxicity, cancer/mutagenicity, cardiotoxicity, cytotoxic effects, neurotoxicity, radiotoxicity, skin irritation/sensitization, and phytotoxicity [78]. The studies usually conclude the mortality, behaviour, reproductive status or physiological and biochemical changes. In general toxicity studies are divided into in vivo, in vitro and in silico approaches [79]. With the increasing cost of experiments and ethical issues over animal studies, gradually such studies are drifting away from in vivo to in silico [80].

The structure-activity relationship-based models can provide both qualitative and quantitative toxicity endpoint data by molecular descriptors and making predictive algorithms [81, 82]. For this purpose, linear method-based models such as multiple linear regression (MLR), partial least squares (PLS) and linear discriminant analysis (LDA) to non-linear methods, such as k-nearest neighbours (KNN), artificial neural networks (ANN), decision trees and support vector machines (SVM) can be used [83]. Recent years have seen deep learning with rectified linear unit (ReLU) activation function and architectures such as recurrent neural networks (RNN) and convolutional neural networks (CNN). These have emerged as the most important tools for in silico toxicity [84–87].

In 2016, Mayr et al. created a DeepTox pipeline for predicting toxicity using artificial intelligence. The model was ascertained by a large number of data inputs of important chemical features representing particular compounds. They proved that DL outshined other AI approaches such as naïve Bayes, SVM and RF [88]. Allen et al. demonstrate that adverse outcome pathway uses a logical sequence of trials within its biological system that can understand toxicity. The same framework is applied in examining the molecular initiating event (MIE) drugs. Studies proved that MIEs can be identified and characterized despite lack of detailed reports, even for some of the most studied molecules in toxicology [89].

In 2018, Ambe et al. used DL, RF and SVM to create predictive classification models of

hepatocellular hypertrophy extracting data on rats from two toxicological databases. The study revealed SVM model using the Hazard Evaluation Support System Integrated Platform data set, trained with 251 chemicals and predicted 214 test chemicals exclusive to the applicability domain. The model has a prediction accuracy of 0.76, sensitivity of 0.90 and area under the curve of 0.81 [90].

Recently, Liu et al. performed Critical Assessment of Massive Data Analysis (CAMDA) using chemical structure-based descriptors such as structural fingerprints and predicted protein targets by utilizing binary DILIrank annotations for prediction of drug-induced liver injury (DILI). They concluded that perception based on proteins and their pathways mathematically allied to DILI [89]. Usually, acute oral toxicity (AOT) is specified by median lethal dose, LD50. Xu et al. studied various kinds of in silico methods designed for AOT prediction intending to decrease cost and time. The team developed an upgraded molecular graph encoding convolutional neural network (MGE-CNN) architecture to notion three sorts of superior AOT models: regression model (deepAOT-R), multi-classification model (deepAOT-C) and multitask model (deepAOT-CR) [90]. The study utilized two exterior data sets comprising 1673 (test set I) and 375 (test set II) composites, the R2 and mean absolute errors (MAEs) of deepAOT-R on the test set I were 0.864 and 0.195, and the prediction accuracies of deepAOT-C were 95.5% and 96.3% on test sets I and II, respectively. The two external calculation accuracies of deepAOT-CR were 95.0% and 94.1%, while the R2 and MAE were 0.861 and 0.204 for test set I, respectively. This novel architecture has proven to be superior as compared to all available models for qualitative toxicity prediction.

A recent investigation by Tokarz et al. used computer-assisted image analysis algorithm, enabled by an entirely convolutional network deep learning method, to sense and measure the microscopic structures of progressive cardiomyopathy (PCM) in the hear of a rat. The algorithms which are trained attained high values for accuracy, intersection over union and dice coefficient for each feature [91]. Chang et al. evaluated digoxin (low therapeutic window drug) cardiotoxicity using AI-based electrocardiography (deep learning model constructed on ECG manifestations). The study of 61 ECGs from patients suffering from digoxin toxicity and 177,066 ECGs from various patients indicated that the proposed model is possibly more applicable to patients with heart failure (HF) and without atrial fibrillation (AF) than those without HF and with AF [92].

Allergic or hypersensitive reactions are the most common type of skin toxicities, due to additives of cosmeceuticals or topical preparations. Peiwen et al. studied and proved that an ML-based prediction model can be used to forecast the skin sensitizing probability and effectiveness of compounds. They created and compare various binary and ternary classification models based on a mechanism using the OECD's eChemPortal database and the NICEATM databases [93]. Verma and Matthews investigated possible ocular irritation and safety concerning household and cosmeceutical chemicals using the in silico method as the rabbit Draize test is not permissible as per EU regulations. The research included 21 ANN c-QSAR models (QSAR-21) to envisage ocular toxicity by means of the ADMET Predictor programme and an assorted training data set of 2928 compounds. The findings indicated that the combined quantitative structure-toxicity relationship (QSTR) models by ANN resulted in 88% sensitivity and 82% specificity for EI and 96% sensitivity and 91% specificity for eye corrosion (EC). The developed in silico models for EI/EC using ML approaches and molecular fingerprints collected data manually from X-Mol (http://www.x-mol.com) and ChemIDplus performed a 95% accuracy for EI and 96% for EC [94].

5.10.1 Toxicity for Multiple-Time Point Assays

The early preclinical stages of drug discovery are in need of predictive models using technologies which are better in assessing drug toxicity. Cells representing specific tissues can be utilized for

predicting the toxic effects of drugs. Image analysis techniques such as high-content microscopy are used to observe the drug-induced structural toxicity wherein intracellular structures are labelled. These approaches are comparatively more accurate and precise than human inspection as last minute structural changes may be missed by the human eye with no option for change under live-cell bright-field imaging. AI-based tools are developed for calculating such structural changes in cell-based models using cellular images taken during multiple doses of a drug (reference with vehicle). As input and the output show a metric of changes for each drug dose at the structural level, the result is a high level of sensitivity [95].

5.11 AI for Drug Safety: Recent Advances and Post-Market Surveillance

AI techniques have shown promise in pre-marketing drug safety, particularly in toxicity evaluation. Toxic drugs can be prevented from moving to clinical trials by making preclinical evaluations robust. High toxicity remains a major cause of drug failures and this leads to about two-third of post-marketing withdrawals and one-fifth of clinical trial failures. Animal studies remain the most conventional way for toxicity estimation. This can, however, be constrained by factors such as time, cost and ethical issues [96]. In silico approaches and computational techniques have utility in such cases to predict toxicity by taking into account various features of the drug.

Several ML approaches have been used for QSAR analysis which rely on regression for chemical properties of drug candidates [97], but assumptions on linearity as well the issue of dimensionality affect most QSARs. So, the most common alternative to regression is ensemble or SVM ways which has easy interpretation, high accuracy and robustness [98]. It has been found that SVM outperformed k-NN (k-nearest neighbour), RF and naïve Bayes algorithms in a QSAR model for HDAC1 (histone deacetylase) inhibitor [99]. Ensemble learning methods combine many ML methods into one predictive model which is less prone to bias and overfitting – the two most common challenges of QSARs.

DL, on the other hand, is an extension of artificial neural network (ANN), which uses a hierarchy of ANNs to gather useful information from raw data. DL was introduced to drug design in 2012 by a competition named Kaggle sponsored by Merck [100]. Deep convolutional neural networks (CNNs) are used to predict toxicity from pretreated cells with drugs which predicted a broad spectrum of toxicity mechanisms, cell lines and nuclear stains [101]. Generative adversarial networks (GANs) [102], long short-term memory (LSTM) [103] and autoencoders [104] are some of the other DL methods for predicting drug toxicity.

The FDA maintains FAERS containing complaints about product quality, adverse events and medication error reports, which is a critical source for post-marketing data mining. Venulet algorithm [105], Naranjo algorithm [106] and the WHO-UMC system are the classical methods to evaluate causality. System pharmacology application in detecting adverse events leads to identification of off-target effects and their clinical observations being the most data-rich source for in silico adverse drug reaction mining. Due to availability of a number of open databases, system pharmacology has methods to choose; network approaches and integrate many features. This includes modular assembly of drug safety subnetworks using knowledge base from literature mining; genome-wide association study data, DrugBank and ChEMBL (which assigns phenotype target); and finally training random forest models to predict drugs causing adverse reactions [107]. Integration of drug-gene interaction can be made for different scales by mining literature on drug-drug interactions (DDI) and further training random forest models for prediction of DDIs [108]. Thus, all these methods heavily rely on molecular features linked directly to drugs with open-source data but with limited clinical information.

5.12 Illusions and Reality of AI in Drug Design

We need to make proper decisions relating to compounds while taking them through clinical studies. In order to use AI in drug design, often times the data availability may not be sufficient. So, we need better molecules with right dosing/PK to attain therapeutic efficacy. Usually, 3D models with better predictability are useful in picking safety relevant endpoints [109], which can be later validated in animal models [110]. Again for considering the molecule of interest, its in vivo mode of action is essential so that we can have validated targets [111]. In most complex diseases, chemical data are available in large scale which has been successfully used in ligand design and synthesis. These data are very useful for target validation but a lot is needed for AI application rather than limiting it to ligand discovery alone. Total advancement in the use of AI in drug discovery is possible only when we understand biology and produce single interest-driven data with better efficacy and endpoints. Thus, advancing better candidates to clinics, having better target validation, improving patient requirement and advancement in clinical trials are essential in reflecting the biological aspect of drug discovery upon which AI can be used [5].

5.13 Future of AI in Drug Design

Drug development involves successful decision making such as correct selection of target, drug, patient, dosages, etc., in which AI can support such decision making by taking into account massive multimodal data to predict models. An unparalleled revolution can be expected in the near future by AI and ML as they are capable of making the costly and complex process of drug discovery cheaper, effective and less time-consuming. This can be applied in the current health industry as seen in the exponential increase of using AI in drug development such as Iktos, Exscientia, Schrödinger, Exscientia, etc. [112]. The first AI designed drug from immune-oncology entered Phase I clinical trials in 2020, after 1 year of research as compared to 5–7 years. Halicin, a new antibiotic, has been identified within a short period of time using AI mining from the existing molecules [113].

Experts are of the belief that AI has the potential to change the pharmaceutical industry and the drug discovery process. Efficient drug discovery using AI requires human input to design algorithms and with domain expertise. A closed workspace between medicinal chemist and AI is essential for analysis of huge data sets, algorithm training and optimization [114]. Advances in AI methods particularly DL have prompted consideration of heterogeneous data types and sources in one model such as imaging, clinical data, knowledge bases, omics data, etc. AI has potential use in clinical trial simulation studies. This can be used to test trial design on virtual population prior to actual clinical trial [115].

5.14 Conclusions

The challenges faced by pharmaceutical companies affect the whole drug development process and the overall life of the product; the progress in AI and its tools aim to reduce these challenges. AI has been included in the manufacturing process of pharmaceutical products, expected dose in personalized medicines and other individualized patient needs [77]. Implementing the latest AI-based technologies will help to bring drugs or molecules to the market with improved quality and better safety (128).

In this chapter, we have reviewed the various aspects of drug design and the use of AI in drug toxicity prediction and monitoring. The early stage challenges in drug design were discussed along with essentials for implementing AI. The importance of ligand discovery and the process of gathering big medical data have been discussed. Disease monitoring and the pharmacovigilance aspect of AI have been explained in detail. The predictions of toxicity endpoints using AI and post-marketing surveillance together with the future of AI have been briefly discussed. Thus, herein we highlighted several key points to be taken into consideration when using AI in the fields of medicine, drug discovery and public health.

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6 Therapeutic Drug Monitoring and Optimal Pharmacotherapy with Medicines of Narrow Therapeutic Index

Anthony Kwaw, Arnold Forkuo Donkor, and Kwame Ohene Buabeng

Abstract

Therapeutic drug monitoring (TDM) remains an under-utilized approach in giving life to the practice of personalized medicine, as it is the medical practice of measuring certain medications at intervals to ensure a constant plasma drug concentration and to optimize dosage regimens. It can be used to enhance patient treatment outcomes due to clear dose-response relationships. TDM also reduces the overall cost of healthcare. Unfortunately, most drugs cannot be monitored with TDM due to the cost and turnaround times, but rather those with a narrow therapeutic indices (e.g. digoxin, lithium, phenytoin, vancomycin, etc.) which have a high risk of causing adverse effects. Also, drugs with significant pharmacokinetic variability as well as those with difficult-to-monitor clinical endpoints are TDM candidates. TDM and its associated benefits in optimizing therapy can only be attained if the method is adequately unified with treatment. TDM processes assume that there is a significant relationship between dose and plasma drug concentration and between the latter and pharmacodynamic properties of the drug. TDM takes into consideration the patient indication and other factors such as weight, age, concomitant drug therapy, and organ function in defining an appropriate initial dosage regimen. Also, patient compliance to treatment, pregnancy, and drug interactions are to be considered as they are major sources of pharmacokinetic variability and may have to be considered when interpreting TDM results. In the interpretation of TDM measurements, sampling time with drug dose, dosage history, patient response, and the desired medicinal targets need to be established. Genetic polymorphisms, smoking, drug formulation, and methodology used in testing may also affect the results. TDM also aims to optimize clinical outcomes in patients with various clinical scenarios in terms of appropriate concentrations of difficult-to-manage medications. TDM is thus a multidisciplinary discipline involving scientists, pharmacists, clinicians, and nurses, and collaboration is recommended to ensure that best practice is attained to patients' benefit.

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Keywords

Drug assay · Pharmacokinetics · Plasma concentration · Therapeutic index

6.1 Background

Medicines are used therapeutically based on safety and efficacy, as well as the benefit of the medicines to patient health, which should outweigh their potential adverse effects. However, that may not always be the case with some medicines even if the best evidence suggests that likeliness [1]. There may be uncertainties to patients' safety and clinical outcomes with some individuals as they are exposed to certain medicines, due to individual variability in drug pharmacokinetics and pharmacodynamics [2]. For this reason, whenever possible, plasma or serum concentration should be measured for medicines of that nature, and treatment outcomes monitored to assess safety, tolerability, and therapeutic impact. Monitoring the effects of medicines with narrow therapeutic indices is attainable in diverse scenarios. It is possible subjectively when the patient talks about the effects observed following drug use. However, a more objective approach may be to measure the actual biological effect (e.g. blood pressure control for anti-hypertensives, blood glucose control for anti-diabetic medicines, etc.) and the adverse effects experienced following exposure to various dosing schedules [3]. Other alternatives may be to use a biological marker that acts as a proxy for the therapeutic outcome or adverse effect [4, 5]. If the direct drug effect appears complex or inaccessible, then use can be made of the measured plasma drug concentration and how it is closely related to the effect observed, whether adverse or beneficial [6, 7].

6.2 Therapeutic Drug Monitoring

TDM refers to the monitoring of the biological effects of medicines with a narrow therapeutic window using the plasma drug concentration. TDM is often used during therapy with such medications to optimize therapy and reduce toxicity whilst maintaining the medicine concentrations within established target ranges that are considered safe [6].

Some criteria that are considered in TDM:

- When clinical effects and other pharmacodynamic responses are hard to establish.
- The association between drug concentration in plasma and biological effects should be likely. Phenytoin seems to be the only anti-epileptic drug with an exceptional positive association between its concentration in plasma and biological effects.
- The therapeutic window is narrow or has concentration-dependent pharmacokinetics. An idea about the appropriate dosage alone should be sufficient to forecast whether the drug concentration in plasma is within the therapeutic range [7].

TDM is not supposed to be limited only to establishing plasma drug concentrations, but more importantly the interpretation of the outcome in a clinical sense. Therefore, the drug pharmacokinetics, sampling time, previously taken medication(s), and the disease condition are crucial [6].

6.3 Medicines That Are Known to Require Therapeutic Drug Monitoring

Medicines that may require TDM to assure safety and efficacy include digoxin, used in cardiovascular health therapeutics, and carbamazepine and sodium valproate used in the management of epilepsy and other neurological disorders. Some other medicines used in cardiovascular therapeutics for arrhythmias that may also require TDM include amiodarone, flecainide, and disopyramide. An aminoglycoside such as gentamicin, kanamycin, amikacin, etc. is documented to be used safely and efficiently with TDM for dosage adjustments in the elderly, paediatrics, cardiovascular, and renal impaired patients with infectious diseases including multidrug-resistant infections where the pathogens are susceptible [9, 10].

It has also been established that based on the clinical pharmacology of the class of medicines below, it may be appropriate to do TDM to guide appropriate dosing and use and to minimize the risk of toxicity and achieve improved therapeutic outcomes when used in depression, in epilepsy, in asthma, and for cancer or autoimmune disorders, respectively. These are:

- Antidepressants such as lithium, imipramine/clomipramine, etc.
- Antiepileptic medications such as phenytoin, clonazepam, and ethosuximide, among others
- Bronchodilators like theophylline in asthma management
- Immunosuppressants for autoimmune disorders and medicines for cancer chemotherapy such as cyclosporine, methotrexate, tacrolimus, etc. (Table 6.1)

Unfortunately, it appears resources in terms of equipments/machineries and trained human resources are not readily available in the Ghanaian Health System, and in most countries in sub-Saharan Africa, so the use of TDM to optimize therapy and improve patient safety and outcomes is not common, if not very rare. Our consultations suggest that most of the teaching hospitals in Ghana have seen the need to have the infrastructure and trained staff to initiate the use of TDM efficiently to improve the safe use of the medicines listed in Table 6.1. These medicines are often used in patients with complex health issues and require monitoring to ensure that they are not harmed, nor worsen their state with pharmacotherapy but with improved health status [11].

The indication for a drug may also play a role in determining the target concentration as exemplified in the case of digoxin in Table 6.1.

Table 6.1 Medicines that require therapeutic drug monitoring to optimize pharmacotherapy and the target range are considered to be safe

Drugs monitored frequently in clinical practice	
Drug	Target range (may vary between laboratories)
Phenytoin	10–20 mg/L
Digoxin	0.8–2 microgram/L <0.01 microgram/L in refractory heart failure
Lithium – acute mania – maintenance	0.8–1.2 mmol/L 0.4–1.0 mmol/L
Tacrolimus	5–20 microgram/L (whole blood)
Drugs for which TDM may be beneficial	
Vancomycin	150–300 mg/L
Amiodarone	1–2.5 mg/L
Salicylate	150–300 mg/L
Sodium valproate	50–100 mg/L
Carbamazepine	5–12 mg/L
Vancomycin	Trough 10–20 mg/L

6.4 Medication Therapy That Requires TDM for Optimal Outcomes

Due to the costly nature of most drug assays, the reason for monitoring, as well as other benefits, should be well defined. For example, TDM of vancomycin helps increase efficacy; that of aminoglycosides, cyclosporine, and paracetamol to reduce toxicity; and salicylates to help in salicylate poisoning. TDM can be key in establishing toxicity in undifferentiated clinical syndrome such as a patient on digoxin experiencing unexplained nausea [12, 13]. Drugs with an unpredictable association between a given dose and concentration in plasma and saturable metabolism like phenytoin will benefit from TDM as well as those with steep dose-response curves like theophylline. Drugs with poorly defined end point (e.g. immunosuppressants) and narrow therapeutic windows (e.g. digoxin, phenytoin, lithium) require TDM permit adjustment in dose to avoid toxicity [6]. Impairment in renal function may also play a part in the alteration in the association between drug concentration in plasma and administered dose as in the case of lithium, gentamicin, and digoxin which requires dose adjustment.

Therapeutic drug monitoring is useful after dose adjustments usually when a steady state is realized, and for establishing a suitable loading dose (after initiating therapy with phenytoin). TDM can be used to monitor patient compliance to anticonvulsant medications, drug treatment

failure, and side effects that mimic the underlying pathology. Prophylactic drugs such as immunosuppressants and anticonvulsants can be used to diagnose undertreatment [14].

6.5 Guidelines for Sample Collection

Blood is the most commonly used specimen for TDM, and its use is recommended when definite information is essential. To ensure adequate drug absorption and accurate therapeutic levels, there must be ample time between drug administration and taking of sample. Samples for TDM especially blood can be taken during the peak level or trough level, which should fall within the therapeutic range. Drug trough levels mostly correlate with therapeutic levels, whereas peak levels of drugs correlate commonly with toxic side effects. The best time to take a blood sample for TDM is just before the next dose irrespective of the route [8].

There are usually significant fluctuations in peak and trough levels in patients who receive the drug at a dosing interval longer than the half-life of the drug and vice versa. Peak and trough drug levels for chronically administered oral medications typically ensue 1–2 hours after administration and soon after the dose is administered respectively. However, digoxin peak level is determined after it has had enough time to equilibrate within the tissue, usually 6–10 hours post-administration orally. It is regarded as correct if the trough level is taken at the time the dose is given [7].

For intravenously administered medications, peak and trough drug levels can be established by sampling ½–1 hour post-administration and after dose administration, respectively. For gentamicin, a blood sample can be taken 30 mins post-peak intravenous administration to establish the peak level. This principle applies to intramuscularly and intravenously administered medications in an area with enough blood perfusion. It is therefore imperative that the clinician knows what questions are to be answered by TDM as this may need diverse sampling times as well as different numbers of samples. For instance, a single sample may be used to answer suspicion of toxicity (e.g. lithium, carbamazepine), whereas establishing a half-life may necessitate not less than two samples drawn at the appropriate time after drug administration [15].

6.6 Timing of Plasma Sample for TDM

Samples for TDM are to be taken at a steady state, which is about 4–5 half-lives after beginning pharmacological therapy. However, for concerns about toxicity or loading dose, it can be taken earlier before steady-state concentration is attained. Sampling time is essential in TDM because the serum drug concentration may alter along with the dosing interval with the least variable point in the dosing interval being just before the next dose. Specimens can, therefore, be taken at any point in the dosage interval for drugs with long half-lives like amiodarone and phenobarbital [6].

Plasma drug concentrations may peak 1–2 hours after oral administration. Notwithstanding, certain factors like erratic absorption and distribution can delay the time to reach peak plasma concentrations. For instance, TDM for digoxin can only be performed 6 hours after a dose, since it will be undergoing distribution.

In the case of aminoglycoside antibiotics, the time for taking a specimen is occasioned by the technique used for the TDM if it's a once-daily dosage regimen. The sample is therefore taken after 6–14 hours when using a nomogram, or two samples within the dosing interval to compute the area under the drug concentration-time curve. However, when given in divided doses in the treatment of enterococcal endocarditis, the samples for trough levels are to be measured to limit toxicity and evaluate if serum concentrations are within the acceptable therapeutic range [6].

Below is the sampling time for certain medications:

- Carbamazepine: trough and peak level collected just after a dose 3 hours later respectively since its half-life is 48 hours after a single dose.
- Theophylline: sampling time is not important for slow-release formulations.
- Gentamicin: pre-dose peak 0.5 hours (intravenous) and 1 hour (intramuscular).
- Phenytoin: due to its long half-life (10–15 hours for IV and 22 hours for PO), TDM may not be useful as it is commonly taken once daily.

6.7 Request for Therapeutic Drug Monitoring

Assaying of medications can be requested for TDM or clinical toxicology purposes (Fig. 6.1). In interpreting the result, the clinician may have to take into consideration the time for taking a sample, the dosage regimen, and the reason for drug monitoring [6].

6.8 Therapeutic Drug Monitoring and Interpretation of Results

Therapeutic ranges of drugs are usually obtained by measuring the clinical responses of the drug in a limited number of patients. The lower limit of the therapeutic range is set to elicit ~50% of the maximal therapeutic response, whilst the upper limit is well-defined by toxicity. Expert clinical interpretation of the result is necessary as therapeutic windows are not absolutes. Background such as the drug therapy duration and time of the last dosage is imperative though it is the unbound form of the drug that interacts with the effector

TDM REQUEST FORM

Patient Name………………………………………………………… Date………………………….

Age…………… Sex………….. Weight……………. Height…………….

Ward……………….. Request by……………………… Phone No………………...

REQUESTED DRUG LEVEL……………………………………………………………………..

REASON FOR REQUISITION:

( ) Compliance ( ) Suspected toxicity

( ) Absence of therapeutic response ( ) Therapeutic confirmation

Please indicate when needed:

( ) stat. ( ) within 1-2 hours ( ) within 24 hours ( ) Others……………….........

Date………………… Route: ( ) IV ( ) IM ( ) SC ( ) PO ( ) Others………………...

Time………………… Dose……………………………… Frequency……………...

DRUG LEVEL USE: SAMPLING TIME:

( ) Trough or pre-dose level Date…………….. Time………………...

( ) Peak level Date…………….. Time…………………

ORGAN-SYSTEM DYSFUNCTION PRESENT?

( ) Cardiac ( ) Hepatic ( ) Renal ( ) GI ( ) Endocrine ( ) Others……………………..

CONCOMITANT DRUG(S):

…………..……………………………………………………………………………………..

DRUG LEVEL AND USUAL THERAPEUTIC RANGE……………………………………….

INTERPRETATION OF RESULT…………………………………….........................

Fig. 6.1 Sample request form for TDM

Table 6.2 Factors that account for low or high drug concentration

Lower than anticipated	Higher than anticipated
Reduce plasma binding Timing of sample Rapid elimination Poor bioavailability Changing hepatic blood flow Patient non-adherence Error in dosage regimen	Increase in plasma protein binding Rapid bioavailability Decrease in renal or hepatic function Slow elimination Error in dosage elimination

site to produce a response [16, 17]. Also, the timing of the sample, when steady state was achieved, and patient compliance to treatment should all be considered. True therapeutic drug monitoring testing considers all factors that affect results and interpretation likewise. Some of these factors are enumerated in Table 6.2.

Drug concentration needs to be understood in the context of each patient without necessarily adhering to the target range. For instance, a patient on an anticonvulsant with serum drug concentration just below the target range and not having seizures requires no increase in dose. In the case of digoxin, serum potassium levels should be monitored when interpreting results as toxic since toxicity can as well occur at therapeutic concentrations in the presence of hypokalaemia [3, 6].

In TDM, plasma or serum may be used to measure both bound and unbound drugs. Therefore, in patients with altered binding capacity due to disease states like renal failure, drug interactions, or non-linear binding, the protein binding effect on drug concentration needs to be considered when interpreting results. This is relevant for phenytoin such that should its unbound portion double from 10% to 20%, the target therapeutic range as a result of total phenytoin concentration needs to be halved, which if not adhered to may lead to toxic adverse effects [6, 18, 19].

Some drugs whose metabolites may be active are not measured, though they add to the therapeutic effect of the parent drug. Therapy with primidone may be monitored by determining phenobarbital, an active metabolite; nonetheless, primidone together with other metabolites such as phenylethylmalonamide is also therapeutically active [20–22].

6.9 Medications That May Benefit from Therapeutic Drug Monitoring

TDM may not be suitable for the listed medications for unique reasons:

- Medications with a wide margin of safety or therapeutic index.
- Medications whose clinical responses are obtained with practical investigations: warfarin.
- Medications with plasma concentration not associated with a clinical response: warfarin.
- Medications like penicillin whose toxicity is not a realistic concern except for anaphylactic reactions in those who are hypersensitive to the drug.
- The association between the plasma concentration and clinical response is not clearly defined: antidepressants.
- Hit and run drugs: Monoamine oxidase inhibitors and the proton pump inhibitor, omeprazole [23].

6.10 Techniques for Measurement of Therapeutic Drug Monitoring

Several separation techniques are available for use in TDM as described in Table 6.3 [24, 25].

6.11 Practical Use of Therapeutic Drug Monitoring

Drug assays ought to be done within a clinically beneficial timeframe with the appropriate biological specimen to be able to make clinical relevant interpretations and decisions. Absorption after oral drug administration delays some drugs, and this requires the specimen to be taken in the elimination phase rather than in the absorption or

Table 6.3 Separation techniques for therapeutic drug monitoring

Technique	Description
High-pressure liquid chromatography (HPLC)	It is a technique based on the relative distribution of the constituents of a mixture between a mobile and stationary phase
Liquid chromatography-mass spectrometry (LCMS)	The mobile phase moves the sample along with the stationary phase, which leads to the separation of the sample constituents
Gas chromatography-mass spectrometry (GC/MS)	A technique that uses very high temperatures to cause vaporization of the sample, which is then passed through an electrical field The molecules can be separated based on molecular weight since the pattern of separation is unique to each drug
Enzyme immunoassay (EIA)	A non-radioactive enzyme label technique with assays performed in a single step
Radioactivity (RIA)	It uses radioactivity to detect the existence of an analyte
Particle-enhanced turbidimetric inhibition immunoassay (PETINIA)	A technique that separates sample constituents by the creation of light scattering particles to quantify drug levels
Enzyme-multiplied immunoassay technique (EMIT)	Separation technique based on competition for the target analyte antibody binding sites
Fluorescence polarization immunoassay (FPIA)	A technique that makes use of a fluorescent molecule as the label and is very sensitive
Chemiluminescence:	A chemical reaction produces light, which when combined with immunoassay technology specifies the amount of analyte in a test sample
Affinity chrome-mediated immunoassay (ACMIA)	A technique for measuring plasma drug concentrations where both bound and unbound drug-antibody-enzyme conjugates are separated using magnetic particles called chrome
Cloned enzyme donor immunoassay (CEDIA)	A technique that uses recombinant DNA technology

distribution phases. Slow drug absorption among others may affect the time to reach peak drug levels after an oral dose. The measured drug concentrations can then be compared to published therapeutic ranges, so as relate them to the pharmacodynamic responses. However, drugs with virtually no individual variability in plasma concentration will not benefit from TDM assays. For drugs with therapeutically active metabolites, both parent drug and active metabolites should be determined to establish an inclusive representation of the association between the total plasma concentration of all the active molecules and the biological response, but it is advisable in routine therapeutic monitoring. The results should be ready if possible within 24 hours of sample receipt. The most vital consideration in interpreting the plasma concentration of a drug is the individualization of therapy. The clinician, therefore, should consider the patient's clinical presentation which may adversely affect the association between plasma drug concentration and clinical response [8, 15].

There must be cost-effectiveness in measuring drug levels in clinical samples. The use of clinical pharmacokinetic principles in offering TDM services offers enormous benefits as significant reduction in adverse reactions, shorter stay in the ICU, and shorter overall hospital stay [24, 25].

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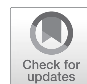

7 Therapeutic Drug Monitoring (TDM) and Toxicological Studies in Alternative Biological Matrices

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Abstract

Recent technology advancements have sparked renewed interest in individualized medicine. Some approaches in attaining individualized therapy such as therapeutic drug monitoring (TDM) have, on the other hand, remained underappreciated. TDM has the potential to enhance patient outcomes while also lowering healthcare expenditures due to its unambiguous dose-response correlations. Two new approaches to TDM have emerged in recent years: target concentration intervention (TCI) and a priori TDM (by combining TDM with pharmacogenomics). Alternative matrices are increasingly being used in toxicological analyses in clinical and forensic contexts. Alternative specimens to blood and urine can provide supplementary evidence about drug exposure and analytical benefits. Several analytical techniques such as laser diode thermal desorption-tandem mass spectrometry (LDTD-MS-MS), ultra-high performance liquid chromatography-tandem mass spectrometry, liquid chromatography-electrospray mass spectrometry, enzyme immunoassay and gas chromatography/mass spectrometry, gas chromatography-surface ionization organic mass spectrometry, chemical ionization mass spectrometry with ammonia, chromatography coupled with electrospray-ionization mass spectrophotometry (LC-ESI-MS), etc. have been used for the detection of drugs of exploitation in the above-mentioned biosamples. This chapter will provide an overview of TDM, including its

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history, current developments, and prospects, as well as toxicological research using various analytical techniques in various biological matrices.

Keywords

Therapeutic drug monitoring · A priori TDM · Thermal desorption-tandem mass spectrometry (LDTD-MS-MS) · Alternative matrices · Liquid chromatography coupled with electrospray-ionization mass spectrophotometry (LC-ESI-MS) · Target concentration intervention (TCI) · Biochips

7.1 Introduction

Therapeutic drug monitoring (TDM) is the measurement of a chemical parameter in a clinical laboratory that, when combined with competent medical interpretation, directly impacts drug prescribing procedures [1]. TDM, also refers to the individualization of drug dosage by keeping drug concentrations in the plasma or blood within a therapeutic range or window [2]. TDM combines pharmaceutics, pharmacokinetics, and pharmacodynamics knowledge to analyze a medication's efficacy and safety in a variety of clinical scenarios [3–7]. This process tries to tailor therapy regimens for the best possible outcomes for patients. TDM is typically described as the measurement of drug concentrations in various biological fluids and their interpretation in terms of therapeutically relevant parameters. Clinical pharmacists and pharmacologists employ pharmacokinetic ideas to assess these interpretations. In the 1960s, the science of TDM brought a new dimension to clinical practice with the publication of the first pharmacokinetic studies linking mathematical theories to patient outcomes [3]. In the late 1960s and early 1970s, clinical pharmacokinetics became a topic. Pioneers in drug monitoring in the 1970s focused on adverse drug reactions, proving that by constructing therapeutic ranges, the incidence of toxicity to drugs such as digoxin [8], phenytoin, lithium, and theophylline [9] could be reduced [10]. The development of clinical pharmacokinetic monitoring was supported by increasing awareness of drug concentration-response relationships, the mapping of drug pharmacokinetic properties, the introduction of high-throughput computerization, and developments in analytical technology [11].

Historical Perspectives on TDM

The first PK study was published in the 1960s, and the significance of PK was proven [12]. Another historical milestone was a 1965 publication [13] that was the first formal review on the importance of "drug monitoring." With advancements in chromatographic techniques, these inquiries received even more traction. The years that followed were a golden age for drug surveillance. Gas chromatography (GC), HPLC, and mass spectrometry were used to determine the concentrations of various medications (MS). The development in the field of immunoassays changed the notion by enhancing the viability of the execution of assays [14], which was another significant milestone. Simultaneously, previously proposed concepts like the relationship between dose and toxicity, PK, and drug-protein binding were thoroughly studied, leading to improvements in sampling procedures and analysis.

The trend in chromatographic methods during the 1990s shifted to software programs for establishing dosage regimens, the idea of noninvasive and reductions in invasive therapies, wearable sensors, and feedback-controlled smart devices.

7.2 Therapeutic Drug Monitoring (TDM)

TDM is the clinical technique of monitoring therapeutic agents in patients. The drug concentration is determined at fixed intervals to find out the desirability and consistency of the amount of drug in blood circulation, allowing individual dose treatments to be optimized [7]. TDM is applied by most pharmaceutical companies to monitor drugs with limited therapeutic ranges, high pharmacokinetic inconsistency, and difficult

target concentrations to monitor and document therapeutic and adverse effects [11].

7.2.1 Purpose of Therapeutic Drug Monitoring (TDM)

TDM necessitates an interdisciplinary approach. The TDM team consists of experts like scientists, doctors, nurses, and pharmacists. They can properly monitor the therapeutically meaningful drug concentrations at the site achieved or not. To guarantee that the best effective monitoring the communication between various team members is necessary, as represented in Fig. 7.1 [15, 16].

Parameters like efficacy, plasma drug concentration, compliance, drug-drug interactions, toxicity avoidance, and therapy discontinuation observation were used as the signs for monitoring purposes [17, 18], though each parameter may not apply equally to every agent. In many cases, physicians monitor the drug concentration then customize the dosage form for an individual during various therapies. This method is particularly crucial in the case of drugs with limited therapeutic ranges, i.e., narrow therapeutic window, such as lithium, cyclosporine, and aminoglycoside antibiotics. It may be highly valuable in cases where changes in dosage regimen are suggested at a later stage, like patines having renal complications. Drug toxicity can be detected clinically in many circumstances. For example, acute phenytoin toxicity is rather straightforward to spot, and testing plasma concentrations is not essential for judgment of disease, but it can be useful in correcting dosage later. Digoxin toxicity can have similar symptoms to heart disease; thus, monitoring the plasma levels in subjects where toxicity is suspected might assist confirm the diagnosis. The extent of plasma digoxin content in 260 subjects treated with *Digitalis lanata* preparations (digoxin, lanatoside C, beta methyldigoxin) allowed Aronson and Hardman to track some outcomes that would not have been visible otherwise. The method's applicability in the diagnosis of digitalis toxicity is limited by the significant connection among "toxic" as well as "nontoxic" plasma concentration levels [19]. The approach can detect digitalis sensitivity in digitalis-treated individuals with toxicity associated with digitalis plasma values less than 2.0 ng/mL. Aronson and Hardman [19] discovered that selecting a dosage regimen after careful monitoring of plasma concentration can reduce digitalis toxicity to less than 4%. This approach is not commonly used currently. Plasma digoxin concentrations should be measured and monitored in digitalis-treated patients with marginal renal function, the elderly, and patients with quick atrial fibrillation who require higher digitalis dosages for heart rate control [20]. When a digoxin-treated patient requires a loading dose of oral amiodarone, the digoxin dose should be decreased first, and digoxin therapy should be adjusted based on any symptoms and signs of digoxin toxicity [21].

7.2.2 TDM: Estimating Plasma Drug Concentration

In general, finding the drug concentration at a steady-state and altering the dose to attain the desired concentration is known to be related to the efficacy of the drug. In this process, the overall contribution of pharmacokinetic unpredictability to changes in quantity needs can be discovered. However, there are significant interindividual pharmacodynamic differences at a specified plasma concentration [22]; it is more common to target instead of a single threshold, a concentration range. The assessment of drug concentration in plasma or blood has become a good replacement gauge of drug exposure in the body for a restricted number of drugs where better association is found among plasma or blood concentration response than dose response [23].

Therapeutic drug measurement is just one aspect of TDM, which also includes expert clinical interpretation of drug concentrations and pharmacokinetic evaluation. To obtain a maximal clinical benefit, expert interpretation of a medication concentration measurement is required. Clinicians take a look at physiological indices of healing responses which include lipid concentrations, blood glucose, blood strain, and coagula-

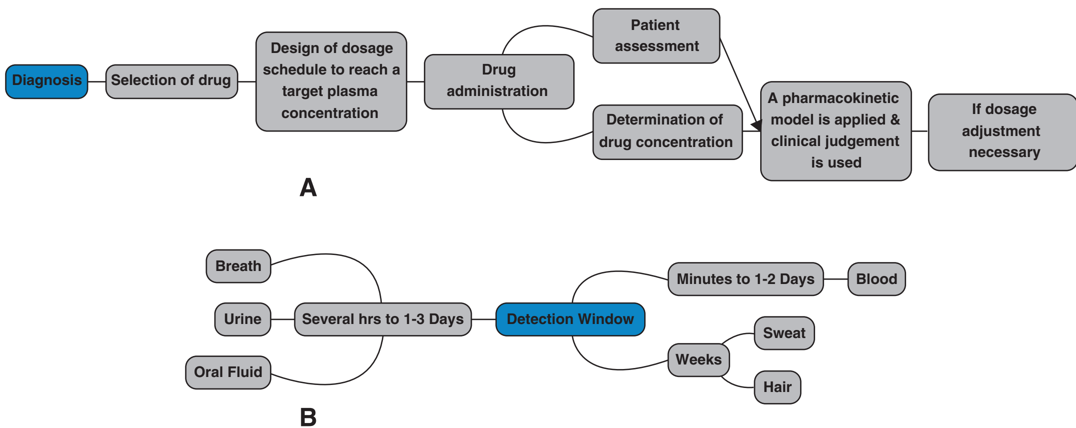

Fig. 7.1 (**a**) Process for dosage decision with TDM. (**b**) Detection window for drugs in biological matrices

tion to monitor drug pharmacodynamics. Many medications have no problems in measuring motion, or the approach is insufficiently sensitive [23]. As a result, TDM is predictable totally from the assumption that there is a distinct relationship among dose and plasma or blood concentration, as well as between blood drug concentration and pharmacodynamic properties [23]. There is no reason to monitor plasma concentrations regularly except when there is a precise cause [24].

7.2.3 TDM Analytical Issues

As previously stated, TDM necessitates the collaboration of various disciplines of sciences, including pharmacokinetics, pharmacodynamics, and laboratory analysis. The method of analysis has a significant impact on the determination of pharmacokinetic parameters, which is often overlooked [25]. The analytical goals established in TDMTherapeutic drug monitoring (TDM) are assessing the nature of the issue to be resolved, choosing the suitable matrixMatrices and scientific method for addressing the problem [26, 27]. The important factors taken into consideration are the timing of blood sampling, the type of blood sample, measuring technology, and data interpretation to make it more expressive. First and foremost, obtaining a blood sample for evaluating medication concentration at the proper time after the dose is critical. The majority of problems in interpreting the data are most likely due to timing issues in the sample [28]. The blood sample can be collected into a heparinized tube or left to clot for most medicines [29, 30].

7.2.4 Practical Issues in TDM

In an ideal world, a high-quality drug assay would be completed in a therapeutically useful amount of time. Many doctors believe that reports from big chemical pathology laboratories, which are operated by skilled scientists and equipped with cutting-edge automated analyzers, will be correct [23]. As a result, analytical laboratories should confirm that methods are in place to get any missing details from the drug assay request that may be needed for proper clinical interpretation of the results, such as dosage regimen and blood sampling time, and that each assay's accuracy, precision, acuity, and specificity are documented and measured regularly. Because the most essential uses of the measures are during dose changes and in detecting toxicity, when prompt judgments must be made, the assay outcomes should be provided rapidly, rather than within 1 day of receipt of the sample [24, 31].

TDM involves a multidisciplinary approach that includes pharmacological, pharmacokinetic, and pharmacodynamic approaches and analyses. TDM takes more than just a simple measurement of a patient's blood drug content and a compari-

son to a target range to be effective. TDM, on the other hand, is critical in the creation of safe and effective therapeutic drugs, as well as their individualization [13, 32]. TDM can also aid in the detection of medication compliance issues in noncompliant patient instances. The sample period about the dose, the dosage history, the patient's response, and the planned clinical targets are all elements to consider when interpreting drug concentration values [33, 34]. This information can be utilized to determine the best dosing regimen for achieving the best response with the least amount of toxicity [35–39].

7.2.5 TDM's Translational Challenges

Because medication concentration in the blood is linked to pharmacological action, concentration is a better indicator of effectiveness or toxicity than dosage. TDM is the clinical practice of determining the concentration of drugs in blood, plasma, or other body fluids that are related to blood drug concentrations. The medication concentration is then used to change the dosing regimen by looking for a therapeutic range, which is a collection of concentrations or exposure intervals. As a result, the analysis method's specificity and sensitivity have a significant impact on TDM reliability. These tests are now performed in clinical TDM using either chromatographic procedures with specific detectors (typically mass spectrometers) or immunoassays. Traditional approaches, on the other hand, have several practical drawbacks for the envisioned large-scale, distributed TDM practice, such as a lack of workflow uniformity, long turnaround times, and high instrumentation costs due to extensive sample preparation. In this sense, recent advances in sensing technology provide a once-in-a-lifetime opportunity to overcome these constraints and fully exploit TDM's promise. Several recent evaluations using biosensing technology [40–43] have properly addressed recent improvements and the current capabilities of such applications.

7.2.6 Recent Advances in TDM Practice

7.2.6.1 Chromatographic Methods

Even though chromatography has been effectively applied in clinical research, there are still several issues in liquid chromatography with tandem mass spectrometry (LC-MS/MS) procedures that need to be solved. Matrix interference, despite its high specificity, can lead to falsely low or high readings in MS [44]. This is because the matrix and co-eluting chemicals can interfere with the ionization process (through ion suppression/enhancement). Furthermore, LC-MS/MS technologies have a lower throughput than traditional immunoassay platforms. Recent research has focused on either fixing these limitations [45, 46] or leveraging or improving the inherent benefits of this strategy [47, 48].

As a result, a substantial effort has been made to boost the throughput of chromatographic procedures [49]. Pioneering multiplex techniques were reported have been all reported for antiretroviral medicines (ARVs) [50], antifungals [51], antineoplastics [52], antibiotics [45, 46, 53], antidepressants [54], and immunosuppressive medications [55] in the previous decade. Recent studies have employed ultra-performance liquid chromatography (UPLC)-MS/MS for simultaneous measurement of antibiotics [56] and ARVs in plasma [50] and breast milk samples [57]. Another area of attention is the use of TDM research to nontraditional samples and sampling. Hair [50, 57], dried blood spots [58], urine [59], sweat [60], saliva [61, 62], and tissue biopsies [63] have all been studied using LC-MS/MS.

To overcome the limitation of LC-MS/MS, like matrix effects, high costs of equipment and analysis, tedious development and optimization, and requirement of a highly skilled operator, one can opt for HPLC methods in combination with simpler detectors such as UV, flame ionization detection (FID), or diode-array detection (DAD). However, in such instances, overlapping peaks across analytes with similar retention periods may be detected, which is exacerbated through the inclusion of unknown components in

complicated sample matrices. Chemometric techniques, which allow the removal of unanticipated signal interferences from the total signal by mathematical modeling [64, 65], are one solution to this problem.

7.2.6.2 Immunoassays

Immunoassays have been extensively studied for the analysis of drugs in laboratories for many years owing to their high affinity, small sample volume requirements, ease of working, good compliance to high-throughput, and cost-effectiveness. The underlying premise is that an analyte is detected by binding to analyte-specific antibodies. For analytes with large and small molecular weights, the competitive and sandwich techniques of assay design are most typically used. The majority of TDM assays use a competitive design, in which limited target drug molecules in the material interact with labeled rivals for a restricted number of binding sites. To put it another way, the signal produced is inversely proportional to the analyte concentration in the tested sample. However, as biopharmaceuticals and biosimilars have become more prevalent, the noncompetitive model has been instigated to play a greater role [66].

Multi-center evaluation studies [42, 67], the development of new reagents, immobilization techniques [68], and signal augmentation approaches to overcoming specificity challenges have all received a lot of attention in recent years. A detailed examination of claimed interferences for digoxin, immunosuppressant, anticonvulsant, and tricyclic antidepressant immunoassays has been provided [42]. Recent reviews [44, 69, 70] have compared immunoassay techniques used for diverse TDM applications, particularly immunosuppressive medications. Studies comparing the effectiveness of commonly available immunoassays to the performance of LC-MS/MS have also been conducted [71, 72].

7.2.6.3 Biosensors

The term “biosensor” refers to a device that analyzes biological samples that use molecular recognition and bioreceptors to turn a biological reaction into a quantifiable signal [73]. These recognition elements have a high binding affinity for a given analyte and can be natural (antibodies, enzymes, membranes) or artificial (molecularly imprinted polymers or aptamers) [74, 75]. The interaction between the analyte and the bioreceptor causes modification in local physicochemical parameters, which translated into a readable signal. Theoretically, this interaction can be utilized to track the concentrations of both the medicine and the biomarker (protein/metabolite) [76].

Biosensors are characterized by their detecting method (optical, electrochemical, thermometric, magnetic, or mechanical), sensing mechanism (direct/label-free or indirect/labeled), functionality (single- or multi-use), or degree of invasiveness. Recent review articles [43, 77] provide greater detail on material selection, identification elements, signal detection algorithms, amplification approaches, and various sensor application domains. Another fascinating point of view is the use of no-wash biosensors for real-time monitoring of tiny compounds [78, 79].

Optical sensing is one of the most widely used methods for drug treatment monitoring. These sensors may function in a variety of spectral bands ranging from ultraviolet to near-infrared, and they can be used with sophisticated analytical techniques like NMR and Raman spectroscopy. Surface plasmon resonance (SPR) and localized surface plasmon resonance (LSPR) are two potent optical sensor technologies that rely on changes in the local refractive indices (RIs) of metal films or nanoparticles. Because molecules of interest in TDM applications are typically tiny, their impact on local RI can be negligible, depending on the analyte size. This is why, in recent SPR research, indirect approaches for signal augmentation, such as those involving nanoparticles, have been adopted. Biosensor-based TDM investigations for anticancer medicines [79, 80], antibiotics [81–84], and therapeutic drug antibodies [85, 86] have all used these sophisticated approaches. SPR has also been used in conjunction with lateral flow tests to assess the therapeutic immunogenicity of infliximab [87].

Surface-enhanced Raman spectroscopy is one of the innovative optical approache utilized in development anticancer drugs [88], antibiotics [89], and antiepileptic drugs [90]. Although optical sensors have fast turnaround times and great sensitivity, they are generally plagued by large background signals, analyte signal attenuation through the matrix, high instrument costs, and inadequate specificity due to nonspecific bioreceptor binding [91].

Another prominent method for TDM is electrochemical sensing [92]. Anticancer medicines [93], antibiotics [94–97], and antifungals have recently been detected using electrochemical biosensors combined with C-nanotubes [98], nanoparticles/doped electrodes [99], and various bioreceptors such as DNA [93], antibodies [94, 95], membranes [100], and aptamers [96, 97]. Because of the fundamental nature of bioreceptors, the issue of nonspecific binding still exists in electrochemical sensors, as it does in optical sensors, and it must be addressed.

7.2.6.4 Biochips

Computer science, electronics, and biology have combined to create biochip, the most fascinating future technology. The potential applications are numerous, both for research and therapeutic usage, with a sizable market [101]. One of the most important parts of immunosuppression in transplanted patients is the proper dosage of immunosuppressants, which have the key role of preventing transplant rejection by partially inhibiting the body's immunological reaction to the donated organ [102].

According to recent clinical investigations, the area under the concentration-time curve (AUC) of immunosuppressant concentrations correlates better with immunosuppressive medication efficiency and adverse effects than the classic TDM of trough concentrations before the next dosage. Clinicians altering immunosuppressant doses for patients in the initial phase after transplantation could be interested in a unique POCT (point-of-care testing) equipment that allows for drug AUC monitoring [103].

TDM is critical in the management of transplanted patients because it allows for the accurate assessment of drug dosages with restricted therapeutic windows. TDM of immunosuppressants usually entails drawing blood before the next dose to establish the lowest drug concentration, known as the trough level [104]. Preliminary tests on the bioassay implementation for drug detection in transplanted patients were successful, and a novel therapeutic drug monitoring (TDM) point-of-care testing (POCT) biochip for immunosuppressant detection in transplanted patients was created [105].

7.3 Science of Toxicology

The study of chemicals that affect living organisms is known as toxicology. Toxic exposure can happen transdermally through skin contact, orally, or through inhalation [106]. Toxicity testing is required to establish a foundation for the control of substances with which humans and other living things may come into contact, whether intentionally or unintentionally. Cosmetics, pharmaceuticals, food additives, pesticides, chemicals, additives, and consumer products are all tested for safety. A toxic impact can be caused by natural or man-made material, and it can cause a wide range of symptoms, both short and long term [107]. As a result, toxicity testing employs a variety of procedures and rates of exposure to the test organism to develop a more precise estimate of the risk of harm that the test material may pose to human health and the environment. Animal research provides the majority of human information about the toxicity of various compounds, albeit it is primarily used to extrapolate expected human physiological responses [108, 109].

Serum, blood, and urine are used as specimens in the great majority of toxicological tests done in clinical laboratories. Blood is frequently employed as a surrogate for measuring a drug's concentration at the site of action; however, it may not accurately reflect the concentration at the site of action [110]. Drugs' exact sites of

action range from nerve endings to receptors on cells all over the body. Blood is chosen as a monitoring specimen because sampling from these areas is rarely possible. Blood is usually the transporter of the medicine from the place of absorption to the site of action, according to logic. Because most medications are water-soluble, monitoring plasma or serum without the influence of red cells is a sensible choice [111]. Other specimens may, however, be tested and, in certain situations, may be required to provide the desired clinical information. Because of the increased sensitivity of contemporary technology, most of this testing has only become achievable in recent years. Many of these alternative specimens have extremely low quantities of pharmaceuticals, drug metabolites, or other poisons that are undetectable using traditional methods [112, 113]. Liquid chromatography-tandem mass spectrometry (LC-MS) has been an analytical technique that has opened the door for the testing of alternative specimens, particularly in the drug industry. This review looks at these specimens but does not examine them for infectious diseases, which is another rationale for looking at different specimen matrices [114].

7.3.1 Toxicology: Biological Sampling and Use of Different Analytical Techniques

Toxicology is a sophisticated scientific area that makes use of a wide range of analytical methods like laser diode thermal desorption-tandem mass spectrometry (LDTD-MS-MS) [115], hyphenated liquid chromatographic techniques [116], chromatography using silica-gel chromatograms [117], ultra-high performance liquid chromatography-tandem mass spectrometry [118], DNA typing [119], and capillary electrophoresis [120] Pesticides, pharmaceuticals, natural products, industrial chemicals, metals, and contaminants are among the forensic discoveries that can be made using these procedures [119]. Toxicological testing can be performed on a variety of samples obtained from the persons under inquiry. Specimens such as blood, urine, nails, hair, bile, gastric contents, liver, and brain tissue can all be valuable [115, 116]. The demand for advanced analytical techniques used in toxicology to resolve conflicts is gradually increasing. For toxicological analysis, many procedures are employed to determine numerous medicines from a variety of biological materials [117]. Table 7.1 outlines the analytical methodologies utilized in various drug analysis investigations, as well as the types of biological matrices used.

7.3.1.1 Reasons for Testing

Toxicology screening (safety assessment) is a way of figuring out how a drug of the issue has a negative impact on the biological activity of an organism, given a certain duration, route of the exposure, and concentration. Various reasons for toxicological screening are described below.

7.3.1.2 Pharmacologic Reasons

Saliva was once one of the additional specimens evaluated to determine the “free” portion of a therapeutic medication or hormone. The amount of drug that could bind to the receptor was better reflected when saliva-free drug concentrations were used [14]. Alternative specimens are frequently utilized in drug misuse testing and therapeutic drug management testing. Saliva was first used to monitor free drugs like phenytoin, primidone, and ethosuximide [14, 144].

7.3.1.3 Pharmacokinetic Reasons

The length of time a medicine stays in the body varies depending on the samples. The amount of medicine that circulates throughout the body is reflected in the blood, which is a decent indication of the period when the drug reaches its intended target for treatments [145]. Urine measurements can assist in defining the elimination parameters and measuring the duration of action. For some medications, other specimens like saliva, sweat, hair, nails, etc. may offer particular concentrations at the site of action or metabolism data [146].

Table 7.1 Different biological samples and analytical techniques for toxicological studies

Sr. no.	Samples	Method used	References
1	Urine and blood	Laser diode thermal desorption-tandem mass spectrometry (LDTD-MS-MS)	[115]
		Ultra-high performance liquid chromatography-tandem mass spectrometry	[118]
		Liquid chromatography-electrospray mass spectrometry	[121]
		Enzyme immunoassay and gas chromatography/mass spectrometry	[122]
		Liquid chromatography-mass spectrometry	[91]
2	Biological samples	Gas chromatography-surface ionization organic mass spectrometry	[123]
		Chemical ionization mass spectrometry with ammonia	[124]
		High-resolution mass spectrometry	[125]
		Liquid chromatography-mass spectrometry	[126]
3	Hair	Gas chromatography-negative chemical ionization tandem mass spectrometry	[127]
		Matrix-assisted laser desorption/ionization (MALDI) combined with imaging mass spectrometry, gas chromatography-mass spectrometry (GC-MS)	[128–131]
		Liquid chromatography coupled with electrospray-ionization mass spectrophotometry (LC-ESI-MS)	[130]
4	Blood, plasma, serum, or urine	Liquid chromatography-mass spectrometry	[132]
5	Nails	Chromatography coupled with electrospray-ionization mass spectrophotometry (LC-ESI-MS)	[133]
6	DNA, biological sample	Polymerase chain reaction (PCR)	[134]
7	Blood samples	HPLC and capillary zone electrophoresis (CZE) techniques	[135]
8	Sweat	GC-MS, LC-MS, enzyme-linked immunosorbent assay (ELISA)	[136, 137]
10	Oral fluid	(GC-MS), liquid chromatography coupled to mass spectrometry (LC-MS)	[133–135]
11	Vitreous humor (VH)	GC-MS, gas chromatography with nitrogen-phosphorus detector (GC-NPD), gas chromatography coupled to tandem ion trap mass spectrometry (GC-MS/MS)	[138–140]
12	Meconium	ELISA, GC-MS, LC-MS, enzyme-multiplied immunoassay technique (EMIT)	[141–143]

7.3.1.4 Availability of Specimens

The types of specimens available in clinical toxicology are frequently limited by case circumstances. The most appropriate samples for detecting recent oral ingestions are gastric contents, although this sample may not be readily obtainable due to patient condition constraints or treatment. The ability to get a urine sample may be hampered by the patient's medical state [147, 148].

7.3.1.5 Ease of Collection

Hair, saliva, and perspiration are examples of noninvasive specimens that can be collected by non-skilled workers. Samples gathered like this are used in instances when obtaining patient or subject approval is more difficult. Biomonitoring and family toxicology studies are two recent instances of this [149, 150].

7.4 Alternative Matrices in Toxicology

Alternative biological matrices have been studied in toxicological testing for many years, owing to their advantages over traditional matrices [151–154]. These benefits include the ability to gather specimens more easily and with less invasiveness, as well as bigger detection windows in some circumstances [153]. Furthermore, these matrices can be employed when blood samples are unavailable, deteriorated, or influenced by postmortem redistribution, as well as when drug consumption is delayed [155]. However, drug levels in some of these matrices may be lower than in urine or blood due to inherent features and toxicokinetics. As a result, contemporary instrument technology has enabled the investigation and analysis of other

matrices; sophisticated and sensitive instruments are allowing the identification of lower amounts of medicines and toxins in some of these alternative specimens [151]. Saliva and hair are already well-known and have been used by several labs for drug testing. Oral fluid, hair, sweat, meconium, breast milk, and vitreous humor are some common alternative matrices in drug testing, based on their features, advantages, and limitations [135].

In toxicological assessments, selecting specimens is a crucial step. To pick the best biological fluid/tissue for the investigation, it's critical to understand the qualities of both the target analyte and the matrix. Biological fluids/tissues are alternative biological matrices that can provide extra information and advantages over blood and urine testing in a variety of ways, including sample collection/preparation/analysis complexity and detection window [134]. Furthermore, when blood and urine are not accessible, these matrices can be collected and examined. However, each of these alternate matrices has its own set of qualities, benefits, and drawbacks that must be evaluated. According to toxicology, alternative matrices will be examined more frequently in the future [156].

7.5 Different Approaches in Various Biological Matrices, with an Emphasis on Toxicology

The application of alternative matrices has been an upsurge in forensic toxicology. Conventional biological fluids like blood, plasma, serum, and urine specimens are normally used for drug testing in forensic toxicology [151]. For the last few years, enormous research work has been done in this field to investigate different types of drug and their metabolites in biological samples [157]. Alternative biological matrices are more compelling in toxicological testing for their benefits over conventional matrices in terms of larger detection windows and the requirement of fewer samples [158, 159]. However, insufficient drug levels have been found in some of these alternative matrices used for toxicological studies. Thus, more advanced instruments and better techniques are required for the analysis of alternative matrices to detect the lower concentrations of drugs in the sample of interest [151].

7.5.1 Microextraction Techniques in Different Biological Matrices

Microextraction procedures concede high recovery of target analytes with independence of the matrices. Preparation of the samples is the most critical step in the analysis of analytes of interest. Conventional procedures for preparing samples like solid-phase extraction (SPE) and liquid-liquid extraction (LLE) are used over the years. For the last few decades, the microextraction technique has taken the attention due to the presence of various drawbacks with these classical sample preparation techniques. The major classification of microextraction techniques is liquid-phase microextraction (LPME), solid-phase microextraction (SPME), microextraction by packed sorbent (MEPS), and fabric-phase sorptive extraction (FPSE) [158, 159].

SPME is the simple, minimized number of steps involved in sample preparation, reduced volume solvent, and cost-effective process with reduced analysis time [152, 160]. Microextraction methods were developed to predict cannabinoids in various matrices by LPDE or SPME [161]. LPME is beneficial for water analysis of several pesticide groups in the environmental analysis [162]. Moreover, these techniques are useful for pharmacokinetics study, direct in vivo sampling, and chip-based microfluidic systems.

7.5.2 Other Green Extraction Technique Application

Besides SPME and LPME, other important green extraction techniques are used in the specimen preparation of alternative biosamples. Amphetamines and methadone in saliva matrices were evaluated by Meng and Yang with the application of small-volume LPE techniques [163].

FPSE is first introduced in 2014. These techniques integrate principles typical of SPME and SPE and use a flexible fabric surface of different materials [164].

7.5.3 Applications of Mass Spectrometry Techniques in Alternative Biological Matrices

There are different types of mass spectrometers available for different applications. Coupling of different chromatography techniques to mass spectrometry is used for the identification and quantification of metabolites from an unknown drug, i.e., LC-MS and GC-MS [165, 166]. Looking forward to the precise estimation and analysis of compounds for their pure structural information, one would opt for MALDI (matrix-assisted laser desorption ionization) coupled to time of flight (TOF) or quadrupole coupled to time of flight [167]. Endogenous matrix components, metabolites, degradation products, exogenous xenobiotics, and other interfering chemicals could be present in the alternative biological matrix. Samples spiked with calibration (reference) standards are used to analyze drugs in the biological matrix. Furthermore, hallucinogens such as LSD and its metabolites are quantified using an ultrafast and sensitive microflow liquid chromatography-MS (MFLC-MS/MS) [167].

7.6 Artificial Intelligence (AI) in Toxicity and Drug Discovery

“Artificial intelligence” was first coined by John McCarthy in 1956 [168]. AI is the set of theories and techniques used to create machines that are capable of stimulating intelligence and performing functions, executed by humans, and that can mimic human intelligence [169]. The first step in drug discovery is the identification of new chemical entities, which have desired biological activity. For the treatment of a particular disease, the active learning algorithms can identify the potential lead compound. For preclinical safety evaluation and toxicity, the quantitative-structure activity relationship (QSAR) method is used, which creates a link between chemical structure and biological activities [169]. AI has an important role in omics study, which are helpful to identify disease pathogenesis by finding the new biomarkers and integrating drugs with the disease. AI-based models have been developed for COVID-19 drug discovery and vaccine development [170]. Drug target interaction and pharmacological properties of the potential drug compound can be predicted by the use of AI (Fig. 7.2a) [171].

Chemical toxicity is defined as the adverse effect produced by exposure to chemical agents, which can be referred to by LD50, moderate, and high toxicity [171]. Evaluation of toxicity of the chemical agents can be done in animal models. The first principle (replacement) of three “R” principles (replacement, reduction, and refinement), refers to the use of alternative methods over the use of animal models [172]. Most of the newly discovered drug molecules are excluded based on toxicological studies. In terms of ethical concern and result reproducibility, the researcher found more reliable findings with the use of AI-based models compared to exchange traditional in vivo toxicity models. In food technology, to determine the concentration of harmful compounds, spectroscopic techniques include hyperspectral imaging, fluorescence spectroscopy, near-infrared (IR) spectroscopy, Fourier transform IR (FTIR), and Raman spectroscopies; biosensors are used which are based on AI algorithms, i.e., machine learning (ML), deep learning (DL), artificial neural networks (ANNs), deep neural networks (DNNs), and convolutional neural networks (CNNs) (Fig. 7.2b) [171, 172].

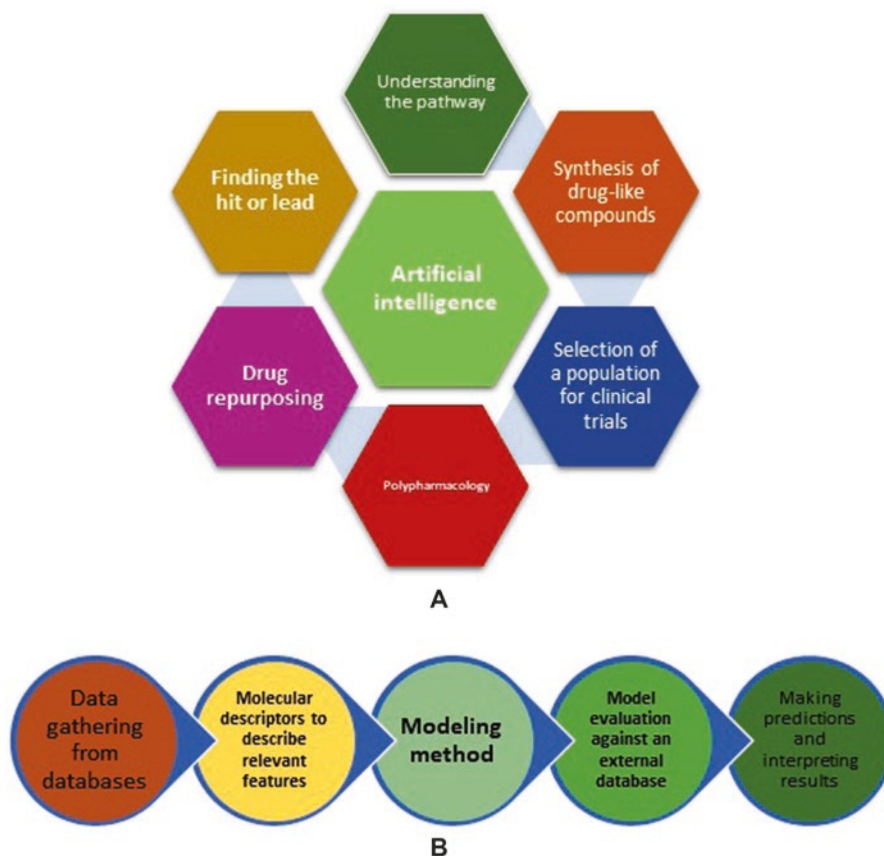

Fig. 7.2 (**a**) Artificial intelligence in drug discovery. (**b**) Stages making up in silico toxicology

7.7 Promises and Pitfalls of Alternative Matrices

Many labs are increasingly analyzing fit-for-purpose alternative sample matrices such as oral fluid, hair, and exhaled breath to detect an expanding range of drugs faster and with greater certainty, as well as to make sample collection as easy, noninvasive, and trustworthy as possible [173]. These analyses are becoming more common because of advances in toxicokinetics (TKS) research and new analytical technology [174].

Window of Detection

The "detection window" for a drug and its metabolites in distinct biological matrices is a crucial concept in PKS and TKS. A drug's detection window must be long enough for sampling to occur to identify it [175]. Today it tends to look at the optimal sample matrix for the application. Whether it's urine, blood, hair, oral fluid, breath, or sweat, each matrix has its own set of benefits and drawbacks, as well as unique information that can be utilized to interpret drug usage and the cause of impairment or death. One factor to consider is the detection window, or how long evidence of drug usage may be discovered in a given matrix. Analysts can, for example, determine use days, weeks, and even months in the past based on hair length (Fig. 7.1b). Urine and blood offer data on drug usage over a significantly shorter period [176].

Recent advancements in sample preparation, chromatography, and MS technologies are assisting labs in overcoming the challenges of adopting alternate sample matrix analysis. Alternative, purpose-built sample matrices are attracting a lot of attention because they have the potential to

make sample collection more convenient, noninvasive, and dependable [177]. Though interpreting drug concentrations in alternative sample matrices is still a hot research area, advances in analytical technologies have made the testing of alternative, fit-for-purpose sample matrices a feasible reality. Few detection methods were sensitive enough to measure the target medicines and metabolites at the levels required when other matrices were introduced [174].

7.8 Artificial Intelligence (AI) in Toxicology and TDM

7.8.1 Artificial Intelligence for Clinical Toxicity and Patient Safety

Bringing novel medications to market poses a significant challenge in terms of drug safety. To solve the problem of drug safety there are two systems. Before the approval of the drug for patient use, clinical trials are performed to ensure the safety and efficacy of its intended use. AI approaches are increasingly routinely optimized to enhance patient care, diagnosis, therapy, and patient follow-up. Machine learning (ML) and deep learning (DL) are two AI subfields. After the drug is marketed, the agencies collect the adverse event reports for performing analyses on follow-ups but can be complicated for rare events like drug-drug interaction [178]. Researchers thus now focus on various statistical and computational tools to minimize the loopholes and act as an add-on to the pharmacovigilance toolbox [179, 180]. Individual case safety reports (ICSRs), which are self-reported, have been a primary data collection method for post-marketing drug safety research. The Naranjo algorithm and Venulet algorithm are the most well-known methods for determining causation [181, 182]. Consequently, dataData mining techniques were able to detect statistical connections between medications and adverse events using spontaneous reporting systems (SRSs) such as FDA Adverse Event Reporting System (FAERS) [183, 184]. Another approach is the application of system pharmacology which focuses on target effects and clinical adverse events. Furthermore, in silico ADR mining contains a massive data pool for medication safety. Databases like DrugBank, SIDER, ChEMBL, ChEBI, PubChem, Reactome, and KEGG are available for data mining. Recently, researchers proposed modular assembly of drug safety subnetworks in which using knowledge bases connected to literature searches, genome data generated a network of proteins. Further, it is compiled into network metrics for the prediction of adverse effects in new drugs [185]. Another approach is based on drug-drug interaction by literature mining and integration of drug-gene interaction and creating a model for prediction of drug-drug interaction focused on skin diseases as represented in Fig. 7.3 [186].

7.8.2 Artificial Intelligence (AI) in the Prediction of Adverse Drug Reaction (ADR) and a Case Study

Adverse drug reactions are pharmacological occurrences caused by the interaction between drugs which can be caused by a variety of factors like drug-drug interaction. Anticipating and reducing ADRs upfront in the drug development process can improve drug safety while also lowering costs. Few researchers developed an innovative and effective computational methodology for accurately predicting adverse drug reactions (ADRs) for trial drugs by combining protein-protein interaction (PPI) networks, drug target data, and gene ontology (GO) annotations with clinical observation data, and the results also depict the importance of including prior knowledge of gene annotations and gene networks, for better estimation of ADR [187].

Deep learning (DL) is an artificial neural network (ANN) extension that learns relevant attributes from unprocessed data using a cascade of ANNs. To predict activity levels for distinct compounds, DL is used with a set of varied QSAR datasets [188]. Recently researchers employed DL to develop drug-induced liver injury predic-

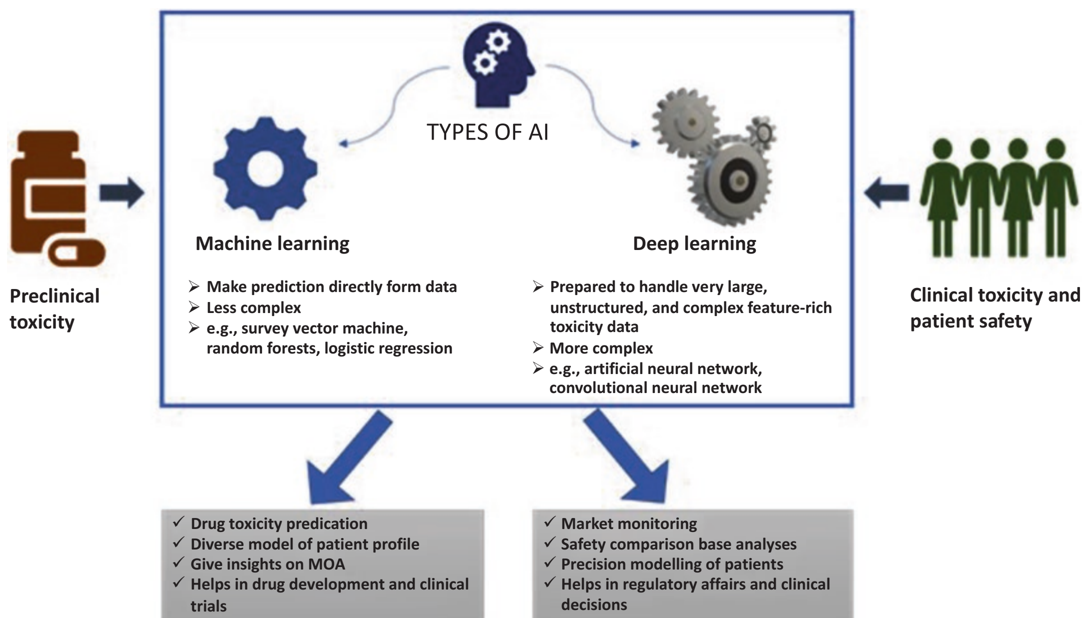

Fig. 7.3 Role of artificial intelligence (AI) in drug toxicity and patient safety

tion models with structural data for measuring hepatotoxicity [189]. CNNs are a type of deep convolutional neural network that generates interpretations of raw pictures from pixel data as a stack of images from which attributes may be retrieved and then used to identify complicated patterns. Images of cells that were pre-treated with a series of medications were used to train CNNs to estimate toxicity. In this method, different medications, nuclear stains, and cell lines can all be used to predict a wide range of toxicity pathways [190]. Another group of researchers created machine learning algorithms, along with a deep learning model, that can anticipate ADRs and detect the chemical substructures linked with those ADRs without having to define the substructures ahead of time. The authors evaluated the performance of the model with ten other fingerprint models. It was discovered that neural fingerprints performed the best among all methods of predicting ADRs [191]. Some researchers utilized a selection strategy to find important features and machine learning algorithms to create computational approaches that could predict neurological ADRs before preclinical toxicity studies [192]. Toxicologists, clinicians, and AI programmers should work together to reduce the adverse effects with the help of AI.

AI has been also used to interpret plasma concentration in anti-retroviral for TDM. The objective of the study was to create a software-based system to interpret plasma concentration in anti-retrovirals for therapeutic drug monitoring [193].

From 199 HIV-positive patients in a TDM study (CCTG 578), data were extracted. Pharmacokinetic parameters of lopinavir and efavirenz were modeled using a Bayesian approach and interpreted by a specialized committee having expertise in HIV and pharmacologists provided TDM suggestions. The pharmacokinetic models acted as the base to form the artificial intelligence (AI) system which could predict the exposure of drugs and data interpretation of the pharmacokinetic parameters and initiate TDM suggestions. The recommendations from the expert committee and modeled pharmacokinetic exposure proven to be optimal for validation of the results obtained from the AI system [193].

It has been seen that among all the patients, lopinavir was administered to 67 patients and

efavirenz was administered to 46 patients and 3 patients were administered with both the drugs. After 4 hours of administration dose concentration was estimated and a high correlation of lopinavir and efavirenz between the estimate through AI and PK modeled values was obtained ($r > 0.79$; $P < 0.0001$). Considerable difference was seen in the mean predicted 4-hour concentration of lopinavir (7.99 μg/ml against 8.79 μg/ml; $P < 0.001$) and efavirenz (4.16 μg/ml against 3.89 μg/ml; $P = 0.02$). 53 out of 69 lopinavir cases were agreed by the AI and specialized committee TDM suggestions, whereas 47 out of 49 were in the case of efavirenz [193]. The study concluded that the AI system correctly calculated lopinavir and efavirenz through concentrations and was in good agreement with TDM recommendations for efavirenz- and lopinavir-treated patients from the specialized committee [193].

7.9 Importance and Future Perspective of TDM

There are some reasons to be optimistic about TDM's future. Analytical methods are improving in terms of simplicity, operability, and automation, allowing for the use of different matrices such as saliva, interstitial fluid, or dried blood spots, which makes sample collection easier [194]. In a different perspective, fresh analytical methods are being used to produce point-of-care TDM methods [195]. TDM research in other areas is also on the rise, with an increasing number of scientists from other disciplines, particularly biomedical engineers, uncovering this wide field of study. Therapeutic monitoring in its broadest sense will become a mainstay of tomorrow's precision medicine, encompassing not only drug concentrations but also a variety of efficacy and tolerability biomarkers [84]. Progress in medical information technology will soon integrate all of our healthcare systems, removing the challenges of communication and medical use of concentration measurements. TDM pharmacological interpretation will benefit from new computer tools with increased user-friendliness and performance, which are currently being developed [196].

As a result, rather than automatized TDM performed by a computer, we propose computer-assisted TDM by practitioners. TDM's development, diffusion, and oversight will continue to necessitate the involvement of competent clinical pharmacologists who are available to advise in difficult circumstances. Automatically collecting monitoring data, on the other hand, will amass large datasets suitable for intriguing new sorts of medical research [197]. Finally, the global trend toward patient empowerment, aided by appropriate mobile apps, will encourage patients to take an active role in their therapeutic monitoring. Most patients will appreciate being able to see the circulation exposure caused by their medications, and an increasing number of them want to be in charge of self-monitoring.

7.10 Challenges Faced in Managing Patient Health Data

Patient health data management is a complex process. The majority of healthcare businesses only work with digital data. The amount continues to rise, laws are continuously changing, and trading it remains difficult. Because standardization is still absent, the path to healthcare interoperability is riddled with potholes. Fragmentation persists in the absence of interconnected systems. Organizations should create a strategic plan to achieve internal interoperability at the very least. There might be a lot of duplicate information in the system, or it could be erroneous and old and it cannot be even deleted due to the various regulations. The more data in the system increases the strain on the software leading to an increase in the cost of software hosting. There is also the problem of data analytics since the huge data takes time for analysis which makes the process cumbersome.

7.11 Conclusions

TDM is required for just a tiny percentage of medications used in pharmacotherapy, but these drugs must achieve maximal efficacy and avoid

drug toxicity. TDM can also help avoid bad side effects. The old approach of TDM for avoiding drug toxicity needs to be revised due to new developments in information technology, new analytical procedures for less frequently monitored drugs, and new clinical pharmacological expert opinions in the presentation of laboratory medicine results. Rather than establishing the cause of an existing adverse drug reaction, TDM can now be utilized to avoid an adverse drug reaction. TDM has several advantages, including significant cost savings due to the patient's shorter stay in the hospital and the avoidance of costly identification and treatment of an adverse medication event. TDM's success is mostly dependent on physicians, clinical pharmacologists, pharmacists, analytical laboratory scientists, and nurses working together to provide a coordinated and integrated approach to patient care. Alternative matrices are increasingly being used in clinical and forensic toxicological testing. A new study is needed to develop more analytical tools and a better knowledge of drug behavior in various matrices.

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8 Analyzing Data from Therapeutic Drug Monitoring, Pharmacokinetics, and Clinical Toxicology Studies

Abdul Malik Sulley

Abstract

Both individual- and population-based data are useful for optimizing the efficacy of drugs with narrow therapeutic indices, while at the same time, limiting the frequency and severity of adverse effects. Pharmacokinetic, pharmacodynamic, descriptive, and inferential analyses all provide valuable information by which safe dosage regimens can be established for patients at individual and population levels. However, to draw reliable conclusions from such analyses requires an understanding of the best endpoints to assess and what types of data to collect. Furthermore, data needs to be handled with such high standards that the integrity of conclusions drawn from it cannot be disputed. This chapter describes key concepts and provides practical examples on planning, handling, and analyzing data in therapeutic drug monitoring, pharmacokinetics and clinical toxicology studies.

Keywords

Analysis · Endpoint · Data · Pharmacokinetics · Steady state

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8.1 Introduction

Therapeutic drug monitoring (TDM) takes place during routine clinical care as well as in clinical research settings. The aim of data analysis in TDM is to answer research questions about the safety and/or efficacy/effectiveness of a drug with a narrow or not-well-established therapeutic index.

This chapter introduces key concepts and issues surrounding planning, handling, and analyzing data in TDM. Some sections are relevant to routine care for individual patients only, clinical trials only, or both. The chapter is organized in such a way as to address the following concepts and issues:

- Endpoints – what are our measures of safety and efficacy/effectiveness?
- Types of data – how do we tell what type(s) of data we have?
- Data handling – how do we treat our data prior to and during analysis?
- Pharmacokinetics – how does the drug "move" through the body and how do we monitor an optimal plasma drug concentration?
- Pharmacodynamics – how does the body respond to the drug and how do we know the drug is working?
- Sample size – how many participants we need to test a hypothesis?

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- Analysis methods – how do we summarize our data and what statistical methods do we use in hypothesis tests?

8.1.1 Software Setup

Analysis examples are provided throughout the chapter. The examples are written in R code [1]. R is a free statistical programming language and environment. There are 18,000 packages that can be installed to enable conduct of several types of analyses and generate graphics. The examples in this chapter were run using version 4.1.3. Further information about installation and packages can be found through the R-Project homepage (https://www.r-project.org/).

R-Studio/Posit is a free coding environment that makes coding with R much easier and efficient [2]. The examples in this chapter were run using R-Studio version 2022.02.0 Build 443 and can be obtained from https://www.rstudio.com/. Posit is the new name for R-Studio (https://www.posit.co).

The code examples in this chapter use three dummy datasets from the qwickr package [3], namely:

- catdata – a dummy categorical dataset
- pkdata – a dummy pharmacokinetics dataset
- rmdata – a dummy repeated measures dataset

Qwickr package can be installed using the code:

```
> remotes::install_github("qwickmalik/
qwickr")
```

For all examples, command lines begin with >, while output lines do not. Commands that span multiple lines are identified by + after the first line. However, when running your code, do not include > or + at the beginning of your command lines. Comments begin with #. Code that appears after # on the same line will not be evaluated when it is run. Those that occur on the lines below or above this will be evaluated.

```
#This is a comment
```

Other packages used in examples that need to be installed are car [4], PKNCA [5], and DescTools [6]. These can be installed using the commands below.

```
> install.packages("car")
> install.packages("PKNCA")
> install.packages("DescTools")
```

After installing packages, please make sure each package is loaded into the programming environment using the commands below.

```
> library(car)
> library(PKNCA)
> library(DescTools)
```

To find out more information about any package or command, type “?” followed by the package or command name. For example, to learn about the stats package which is automatically installed along with R, run the following line of code:

```
> ?stats
```

8.2 Endpoints

An endpoint is a parameter, measure, or phenomenon by which the safety, efficacy, or effectiveness of an intervention (or drug) is measured. At the individual patient level, the decision of whether an intervention is safe, efficacious, or effective is based on that patient’s data, comprising diagnostic and lab tests and clinical examination. At the population level, it is usually based on a test of hypothesis. The null hypothesis is usually that there is no significant difference between two interventions (placebo could be

considered as an intervention), with respect to that endpoint.

Efficacy can be described as the ability of an intervention to produce an intended desired effect in a controlled experimental setting. Here, participants are carefully selected to limit the effects of external factors on the outcome of the intervention. In effectiveness studies, an intervention is administered in real-life settings where a drug may be used in the presence of other drugs and/or diseases.

In TDM several types of endpoints can be assessed. Examples of these include the following:

(a) Pharmacokinetic profile (including plasma drug concentration) [7–9]
(b) Pharmacodynamic profile [8]
(c) Incidence of an event, e.g., clinical failure, adverse event (skin rash), mortality [9, 10]
(d) Biomarker levels, e.g., c-reactive protein (CRP), procalcitonin [10]
(e) Time to event, e.g., time to infection site negative culture [10]

8.3 Types of Data

Data (singular: datum, although data is used as both singular and plural in some publications) can be described as a collection of information that is usually organized to facilitate analysis and decision-making. The pieces of information can be classified as qualitative or quantitative. Qualitative data often describe types or qualities of a subject or phenomenon. For example, Joe has some money in his wallet, consisting of a $50 note, a $10 note, and a 25¢ coin. Here we are describing the denominations of money. Quantitative data, on the other hand, are numerical measurements by which properties or attributes of a subject or phenomenon are quantified. For example, the total amount of money in Joe's wallet is $60.25. Joe's friends have $55, $80.15, and $77.50 each. Here, we are measuring amount or quantities.

In clinical research, qualitative data can be of two main types: structured (categorical) or unstructured (free text), while quantitative data can also be referred to as continuous data. Categorical and continuous data consist of subtypes as listed below.

(a) Qualitative data
(a) Unstructured data (free text)
(b) Structured or categorical data
    (i) Binary – only two categories or responses often coded 0 and 1, e.g., coin (tail, head), mortality (dead, alive)
    (ii) Nominal – more than two categories, with no specific order between them, e.g., blood type (A, B, AB, O) or eye color (amber, brown, gray, green, blue, etc.)
    (iii) Ordinal – more than two categories with order, although the difference between adjacent categories cannot be quantified, e.g., education level (primary school, high school, bachelor's degree, master's degree, doctorate degree)
(b) Quantitative or continuous data (numeric)
    (a) Discrete – whole numbers often representing counts, e.g., number of cancer patients in a ward.
    (b) Interval – there are order and a meaningful difference between two values with no meaningful zero (0), e.g., Celsius temperature scale,
    (c) Ratio – interval data type with a meaningful zero (0) i.e., zero means an absence of the phenomenon being measured, e.g., hemoglobin levels, plasma drug concentration, and Kelvin temperature scale (0 Kelvin is absence of heat).

8.4 Data Handling

Vast amounts of data are often collected during clinical trials or routine clinical care. Before analysis of clinical data can be done, data needs to be collated (grouped together usually in an analysis-ready dataset) and validated (checked for quality). Quality checks are be conducted to confirm that the analysis dataset is not different from the source, be it case report forms, paper charts, lab reports, electronic medical records, or other datasets.

As much as possible, data editing rules should be prespecified in study protocols, statistical analysis plans (SAPs), or standard operating procedures (SOPs) and should be done in the source document and/or the analysis dataset. For example, a plasma drug concentration measurement that is below the level of quantification (BLQ) may be treated as missing, a zero (0), or half the lower limit of quantification (LLQ), and this needs to be specified. When plasma concentrations are above the upper limit of quantification (ULQ), samples need to be diluted and re-analyzed to ensure that data is accurately captured.

The Clinical Data Interchange Standards Consortium (CDISC) has developed a series of standards for organizing, formatting, and sharing clinical trial data between and among sponsors, contract research organizations (CROs), laboratories, and regulatory authorities. Of these standards, the Study Data Tabulation Model (SDTM) and Analysis Data Model (ADaM) are most often used by biostatisticians. Further information is available on the CDISC website (www.cdisc.org).

8.5 Pharmacokinetics

Pharmacokinetics (PK) is often described simplistically as "what the body does to a drug." However, there is more to this. We are interested in changes across time, in drug concentrations from the time it enters the body, moves through the body as an intact molecule or a metabolite, and ending with its removal from the body. By examining PK properties of a drug, we can understand how it is absorbed, distributed, metabolized, and eliminated from the body. Common routes of administration in PK studies are intravenous (IV), intramuscular (IM), and oral. Depending on the route of administration, there may not be an absorption phase, e.g., IV administration.

In TDM, PK is used in routine clinical care to find drug doses that are optimal for individual patients. Here, the key emphasis is on finding an appropriate balance between safety and effectiveness of a drug with a narrow therapeutic range. For such drugs, there is a small dose range of effectiveness below which the drug is ineffective, and above which the drug can be toxic. Furthermore, a fixed dose may not be effective or safe in every patient; therefore, the ideal dose depends on the individual patient. Finding the appropriate dose not only helps to prevent serious adverse events (SAEs) but can also help to improve compliance to treatment. For example, if a drug causes unbearable adverse drug reaction (ADR) or needs to be taken frequently, patients would be less likely to comply with treatment and therefore not benefit from the drug. Therefore, using PK information, healthcare teams and researchers can find ideal dosages for individual patients or develop controlled-release or extended-release formulations to reduce the frequency of administering the drug. Medical devices can also be developed to automatically administer the drug as needed.

Pharmacokinetic (PK) parameters are properties of a drug by which its absorption, distribution, and elimination are assessed. Table 8.1 lists and describes key parameters commonly used in TDM, although other PK parameters may be explored as well. In Table 8.1, although reference is made to concentrations of drug or metabolite in the body, in practice, concentrations are mostly measured in plasma.

8.5.1 Single-Dose PK Parameters

After a single dose of a drug is administered, its concentration typically rises to a peak in the body and then drops over time as it is eliminated. Non-compartmental analysis (NCA) assumes that the whole body is one compartment, and this provides for simpler methods to calculate PK parameters. All calculations in this chapter are based on this assumption.

Figure 8.1 illustrates how the plasma concentration changes over time after a single dose of an unknown drug is administered orally. The accompanying data is provided in Table 8.2. The concentration-time curve and data are very important in understanding what PK parameters are and in calculating them.

Table 8.1 Pharmacokinetic parameters

PK parameter	Description
C_{max}	Maximum (peak) observed concentration of drug in the body
t_{max}	Time to reach maximum (peak) drug concentration after drug administration
AUC_{0-t} or AUC_t	Area under the concentration-time curve from time zero to time t
AUC_{last}	Area under the concentration-time curve from time zero to the last quantifiable concentration
$AUC_{0-\infty}$ or AUC_{∞}	Area under the concentration-time curve from time zero to infinity
λ	Elimination rate constant
$t_{1/2}$	Terminal half-life
CL	Apparent total clearance of drug from the body
CL/F	Apparent total clearance of drug from the body after non-intravenous administration
F	Bioavailability – fraction or percentage of drug systemically available after administration
V_d	Apparent volume of distribution after non-intravenous drug administration
V_d/F	Apparent volume of distribution after non-intravenous drug administration
C_{last}	Last observed quantifiable drug concentration
C_{ss}	Steady-state plasma concentration of drug during constant rate infusion
$C_{av,ss}$	Average steady-state plasma concentration of drug during multiple dose administration
$C_{max,SS}$	Maximum (peak) steady-state drug concentration within a dosage interval
C_{trough}	Trough plasma concentration, at the end of a dosing interval, immediately before next dose administration
τ	Dosing interval

Given that it is impossible and harmful to patients to collect blood at every minute, it is important to plan blood sampling times based on existing information of the drug's profile. This information can come from animal or human studies, depending on the stage of drug development.

8.5.1.1 C_{max} and t_{max}

The maximum or peak concentration and nominal time at which this occurs are the easiest parameters to estimate, as is evident from Fig. 8.1 and Table 8.2. Careful planning of blood sampling times is critical to obtaining the highest possible blood concentration.

When analyzing data involving several participants, it is more efficient to determine C_{max} and t_{max} without having to graph each participant's concentration-time curve. Example 8.1 illustrates how to do this using the PKNCA package in R. C_{max} is 0.644 ng/mL, while t_{max} is 1 h.

Example 8.1

```
#Cmax
> pk.calc.cmax(conc=patient1$CONC)
>
[1] 0.644
>
#tmax
>     pk.calc.tmax(conc=patient1$CONC,
time=patient1$TIME)
>
[1] 1
```

8.5.1.2 AUC_t

The area under the concentration-time curve gives an indication of how much of a drug was in the body, after administration from time zero (0) to time *t*. It is measured units of concentration-time, e.g., ng.h/mL. There are several ways of calculating this, of which the most common is the trapezoidal method or linear trapezoidal rule. By

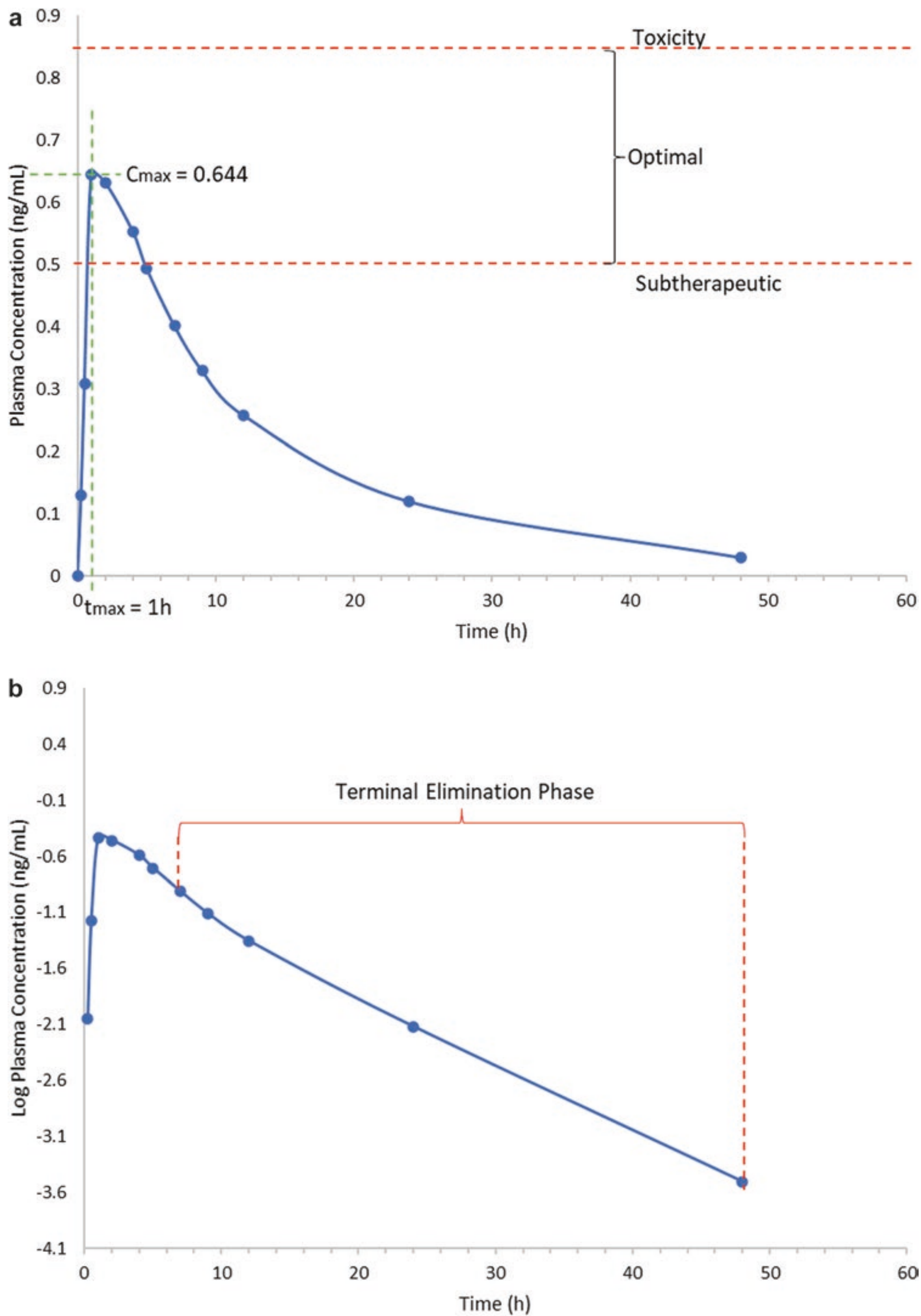

Fig. 8.1 (**a**) Concentration-time curve for an unknown drug. (**b**) Semilogarithmic curve for the same data

this rule, the concentration-time curve is divided into a series of adjacent trapezoids. The area for each trapezoid is calculated and all the calculated areas summed to give AUC_t. The trapezoidal rule is represented by Eq. 8.3, which is obtained by combining Eqs. 8.1 and 8.2:

Table 8.2 How to calculate AUC by hand using a spreadsheet

Sample	Time (*h*)	Concentration (ng/mL)	$C_i + C_{i-1}$	$T_i – T_{i-1}$	AUC_{48}
1	0	0	–	–	–
2	0.25	0.129	0.129	0.25	0.016
3	0.5	0.308	0.437	0.25	0.055
4	1	0.644	0.952	0.5	0.238
5	2	0.632	1.276	1	0.638
6	4	0.553	1.185	2	1.185
7	5	0.494	1.047	1	0.524
8	7	0.402	0.896	2	0.896
9	9	0.33	0.732	2	0.732
10	12	0.258	0.588	3	0.882
11	24	0.12	0.378	12	2.268
12	48	0.03	0.15	24	1.800
Total					9.233

$$\text{Area of a trapezoid}(A) = \frac{(C_2 + C_1)}{2}.(t_2 - t_1) \quad (8.1)$$

$$\text{AUC}_t = A_1 + A_2 + A_3 + \ldots + A_t \quad (8.2)$$

$$\text{AUC}_t = \sum_{i=0}^{T} \frac{(C_i + C_{i-1})}{2}.(t_i - t_{i-1}) \quad (8.3)$$

where:
A is the area of a single trapezoid.
C is the concentration at a given point in time.
t_i is the ith time point.
T is the last sampling time point and is equal to t in AUC_t.

The AUC_t can be calculated by hand using a spreadsheet (Table 8.2) or in R using the PKNCA (Example 8.2).

Example 8.2

```
#Step 1 extract data for patient 1 for
treatment A
#This data will be used for many of the
examples that follow
> patient1 <- subset(pkdata,
+ pkdata$SUBJECTNUM == 1 &
+ pkdata$GROUPING == "Group A")
>
#Step 2 calculate the AUC using trape-
zoid method
> AUC(x=patient1$TIME, y=patient1$CONC,
method="trapezoid")
>
[1] 9.23325
```

A more accurate method for calculating AUC is the integration method, which can be done using the PKNCA package as follows:

Example 8.3

```
> pk.calc.auc.last(conc=patient1$CONC,
patient1$TIME)
>
[1] 8.874462
```

8.5.1.3 λ and $t_{1/2}$

The elimination rate constant (λ) is a value that describes the rate at which a drug leaves the body. Assuming the entire body is a single compartment, deriving the elimination rate constant assumes that there is a direct proportional relationship between the rate of drug elimination and drug concentration. Given that the elimination phase of the curve (terminal/descending arm) is exponential in nature (Fig. 8.1a), a natural log transformation of plasma drug concentrations is necessary to illustrate this linear relationship,

which appears as a straight line through points on the descending arm. The terminal slope of the resulting semilogarithmic graph (Fig. 8.1b) is the elimination rate constant.

The terminal elimination phase must not be influenced by absorption; therefore, a simple way to identify it is that there will be a straight line from its starting point to the last measured concentration [11]. This phase must have at least three (3) points to estimate the elimination rate constant, although more points will increase the reliability of estimates.

Half-life ($t_{1/2}$) is the amount of time it takes for half of a drug to be eliminated from the body. It is related to the elimination rate constant (λ) according to the formula below:

$$t_{1/2} = \frac{\ln 2}{\lambda} \tag{8.4}$$

Both can be calculated in one step using the PKNCA package in R using our patient1 data. The λ is 0.059, while $t_{1/2}$ is 11.65 h.

Example 8.4

```
> pk.calc.half.life(
+ conc=patient1$CONC,
+ time=patient1$TIME,
+ tmax=pk.calc.tmax(conc=patient1$CONC,
time=patient1$TIME),
+ tlast=48)
>
  lambda.z   r.squared   adj.r.squared
lambda.z.time.first
0.05948419 0.9993719 0.9987438 12
lambda.z.n.points clast.pred half.life
span.ratio
3 0.02969165 11.65263 3.089431
```

8.5.1.4 AUC$_\infty$

For drugs that are eliminated relatively quickly, it is possible to measure drug concentrations until the drug is eliminated from the body completely; therefore, the AUC$_t$ provides a picture of the entire exposure. However, for many drugs, elimination is quite slow and nonlinear, and it may be impractical to continue sampling blood over long periods of time. Therefore, blood sampling is often stopped at a prespecified time point t and AUC$_t$ reflects how much of the drug was in the body up till that time point. In this case, the last observed plasma concentration is usually quantifiable or nonzero. To get an estimate of how much drug would have been in the body if sampling was not stopped, AUC$_\infty$ is calculated by extrapolating the concentration-time curve to infinity. The ratio AUC$_t$/AUC$_\infty$ gives an indication of the extent to which this extrapolation went. AUC$_\infty$ is calculated using the formula below (12), where C_{last} is the last measured concentration:

$$\text{AUC}_\infty = \text{AUC}_t + \frac{C_{\text{last}}}{\lambda} \tag{8.5}$$

In Example 8.5, AUC$_\infty$ is calculated in R using λ calculated in the previous example. The same result will be obtained by hand using Eq. 8.5.

Example 8.5

```
>  pk.calc.auc.inf(conc=patient1$CONC,
patient1$TIME, lambda.z = 0.05948419)
>
[1] 9.378798
```

8.5.1.5 CL/F and V_d/F

Clearance (CL) is the rate at which a drug is removed from a reference fluid in the body, which in most cases is plasma or blood. Its unit of measurement is volume of reference fluid cleared of drug per unit time, e.g., mL/min.

Volume of distribution (V_d) is the fluid volume in which a drug circulates in the body.

Clearance is related to the elimination rate constant and volume of distribution according to the equation below:

$$\lambda = \frac{\text{CL}}{V_d} \tag{8.6}$$

Clearance is independent of V_d; therefore, if clearance increases or decreases, V_d will not change, although the elimination rate constant will. Based on this, and comparing Eqs. 8.5 and 8.6, it is evident that CL and V_d directly determine half-life ($t_{1/2}$). In other words, a drug that is

cleared fast will have a shorter half-life compared to a drug that is cleared slowly.

Clearance is also related to AUC according to Eq. 8.7, where X_o is the drug dose and F is bioavailability (fraction of drug that is systemically available). If the drug is administered intravenously, then all of it will be systemically available so $F = 1$.

$$\mathrm{CL} = \frac{X_o.F}{\mathrm{AUC}} \tag{8.7}$$

With Eqs. 8.6 and 8.7, it is possible to determine V_d as follows:

$$V_\mathrm{d} = \frac{X_o.F}{\mathrm{AUC}.\lambda} \tag{8.8}$$

After obtaining V_d, this can be plugged into Eq. 8.6 to obtain CL.

For non-intravenously administered drugs where F is not known, apparent clearance (CL/F) and apparent volume of distribution (V_d/F) can be presented.

Assuming a dose of 10 ng was administered, CL/F and V_d/F can be obtained using the lines of code in Example 8.6.

Example 8.6

```
> # Cl/F
> pk.calc.cl(10, pk.calc.auc.last(conc=patient1$CONC,
patient1$TIME))
>
[1] 1.126829
>
> # Vd/F
> pk.calc.vd(dose=10, aucinf = 9.378798,
lambda.z = 0.05948419)
>
[1] 17.92467
```

8.5.2 Steady-State PK Parameters

The PK parameters discussed so far are based on a single administered dose of a drug. However, most drugs are administered at regular intervals over a period. One of the reasons for this is to ensure that organs or pathogens are exposed to an optimal concentration of the drug over the period, to provide the desired effect. In TDM, an added concern is that plasma concentrations (C_p) should not exceed minimum toxic levels ($C_{min,tox}$).

Steady-state is a situation where the rate at which a drug enters the body is equal to the rate at which it is eliminated. The amount of time it takes to achieve steady state is determined by the elimination half-life of the drug. Approximately 50% steady state is achieved after one half-life. If a dose is administered every one half-life, then 75% steady state is achieved after two half-lives, 85.5% after three half-lives, and about 97% after five half-lives. Therefore, as a rule of thumb, steady state is assumed to be achieved after five half-lives. Figure 8.2 shows concentration-time curve for a drug at steady state after multiple oral doses.

8.5.2.1 $C_{av,SS}$ and C_{SS}

At steady state achieved through multiple dose administration (e.g., oral, intravenous bolus, or intravenous infusion), plasma concentrations rise to a peak ($C_{max,SS}$) after drug administration at the beginning of each dosing interval (τ) and drop to a trough level (C_{trough}) immediately before the next dose is administered. Therefore, a more useful measure of drug exposure is the average plasma concentration ($C_{av,SS}$). One rule to note is that the AUC for each dosing interval at steady state (AUC_τ) is equal to AUC_∞. Since AUC_τ is the total amount of drug in plasma during a dosing interval, we can find the average concentration per unit time according to Eq. 8.10:

$$C_{\mathrm{av,SS}} = \frac{\mathrm{AUC}_\infty}{\tau} \tag{8.10}$$

Example 8.7 In this example, auclast is the AUC_∞ calculated from the single acute dose PK profile. Start and end indicate when a steady-state dosing interval starts and ends:

```
> # Cav,ss
```

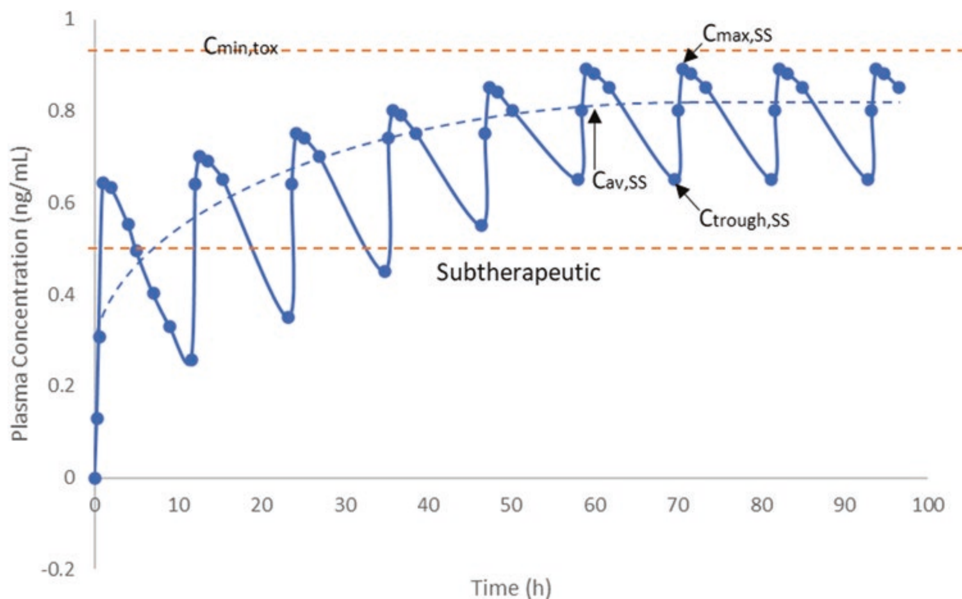

Fig. 8.2 Steady-state concentration-time graph for multiple oral doses of an unknown drug

```
> pk.calc.cav(auclast = 9.378798, start
= 23.2, end =34.8)
>
[1] 0.8085171
```

During *constant rate* infusion, the infusion rate (R_o) can be set based on the rate of clearance (CL), so at steady state, plasma concentration at any point in time would be constant (C_{SS}). Equation 8.11 illustrates this relationship.

$$C_{SS} = \frac{R_o}{CL} \tag{8.11}$$

8.5.2.2 C_{trough} and $C_{max,SS}$

The trough plasma concentration (C_{trough}) is the last concentration measured at steady state, immediately before the next dose administration. As its name implies, $C_{max,SS}$ is the maximum plasma concentration at steady state.

Example 8.8 In this example, conc and time are the variables for plasma concentrations and time, respectively. End is the time for a steady-state dosing interval. In this example 92.8 h is chosen.

```
> # Ctrough
>  pk.calc.ctrough(conc=pkssdata$CONC,
time = pkssdata$TIME, end = 92.8)
>
[1] 0.65
```

With the PK parameters described above, it is possible to calculate other measures such as concentration to dose ratio (C_{trough}/Dose) and metabolite to parent compound ratio [13].

8.6 Pharmacodynamics

Broadly, pharmacodynamics (PD) refers to the effects of a drug on the body. Specifically, it is the effect of drug concentration at the site of action. Since is it not always possible to determine drug concentration at the site of action, plasma concentrations are usually used as a proxy. Where endpoints are blood biomarkers, they can be measured from the same blood samples used to measure drug concentrations for PK analysis. Here, it is quite easy to correlate effects with drug concentrations. On the other hand, if pharmacological effects are assessed using other means,

e.g., cognitive tests, it is very important that these assessments are timed to coincide with blood draws for PK analysis.

8.6.1 PK/PD

PK/PD analysis involves exploring relationships between drug concentrations or PK parameters and PD measurements, or calculating indices based on PK and PD parameters [14]. For example, in TDM of antibiotics, the minimal inhibitory concentration (MIC) is a PD parameter representing the minimum amount of drug exposure that prevents visible microbial growth. PK/PD indices such as C_{max}/MIC and AUC/MIC give an idea of how effective a dose might be [15].

8.7 Descriptive and Inferential Statistics

Broadly, two types of analyses can be performed on group-level data or data from multiple study participants: descriptive (summary statistics) and inferential. Often, both are presented for group-level data.

Descriptive analyses provide an overview of what our data looks like, i.e., describing the data. For categorical data in clinical research frequencies and percentages for each category are usually. For continuous data, summary statistics include arithmetic mean, geometric mean, median, minimum-maximum range, standard deviation (SD), and inter-quartile range (IQR). Descriptive statistics also includes graphical presentation of the summaries mentioned above, for both categorical and continuous data. Sometimes, categorical data are stored as numbers in a dataset for simplicity and ease of analysis; however, this does not make them continuous data. For example, mortality variable may be stored as 1 for "alive" and 0 for "dead" in a database of 100 patients. However, a mean of 0.73 will be meaningless for this variable. Instead, 73% alive and 27% dead are more meaningful.

Inferential statistics provide a means to make inferences or predictions about the population, based on data from a sample taken from the population. Inferential statistics usually involves hypothesis testing and confidence intervals.

8.7.1 Statistical Hypothesis Testing

Hypothesis testing is a means of assessing whether results from one group are different from another group or a prespecified value. It involves stating a null hypothesis (H_o) and an alternative hypothesis (H_a). The H_o is generally considered the status quo, while H_a is the result we suspect and want to confirm or conclude. The H_a is usually the opposite of H_o. For example, if H_o is that the groups or values being compared are not different from each other, H_a will be that the groups or values being compared are different from each other. Similarly, if H_o is that X is smaller than or equal to Y, H_a will be that X is greater than Y.

There are two types of errors to be aware of in hypothesis testing – Type I and Type II errors.

8.7.1.1 Type I Error

Type I error or alpha (α) is the probability of having a false-positive result in a hypothesis test. Type I error is committed when the null hypothesis is rejected although it is in fact true. Type I error must be kept low to increase reliability of results. It is usually set at 5% or 0.05. The smaller the alpha, the larger the sample size.

8.7.1.2 Type II Error

Type II error or beta (β) is the probability of not rejecting the null hypothesis when the null hypothesis is false (false-negative).

Power is the probability of rejecting H_o correctly and is calculated as $1 - \beta$. Type II error must be kept low to increase reliability of results. It is usually set at 10% or 20%, i.e., power is usually 90% or 80%, respectively. The smaller the β, the larger the power and the larger the sample size.

8.7.1.3 Significance Testing and *p*-Value

Tests of significance are statistical methods used to assess the strength of evidence against the null

hypothesis. It enables us to determine if H_o should be rejected in favor of H_a or not.

Tests of significance generally produce a *p*-value, which is the probability that results as extreme as those from a sample could be obtained when the null hypothesis is true.

The *p*-value is usually compared to a prespecified significance level or alpha (α). If the *p*-value is smaller than or equal to α, H_o is rejected, in favor of H_a. If the *p*-value is larger than α, there is not enough evidence to reject H_o, and therefore we fail to reject it.

The *p*-value is influenced by sample size; therefore, for a given endpoint, if the sample size is large enough, it is possible to reject the null hypothesis. Therefore, rather than "accepting" the null hypothesis, it is better to say we "fail to reject the null hypothesis" or "the null hypothesis is supported."

The *p*-value is not an indicator of the magnitude of effect, but rather the strength of evidence.

8.7.2 Confidence Interval (CI)

A confidence interval is a range within which we are confident that the population measure falls. A level of confidence needs to be set, and this is usually $1 - \alpha$ expressed as a percent. Therefore, if α is 5%, our level of confidence will be $(1 - 0.05) \times 100 = 95\%$. This CI will be called a 95% CI.

The CI gives us an idea of how close results from a sample from the population are to the population. Therefore, CIs are a means of assessing if results from samples are generalizable to the population.

Methods for calculating CIs depend on the type of inferential statistics being conducted and are beyond the scope of this chapter. However, in analysis examples provided later in the chapter, they include how to interpret CIs.

8.8 Sample Size and Power

Sample size calculation enables us to determine how many participants need to be studied to reliably answer the research question. The most important endpoint is used to formulate the primary objective of the study, while the others can be grouped into secondary and/or exploratory objectives. Sample size calculations are always based on the primary objective.

To calculate sample size, significance level (alpha) and power need to be set.

The sample size for any clinical study is based on the type of data collected for the primary endpoint. For example, if the data is continuous and the null hypothesis involves a comparison of means, the sample size calculation will be based on the t-test. Using the power.t.test command in R, the sample size for a hypothetical study is 34 per arm as calculated below:

Example 8.9

```
> Sample size calculation for differ-
ence in means
> p_sd = q.pooled_sd(sds=c(2.41, 2.21),
+  ns=c(20, 20))
> power.t.test(n = NULL,
+ delta = 1.6,
+ sd = p_sd,
+ sig.level = 0.05,
+  power = 0.8,
+ type = "two.sample",
+ alternative = "two.sided")
>
>
Two-sample t test power calculation
 n = 33.76509
 delta = 1.6
 sd = 2.312
 sig.level = 0.05
 power = 0.8
 alternative = two.sided
NOTE: n is number in *each* group
```

where:

q.pooled_sd is a command from the qwickr package that calculates pooled standard deviations (SD).

sds contains the SDs of 2.41 and 2.21 for the two study groups (for calculating pooled SD p_sd).

ns contains the number of participants in each group 20 (for calculating pooled SD).

power.t.test is a command from the stats package that calculates sample size for comparison of means.

delta is the difference in mean (0.6).

sd is the pooled SD (p_sd).

n is the number of participants per group (equal to NULL because we want the power.t.test command to calculate it for us).

sig.level is the significance level or Type I error probability.

power is the power of test ($1 - \beta$).

type is the type of t test, here "two.sample" for comparison of two independent groups.

alternative is the alternative hypothesis, here "two.sided" for a two-sided comparison.

Sample size calculations for comparison of means can also be based on other statistical tests such as ANOVA or repeated measures. However, these are more advanced and will not be the focus of this chapter.

For a categorical endpoint where the expected proportion of events (e.g., bleeding adverse event) in group 1 is 50% and group 2 is 30%, the sample size required to detect such a difference in proportions at 80% power and 5% significance level is 93 per arm as calculated below:

Example 8.10

```
> Sample size calculation for differ-
ence in proportions
> power.prop.test(n = NULL, p1 = 0.5,
p2 = 0.3, power = 0.8, sig.level = 0.05)
>
Two-sample comparison of proportions
power calculation
 n = 92.99884
 p1 = 0.5
 p2 = 0.3
 sig.level = 0.05
 power = 0.8
 alternative = two.sided
NOTE: n is number in *each* group
```

8.9 Analysis Methods

Descriptive and inferential statistics can be applied to pharmacokinetic and pharmacodynamic parameters, as well as the other endpoint types listed at the beginning of this chapter. In the remaining sections of this chapter, a selection of analysis methods is presented, with simple examples using R.

8.9.1 Categorical Endpoints/ Comparison of Proportions

8.9.1.1 Chi-Square Test

The chi-square test is used to assess the relationship between two categorical variables. It tests the null hypothesis that there is no statistically significant relationship between the two categorical variables. The main assumption is that for each combination of exposure (or treatment) and outcome variables, there are at least five observations. This can be checked using the table command. The frequencies summarized by the command can be supplied to the chisq.test command to run the test.

Example 8.11 In the example below, we want to find out if there is a significant relationship between Group and Sex.

With a p-value of 0.201, we fail to reject the null hypothesis. Therefore, we conclude that based on our data, there is no significant relationship between study Group and Sex. This is expected in a randomized trial if randomization was done effectively.

```
> # Chi-square test
> ## Cross-tabulate the data
```

```
>   tab   <-   table(catdata$GROUPING, catdata$SEX)
> print(tab)
 Female Male
 A 11 9
 B 6 14
> # Run Chi-square test
> chisq.test(tab)

 Pearson's Chi-squared test with Yates' continuity correction


data: tab
X-squared = 1.6368, df = 1, p-value = 0.2008
```

8.9.1.2 Fisher's Exact Test

When the main assumption in the chi-square test fails to hold, i.e., there is at least one combination of exposure and outcome variables with less than five observations, Fisher's exact test is used.

Example 8.12 In this example, we test the null hypothesis that there is no association between treatment (grouping) and improvement in pain. A cross-tabulation of the two variables shows that one cell has two observations; therefore, chi-square test would not be appropriate. Instead, we conduct Fisher's exact test.

With a *p*-value of 0.235, we fail to reject the null hypothesis. Therefore, there is no association between treatment and improvement in pain.

```
> # Fisher's Exact test
> ## Cross-tabulate the data
>   pain   <-   table(catdata$GROUPING, catdata$PAIN_IMPROVED)
> print(pain)
>
 0 1
 A 2 18
 B 6 14
>
>
> ## Run Fisher's Exact test
> fisher.test(pain)
>
 Fisher's Exact Test for Count Data

data: pain
p-value = 0.2351
alternative hypothesis: true odds ratio is not equal to 1
95 percent confidence interval:
 0.02308176 1.80076397
sample estimates:
odds ratio
 0.2680041
```

8.9.1.3 McNemar Test

The McNemar test is used to test the consistency of an outcome when assessed at two different time points (e.g., before and after an intervention) or assessed using two different methods (e.g., COVID-19 using polymerase chain reaction (PCR) versus a rapid diagnostic test). The key requirement is that the same individual is assessed twice; therefore, the observations are paired. As a result, McNemar test is sometimes referred to as the paired chi-square test. Another requirement is that the outcomes are both binary.

Example 8.13 In this example, we use the repeated measures dataset rmdata from the qwickr package. We want to find out if disease state at visit 1 (before treatment) is the same as that at visit 4 (after treatment) for participants in group A.

With a *p*-value of 0.003, we reject a null hypothesis that disease states before and after treatment are the same.

```
> # McNemar Test
> ## Create a subset of rmdata containing visits 1 and 4 for participants in group A
> mndata <- rmdata[rmdata$VISITNUMBER %in% c(1,4) & rmdata$GROUPING == "A",]
>
>   ##   Create   separate   vectors   for DISEASE_STATE variable at visits 1 and 4
```

```
> before <- mndata$DISEASE_
STATE[mndata$VISITNUMBER == 1]
> after <- mndata$DISEASE_
STATE[mndata$VISITNUMBER == 4]
>
> ## Create cross-tabulation of before
and after data
> crosstab <- table(before, after)
> print(crosstab)
 after
before 0 1
 1 0
 1 11 3
>
> ## Run the McNemar test
> mcnemar.test(crosstab)

 McNemar's Chi-squared test with conti-
nuity correction


data: crosstab
McNemar's chi-squared = 9.0909, df = 1,
p-value = 0.002569
```

8.9.1.4 Logistic Regression

Simple logistic regression is used to assess the probability of occurrence of an outcome. The outcome/dependent variable must be binary, and the independent variable can be categorical or continuous. The null hypothesis is that the probability of a particular value of the binary outcome variable is not associated with the value of the independent variable.

Multiple logistic regression is an extension of simple logistic regression where there are two or more independent variables in the model. Ordinal logistic regression is an extension of simple logistic regression where the outcome/dependent variable has more than two levels and is ordered (ordinal).

Example 8.14 In this multiple logistic regression example, we test the null hypothesis that the probability of having pain improvement is associated with study treatment (grouping) and age. We use catdata from qwickr package. Logistic regression in R is done using a generalized linear model with a binomial distribution for the error term.

From the output below, the odds of having improvement in pain in group B was 1.5 times lower compared to group A. However, with a *p*-value of 0.104, we fail to reject the null hypothesis. Similarly, for each unit increase in age, the odds of having pain improvement reduces by 0.05. However, with a *p*-value of 0.379, we fail to reject the null hypothesis regarding age. This is confirmed by the 95% confidence intervals (CIs) for study group and age. Both CIs include an odds ratio of 1, suggesting that we are 95% confident that the true population odds of pain improvement could increase or decrease or remain the same with change in study group and age. In summary, the results from our sample are not strong enough to reject the null hypothesis.

```
> # Multiple logistic regression
> multiplelogistic <- glm(PAIN_IMPROVED
~ GROUPING + AGE,
+ data = catdata,
+ family = "binomial")
>
> ## Display the regression output
> summary(multiplelogistic)
>
Call:
glm(formula = PAIN_IMPROVED ~ GROUPING
+ AGE, family = "binomial",
 data = catdata)

Deviance Residuals:
 Min 1Q Median 3Q Max
-2.3075 0.3541 0.4578 0.7542 1.0194

Coefficients:
 Estimate Std. Error z value Pr(>|z|)
(Intercept) 5.00185 3.35975 1.489 0.137
GROUPINGB -1.52362 0.93767 -1.625 0.104
AGE -0.04551 0.05173 -0.880 0.379

(Dispersion parameter for binomial fam-
ily taken to be 1)
```

```
 Null deviance: 40.032 on 39 degrees of
freedom
Residual deviance: 36.673 on 37 degrees
of freedom
AIC: 42.673

Number of Fisher Scoring iterations: 5
> ## Generate 95% CI
> confint(multiplelogistic)
Waiting for profiling to be done...
 2.5 % 97.5 %
(Intercept) -1.3888665 12.18110393
GROUPINGB -3.6594346 0.17225183
AGE -0.1503426 0.05888971
```

8.9.2 Continuous Endpoints/ Comparison of Means

8.9.2.1 One-Sample T-Test

The one-sample t-test is used to test the null hypothesis that the mean for a normally distributed variable (interval or ratio) is not statistically significantly different from a prespecified value. One key assumption is that the data must be normally distributed. This can be checked using a histogram and/or the Shapiro-Wilk test [16]. The null hypothesis for the Shapiro-Wilk test is that the data is normally distributed.

Example 8.15 In the example below, the Shapiro-Wilk test gives a *p*-value of 0.719; therefore, we fail to reject the null hypothesis that the data is normally distributed. With this, we proceed to test the null hypothesis that the mean biomarker level is 57 units. From the output of the one-sample t-test, the mean is 79.7 units, 95% confidence interval (CI) is 75.6 units to 83.9 units, and *p*-value is <0.001. Therefore, we reject the null hypothesis. We conclude that the mean age of our sample is not equal to 60 units.

```
> # One-sample t-test
> ## Create a subset of rmdata contain-
ing visit 1 for participants in group A
> mndata <- rmdata[rmdata$VISITNUMBER
%in% c(1) & rmdata$GROUPING == "A",]
>
> ## Shapiro-Wilk test
> Shapiro.test(mndata$BIOMARKER)

 Shapiro-Wilk normality test


data: mndata$BIOMARKER
W = 0.96155, p-value = 0.7193

>
> ## t-test
> t.test(BIOMARKER ~ 1, mu = 56, data =
mndata)

 One Sample t-test


data: BIOMARKER
t = 12.375, df = 14, p-value =
6.295e-09
alternative hypothesis: true mean is
not equal to 56
95 percent confidence interval:
 75.62886 83.85931
sample estimates:
mean of x
 79.74408
```

8.9.2.2 One-Sample Wilcoxon Test

When the assumption of normality fails to hold, continuous data cannot be analyzed using the parametric one-sample t-test. Instead, the non-parametric one-sample median or Wilcoxon or Mann-Whitney test is used. The null hypothesis is that the sample median is equal to a specified number.

Example 8.16 In the example below, the Shapiro-Wilk test gives a *p*-value of 0.026; therefore, we reject the null hypothesis that the data is normally distributed. With this, we proceed to use the one-sample Wilcoxon test for the null hypothesis that the median age is equal to 64 years. With a *p*-value of <0.001, we reject this null hypothesis and conclude that the median age is not equal to 64 years.

```
> # One-sample Wilcoxon Test
> ## Shapiro-Wilk test
> Shapiro.test(catdata$AGE)

 Shapiro-Wilk normality test


data: catdata$AGE
W = 0.93607, p-value = 0.02554

> ## Wilcoxon test
> wilcox.test(AGE ~ 1, mu = 64, data =
catdata)

 Wilcoxon signed rank test with conti-
nuity correction


data: AGE
V = 146.5, p-value = 0.0004034
alternative hypothesis: true location
is not equal to 64
```

8.9.2.3 Independent T-Test

The independent (or two sample) t-test is used to compare (interval or ratio) data between two independent groups or samples of participants or observations. The null hypothesis is that there is no statistically significant difference in means between the two independent groups. In other words, the difference in means of the two groups is equal to zero (0).

This test is based on four assumptions as follows: (1) data are normally distributed; (2) variances (or standard deviations) of the two groups are "equal" or homogeneous; (3) data for the two groups are independent, i.e., no participant belongs to both groups; and (4) the two groups are randomly sampled.

The independent t-test is robust to violations of normality, especially when the groups have equal sizes and variances are homogeneous [17]. Furthermore, according to the central theorem, as sample size increases, the distribution of data approaches normality. By convention, when $n \geq 30$, normality can be assumed. However, for the purposes of learning, most of the examples in this chapter involve $n < 30$ so the test for normality will be done where necessary.

Homogeneity of variances can be checked using Levene's test, whose null hypothesis is that the variances are equal [18]. Therefore, a statistically significant test means that the variance of one group is different from the other. Welch's t-test does not assume homogeneity of variances, and this is conducted by default using the t.test command in R as illustrated below.

Example 8.17 In this example, we create a subset of rmdata at visit 4 for participants in groups A and B. We want to find out if their mean biomarker levels are significantly different. As confirmed by the Shapiro-Wilk test, the data are normally distributed; therefore, we proceed with a two-sample t-test.

Although the means of groups A and B are 75.65 units and 78.19 units, respectively, the null hypothesis is supported with a p-value of 0.366. This is confirmed by the 95% CI. We are 95% confident that the true population difference in means lies between −8.22 units and 3.14 units, which includes 0 units.

```
> # Independent t-test
> ## Create a subset of rmdata contain-
ing visits 4 for participants in groups
A and B
> itdata <- rmdata[rmdata$VISITNUMBER
== 4,]
> ## Test for normality
> Shapiro.test(itdata$BIOMARKER)

 Shapiro-Wilk normality test


data: itdata$BIOMARKER
W = 0.96112, p-value = 0.3308

>
> ## Run the t-test
> t.test(BIOMARKER ~ GROUPING, data =
itdata)

 Welch Two Sample t-test


data: BIOMARKER by GROUPING
```

```
t = -0.92031, df = 25.208, p-value =
0.3661
alternative hypothesis: true difference
in means between group A and group B is
not equal to 0
95 percent confidence interval:
 -8.218544 3.140587
sample estimates:
mean in group A mean in group B
 75.65129 78.19027
```

8.9.2.4 Wilcoxon Rank Sum (Mann-Whitney U) Test

When data from two independent samples are not normally distributed, the Wilcoxon Rank Sum test is a nonparametric alternative for analyzing the data. As such the data is ranked (ordinal) and the null hypothesis is that the medians of the two samples are equal.

Example 8.18 In this example, since age is not normally distributed, we conduct the Wilcoxon Rank Sum (Mann-Whitney U) test. The null hypothesis that the median age for the two groups is equal is supported, given a *p*-value of 0.533.

```
> # Wilcoxon Rank Sum (Mann-Whitney U)
Test ####
> ## Shapiro-Wilk test
> Shapiro.test(catdata$AGE)

 Shapiro-Wilk normality test


data: catdata$AGE
W = 0.93607, p-value = 0.02554

>
> # Wilcoxon test
> wilcox.test(AGE ~ GROUPING, data =
catdata)

 Wilcoxon rank sum test with continuity
correction


data: AGE by GROUPING
W = 223.5, p-value = 0.5331
alternative hypothesis: true location
shift is not equal to 0
```

8.9.2.5 Paired T-Test

The paired t-test is used when the two samples being compared are not independent. This often arises when repeated measurements are done for the same participant, e.g., blood pressure measured before treatment and after treatment. Here, participants serve as their own controls. The paired t-test assumes that the differences between paired observations are interval and normally distributed.

Example 8.19 In this example, since the biomarker is normally distributed (Shapiro-Wilk *p*-value = 0.223), we proceed with the paired t-test. With a *p*-value of 0.112, the null hypothesis that the mean difference in biomarker levels between visits 1 and 4 is equal to 0 is supported. This is confirmed by the 95% CI. We are 95% confident that the true population mean difference lies between −1.02 and 9.21 units, which includes 0 units.

```
> # Paired t-test ####
> ## Create a subset of the data for
visits 1 and 4 for group A
> mndata <- rmdata[rmdata$VISITNUMBER
%in% c(1,4) & rmdata$GROUPING == "A",]
>
> ## Shapiro-Wilk test
> Shapiro.test(mndata$BIOMARKER)

 Shapiro-Wilk normality test


data: mndata$BIOMARKER
W = 0.95451, p-value = 0.2229

>
> ## Run paired t-test
> t.test(BIOMARKER ~ VISITNUMBER, data
= mndata)

 Welch Two Sample t-test
```

```
data: BIOMARKER by VISITNUMBER
t = 1.641, df = 27.086, p-value = 0.1124
alternative hypothesis: true difference
in means between group 1 and group 4 is
not equal to 0
95 percent confidence interval:
 -1.02389 9.20948
sample estimates:
mean in group 1 mean in group 4
 79.74408 75.65129
```

8.9.2.6 Wilcoxon Signed Rank Test

The Wilcoxon Signed Rank test is used when the assumption of interval (or ratio) normally distributed differences in paired observations fails to hold. As such, the data is ranked (ordinal) and the null hypothesis is that the medians of the two samples are equal.

Example 8.20 In this example, since the gait length is not normally distributed (Shapiro-Wilk *p*-value = <0.001), we proceed with the Wilcoxon Signed Rank test to assess if the medians in this paired assessment (before/visit 1 versus after/visit 4) of gait length are equal.

The null hypothesis that median gait lengths before and after treatment are equal is rejected, given a *p*-value of <0.001.

```
> # Wilcoxon Signed Rank test ####
> ## create a subset of the data for
visits 1 and 4 for group A
> mndata <- rmdata[rmdata$VISITNUMBER
%in% c(1,4) & rmdata$GROUPING == "B",]
>
> ## Shapiro-Wilk test
> Shapiro.test(mndata$GAIT_LENGTH)

 Shapiro-Wilk normality test


data: mndata$GAIT_LENGTH
W = 0.81633, p-value = 0.000132

>
> ## Run paired t-test
> wilcox.test(GAIT_LENGTH ~ VISITNUMBER,
data = mndata, paired = T)

 Wilcoxon signed rank test with conti-
nuity correction


data: GAIT_LENGTH by VISITNUMBER
V = 0, p-value = 0.0007211
alternative hypothesis: true location
shift is not equal to 0
```

8.9.2.7 Linear Regression

Simple linear regression is used to test the relationship between a continuous predictor and a continuous outcome variable. It is useful in predicting what the value of one variable will be based on the value of the other variable. When the more than one predictor is used, it is called multiple regression. Here, the predictors could include categorical variables. In this case, each categorical variable is recoded to have numeric values such that each level of the categorical predictor is treated as if it was a binary variable by itself.

Linear regression is based on four assumptions. First, the relationship between the dependent and independent variable is linear. Figure 8.3 is an example showing a simple linear regression line and associated equation. Equation 8.12 is the general form of a multiple regression equation.

$$y_i = a + b_1 x_{1i} + b_2 x_{2i} + \ldots + b_z x_{zi} + e_i \quad (8.12)$$

where

y_i is the outcome/response for the ith participant.

a is the intercept or the value of y when all predictors have a value of 0.

x_i is the value for predictor x for the ith participant.

b is the coefficient for each respective predictor. It is the slope of the regression line and is interpreted as the magnitude of change in the response variable for each unit change in the respective continuous predictor or the difference between levels, for a binary predictor. This value is adjusted based on the effects of other predictors.

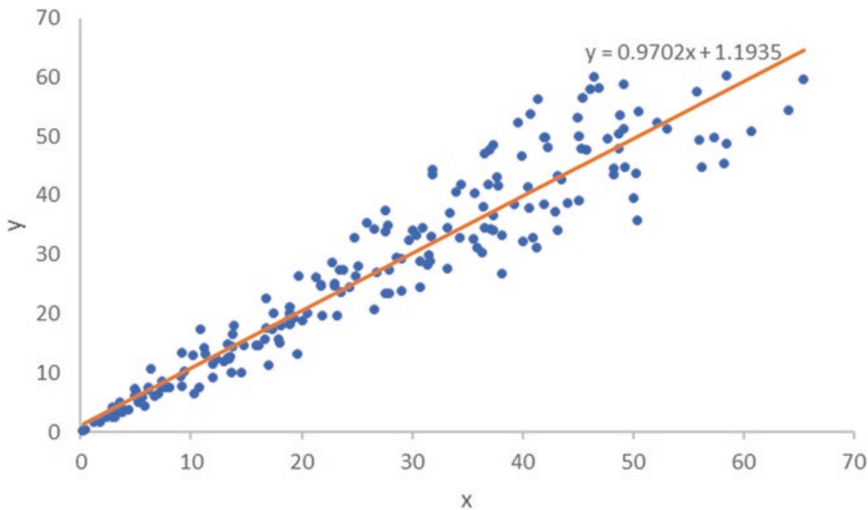

Fig. 8.3 Scatterplot with a regression line

e_i is the residual or error term, which represents other effects on y that are not accounted for by the predictors in the model.

The second assumption is that (residuals of) both outcome and continuous predictor variables are normally distributed. Based on the central theorem, if the sample size is about 30 or more, the residuals can be assumed to be normal. Third, observations are independent of each other. Lastly, variances of the residual are homogeneous for each value of the predictor. When sample sizes for groups being compared are approximately equal, homogeneity of variances is not of much concern.

Example 8.21 In this simple regression example, biomarker is not a statistically significant predictor of gait length at visit 1 ($p = 0.704$). The R-squared is a measure of the amount of variance in biomarker explained by gait length, and in this case, it is only about 0.5%. The adjusted R-squared excluded variability that is due to chance, and being negative, it suggests that the model does not fit the trend in the data.

```
> # Linear Regression ####
> ## create a subset of the data for
visit 1 for groups A and B
> lmdata <- rmdata[rmdata$VISITNUMBER
== 1,]
>
>
> ## Create a linear regression model
> mod <- lm(GAIT_LENGTH ~ BIOMARKER,
data = lmdata)
>
> ## Test of normality for residuals
> shapiro.test(resid(mod))

 Shapiro-Wilk normality test


data: resid(mod)
W = 0.97715, p-value = 0.7458

>
>
> ## Display linear regression output
> summary(mod)

Call:
lm(formula = GAIT_LENGTH ~ BIOMARKER,
data = lmdata)

Residuals:
 Min 1Q Median 3Q Max
-6.2170 -2.3407 -0.3289 2.6034 8.3873
```

```
Coefficients:
 Estimate Std. Error t value Pr(>|t|)
(Intercept)  71.05733  6.63726  10.706
2.09e-11 ***
BIOMARKER -0.03153 0.08216 -0.384 0.704
---
Signif. codes: 0 '***' 0.001 '**' 0.01
'*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 3.702 on 28
degrees of freedom
Multiple R-squared: 0.005233, Adjusted
R-squared: -0.03029
F-statistic: 0.1473 on 1 and 28 DF,
p-value: 0.704
```

Example 8.22 In this multiple regression example, for visit 1 (reference visit) gait length increases by 2 cm for group B compared to group A (reference group), keeping biomarker constant ($p = 0.006$). Furthermore, for group A (reference group), gait length increases by 9.12 cm at visit 4 compared to visit 1, keeping biomarker constant ($p < 0.001$). The regression model explains about 50% (adjusted to 48%) of the variance in gait length. An overall p-value of 1.142e-15 for the model indicates that the model is better than a model consisting of only the intercept.

```
> # Multiple Regression ####
> ## Create a linear regression model
> mmod <- lm(GAIT_LENGTH ~ GROUPING +
VISITNUMBER + BIOMARKER, data = rmdata)
>
> ## Test of normality for residuals
> shapiro.test(resid(mmod))

 Shapiro-Wilk normality test


data: resid(mmod)
W = 0.99413, p-value = 0.8996

>
> ## Display linear regression output
> summary(mmod)

Call:
lm(formula = GAIT_LENGTH ~ GROUPING +
VISITNUMBER + BIOMARKER,
 data = rmdata)

Residuals:
 Min 1Q Median 3Q Max
-9.982 -2.558 -0.072 2.663 10.235

Coefficients:
 Estimate Std. Error t value Pr(>|t|)
(Intercept) 67.718131 4.182362 16.191 <
2e-16 ***
GROUPINGB   2.035633   0.725162   2.807
0.00588 **
VISITNUMBER2  0.509900  1.027276  0.496
0.62060
VISITNUMBER3  1.410830  1.026279  1.375
0.17192
VISITNUMBER4  9.124217  1.040061  8.773
1.97e-14 ***
BIOMARKER  -0.002646  0.050931  -0.052
0.95866
---
Signif. codes: 0 '***' 0.001 '**' 0.01
'*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 3.97 on 114
degrees of freedom
Multiple R-squared: 0.4972, Adjusted
R-squared: 0.4752
F-statistic: 22.55 on 5 and 114 DF,
p-value: 1.142e-15
```

8.9.2.8 Analysis of Variance (ANOVA)

A one-way analysis of variance (ANOVA) is a special case of simple linear regression with an interval (or ratio) response variable and categorical predictor variable. It is called one-way because there is only one predictor.

When the categorical predictor has two levels, the result is identical to a two-sample independent t-test. When additional continuous predictors are added to the model, these predictors are called covariates, and the test is called an analysis of covariance (ANCOVA). When additional categorical predictors (factors) are added to an

ANOVA, it is called factorial ANOVA. Depending on the number of factors included in the model, it can also be called a two-way, three-way, etc. ANOVA or ANCOVA.

ANOVA and ANCOVA have similar assumptions as linear regression and are robust against minor departures from normality and unequal variances of residuals, especially when group sizes are equal and large.

ANCOVA is used to assess the effects of a variable while controlling for the covariates. When the grouping variable has three or more groups, the ANOVA/ANCOVA output does not give information about which pairs of groups are significantly different from each other. This information is obtained using post-hoc tests.

Example 8.23 In this two-way ANCOVA example, we want to compare means of gait length for a combination of factors (group and visit number), while controlling for biomarker. Therefore, group and visit number are included in the model as an interaction (denoted by * between them), while biomarker is controlled for (included in the model additively). We use the Anova command from the car package, because it is better suited for models with interactions.

Since Grouping*Visit Number is statistically significant, we interpret that result and ignore the main effects of GROUPING and VISITNUMBER. Where the interaction term is not significant, we interpret the main effects. The ANCOVA output tells us that there is a significant interaction between the effects of group and visit number on gait length while controlling for biomarker [F(3, 111) = 36.30, $p < 0.001$].

```
> # Analysis of variance ####
> ## Create a linear regression model
> mmod <- lm(GAIT_LENGTH ~ GROUPING *
VISITNUMBER + BIOMARKER, data = rmdata)
>
> ## Run ANOVA
> Anova(mmod)
Anova Table (Type II tests)
Response: GAIT_LENGTH
 Sum Sq Df F value Pr(>F)
GROUPING 124.22 1 15.2003 0.0001659 ***
VISITNUMBER 1605.00 3 65.4659 < 2.2e-16
***
BIOMARKER 11.89 1 1.4549 0.2303084
GROUPING:VISITNUMBER 889.97 3 36.3006 <
2.2e-16 ***
Residuals 907.12 111
---
Signif. codes: 0 '***' 0.001 '**' 0.01
'*' 0.05 '.' 0.1 ' ' 1
>
```

Given the significant interaction effects we have, we want to know (1) how gait length differs between groups for each visit, and (2) how gait length differs between visits for each group. We will answer these questions by performing post-hoc comparisons.

First, we generate the estimated marginal means from the model, then we run pairwise comparisons. Estimated marginal means are mean values for the response variable (gait length) for each level of categorical predictor (factor) or average continuous predictor (covariate). We use the emmeans package to generate the estimated marginal means.

1. How does gait length differ between groups for each visit?

Estimated marginal means for gait length are different between groups A and B for each visit. The largest difference of 7.43 is observed at visit 4 ($p < 0.001$). This can be confirmed by finding the difference in emmeans at visit 4. It is important to note the direction of these differences. At visits 1 through 3, gait length was smaller in group A compared to B. Gait length in group A increased gradually over the study period until it became larger than that for group B at visit 4, suggesting that the treatment provided for group A improves gait length.

```
> ## (1) Estimated marginal means by
visit number
> library(emmeans)
```

```
> EM <- emmeans(mmod, ~ GROUPING, by=c("VISITNUMBER"))
> EM
VISITNUMBER = 1:
 GROUPING emmean SE df lower.CL upper.CL
 A 65.7 0.739 111 64.3 67.2
 B 71.2 0.742 111 69.7 72.6

VISITNUMBER = 2:
 GROUPING emmean SE df lower.CL upper.CL
 A 66.2 0.745 111 64.7 67.7
 B 71.8 0.744 111 70.4 73.3

VISITNUMBER = 3:
 GROUPING emmean SE df lower.CL upper.CL
 A 67.6 0.738 111 66.2 69.1
 B 72.2 0.738 111 70.7 73.7

VISITNUMBER = 4:
 GROUPING emmean SE df lower.CL upper.CL
 A 81.5 0.748 111 80.0 82.9
 B 74.0 0.739 111 72.6 75.5

Confidence level used: 0.95
>
> ## Pairwise comparisons by visit number
> pairs(EM)
VISITNUMBER = 1:
 contrast estimate SE df t.ratio p.value
 A - B -5.43 1.04 111 -5.197 <.0001

VISITNUMBER = 2:
 contrast estimate SE df t.ratio p.value
 A - B -5.64 1.06 111 -5.312 <.0001

VISITNUMBER = 3:
 contrast estimate SE df t.ratio p.value
 A - B -4.58 1.04 111 -4.386 <.0001

VISITNUMBER = 4:
 contrast estimate SE df t.ratio p.value
 A - B 7.43 1.05 111 7.087 <.0001

> ### Another method of getting the same result
> ### contrast(EM, "pairwise", adjust = "Tukey" )
```

2. How does gait length differ between visits for each group?

   For group A, estimated marginal means for gait length are significantly different between visits 1 and 4, 2 and 4, and 3 and 4. This is consistent with findings from the first pairwise comparison. For group B, the means are only different between visits 1 and 4.

```
> ## (2) Estimated marginal means by grouping
> EM2 <- emmeans(mmod, ~ VISITNUMBER, by=c("GROUPING"))
> EM2
GROUPING = A:
 VISITNUMBER emmean SE df lower.CL upper.CL
 1 65.7 0.739 111 64.3 67.2
 2 66.2 0.745 111 64.7 67.7
 3 67.6 0.738 111 66.2 69.1
 4 81.5 0.748 111 80.0 82.9

GROUPING = B:
 VISITNUMBER emmean SE df lower.CL upper.CL
 1 71.2 0.742 111 69.7 72.6
 2 71.8 0.744 111 70.4 73.3
 3 72.2 0.738 111 70.7 73.7
 4 74.0 0.739 111 72.6 75.5

Confidence level used: 0.95
>
> ## Pairwise comparisons by grouping
> pairs(EM2)
GROUPING = A:
 contrast estimate SE df t.ratio p.value
 1 - 2 -0.469 1.05 111 -0.448 0.9698
 1 - 3 -1.882 1.04 111 -1.803 0.2775
 1 - 4 -15.718 1.06 111 -14.898 <.0001
 2 - 3 -1.413 1.05 111 -1.350 0.5334
 2 - 4 -15.249 1.07 111 -14.286 <.0001
```

```
 3 - 4 -13.836 1.05 111 -13.127 <.0001

GROUPING = B:
 contrast estimate SE df t.ratio p.
value
 1 - 2 -0.675 1.06 111 -0.638 0.9194
 1 - 3 -1.030 1.05 111 -0.985 0.7582
 1 - 4 -2.860 1.05 111 -2.726 0.0369
 2 - 3 -0.355 1.05 111 -0.339 0.9865
 2 - 4 -2.185 1.05 111 -2.089 0.1629
 3 - 4 -1.830 1.04 111 -1.751 0.3025

P value adjustment: tukey method for
comparing a family of 4 estimates
```

8.9.2.9 Kruskal-Wallis Test

A Kruskal-Wallis test is a nonparametric test used in place of ANOVA when the assumptions are grossly violated. The dependent variable is treated as ordinal just like in a Wilcoxon Rank Sum test, with the additional benefit of being able to handle categorical independent variables with two or more levels.

Example 8.24 In this example, we compare gait length between study groups. With a *p*-value <0.001, we reject the null hypothesis that there is no difference in gait lengths between study groups.

```
> # Kruskal-Wallis test ####
> ## create a subset of the data for
visit 4 for groups A and B
> kwdata <- rmdata[rmdata$VISITNUMBER
== 4,]
> ## Run KW test
> kruskal.test(GAIT_LENGTH ~ GROUPING,
data = kwdata)

 Kruskal-Wallis rank sum test


data: GAIT_LENGTH by GROUPING
Kruskal-Wallis chi-squared = 16.021, df
= 1, p-value = 6.264e-05
```

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9 Reducing Toxicity in Critically Ill Patients by Using Therapeutic Drug Monitoring

Zalak Panchal, Khushboo Faldu, and Jigna Shah

Abstract

Nowadays, the healthcare system encounters high morbidity rates which are related to severe infections in critical illnesses. However, by dose individualization, we can minimize the toxicity in critically ill patients. These days, the patient and organism complexity demand precision dosing which can be achieved through therapeutic drug monitoring (TDM) services. TDM has proven to be an effective approach for dose individualization. Conventionally, TDM of antifungal, antibiotics, antiviral, and antimicrobial classes of drugs essentially involve quantitative drug measurement in the plasma of the patients to reduce toxicity risks associated with agents of narrow therapeutic indices. But it has been observed that there is significant institutional variation related to TDM-specific criteria like the selection of patients, concentration monitoring, time of sampling, assay techniques, pharmacokinetic/pharmacodynamic (PK/PD) target selection, and dosage optimization procedures. The goal of this chapter is to examine available information on TDM practices for different drug classes and to illustrate how TDM might be useful in the reduction of toxicity in critically ill patients with serious infections.

Keywords

Therapeutic drug monitoring · Critically ill patients · Toxicity · Pharmacokinetics · Pharmacodynamics · Antimicrobial · Antibiotic · Antifungal · Antiviral · Anticoagulant

Abbreviations

5-FC	5-Fluorocytosine
ABCDE	Airway, breathing, circulation, disability, exposure
ADME	Absorption, distribution, metabolism, excretion
AIS	Abbreviated Injury Score
APACHE	Acute Physiology and Chronic Health Evaluation
ARC	Enhanced renal clearance
AUC	Area under the curve
BMD	Bone mineral density
CL	Drug clearance
CLSI	Clinical and Laboratory Standards Institute
CRRT	Continuous renal replacement therapy
DOACs	Direct oral anticoagulants

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EC50	Half maximal effective concentration
ECMO	Extracorporeal membrane oxygenation
ESBL-PE	Extended-spectrum ß-lactamase-producing *Enterobacteriaceae*
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FLC	Free light chains
HCV	Hepatitis C virus
HIT	Heparin-induced thrombocytopenia
HPLC-UV	High-performance liquid chromatography with ultraviolet detection
HSV	Herpes simplex virus
IATDMCT	International Association of Therapeutic Drug Monitoring and Clinical Toxicology
ICU	Intensive care unit
ISS	Injury Severity Score
IV	Intravenous
LC-MS	Liquid chromatography-mass spectrometry
LMWH	Low molecular weight heparin
MAP	Mean arterial pressure
MELD	Model for end-stage liver disease
MIC	Minimum inhibitory concentration
MRSA	Methicillin-resistant *Staphylococcus aureus*
NFB	Non-fermenter bacilli
PAE	Post-antibiotic effect
PD	Pharmacodynamic
PDDIs	Possible drug-drug interactions
PK	Pharmacokinetic
RRT	Renal replacement therapy
SAPS	Simplified Acute Physiology Score
SOFA	Sepsis-related organ failure assessment
TDM	Therapeutic drug monitoring
TISS	Therapeutic Intervention Scoring System
UFH	Unfractionated heparin
Vd	Volume of distribution
VRE	Vancomycin-resistant enterococci
VTE	Venous thromboembolism

9.1 Introduction

9.1.1 Critically Ill Patients

Critically ill patients are at-risk patients who can easily acquire infections as a result of multiresistant organism which could lead to significant morbidity or mortality [1–3]. These patients are generally victims of motor vehicle accidents, violence, burns, drowning, falls, or patients with cardiovascular and cerebrovascular comorbidities [3]. The main clinical indications that define a critical state in patients are as follows: hypotension, tachycardia, tachypnea, a decreased volume of urine output, and altered consciousness [4]. When these observations are analyzed collectively, their sensitivity and specificity for critical illness are considerably increased [4]. Therefore, the management of critically ill patients should be thoughtfully planned. Additionally, the evaluations of critically ill patients must be carried out by a qualified clinician using a standardized ABCDE (airway, breathing, circulation, disability, and exposure) format [2, 5]. The classification of patients into risk groups is based on their severity and identifying discrepancies between various units or medical centers [6]. In addition to the required clinical estimate, score systems are employed at various stages of in-hospital treatments for acutely or potentially critically ill patients [6, 7]. The scoring methods use morphological, physiological, and biochemical factors to quantify the severity of critically ill/injured patients and assign them to a risk group [6, 7]. The existing scoring system includes:

1. Organ-based scoring which is like therapeutic scoring. It is based on the observation that as the health of the patient deteriorates, the organs get affected and can malfunction or head into failure. One of the assessments is sepsis-related organ failure assessment (SOFA) [6, 7].
2. Simple scale: It is based on clinical judgment (e.g., survive or die) [7].

3. Anatomy-based scoring is based on the involvement of anatomical areas and is utilized mainly in trauma patients. Examples include Injury Severity Score (ISS) and Abbreviated Injury Scale (AIS) [7].
4. Physiological assessment is based on the degree of derangement of routinely measured physiological variables, e.g., Acute Physiology and Chronic Health Evaluation (APACHE) and Simplified Acute Physiology Score (SAPS) [6, 7].
5. Assessment based on the disease-like model for end-stage liver disease (MELD), Child-Pugh for assessment of liver failure, subarachnoid hemorrhage scoring by World Federation of Neurosurgical Societies, and Ranson's assessment for acute pancreatitis [7].
6. Therapeutically weighted scoring like the Therapeutic Intervention Scoring System (TISS) utilizes the assumption that critically ill individuals require multiple complex therapeutic interventions and procedures in comparison to comparatively healthy or recovering individuals [7].

9.1.2 Therapeutic Drug Monitoring

Therapeutic drug monitoring (TDM) is an effective approach for dose individualization [8]. In general, TDM deals with determining drug concentration in the bloodstream and adjusting doses within a targeted therapeutic window [8, 9]. The reliability of TDM is predominantly due to the specificity and sensitivity of the analytical methods [10]. Moreover, the International Association of Therapeutic Drug Monitoring and Clinical Toxicology (IATDMCT) defines TDM as "a multidisciplinary clinical specialty aimed at improving patient care by individually adjusting the dose of drugs for which clinical experience or clinical trials have shown improved outcomes in the general or special populations" [11]. TDM concedes the evaluation of drug efficacy and safety in a range of clinical contexts by a collective understanding of pharmaceutics, pharmacokinetics, and pharmacodynamics [12]. Thus, TDM helps to improve the clinical effects of the drug along with patient management.

TDM is therapeutically relevant and requires a good correlation between the plasma concentration of the drug and clinical efficacy [12]. For drug concentration measures to be effective in treating patients, they must be strongly related to the drug action, toxicity, or both [12]. This allows for the definition of an effective therapeutic window – the concentration range between the minimum effective concentration and the concentration at which noxious effects begin to appear – as well as dose titration to attain effective concentration within that window [10, 12]. Therapeutic drug monitoring engages in determining drug concentrations alongside interpreting the results clinically. This necessitates an understanding of pharmacokinetics, pharmacodynamics, sample time, drug history, and the clinical status of the patient [9]. TDM is essential because pharmacokinetics and pharmacodynamics (PK/PD) are highly variable due to increased patient and organism complexity (Fig. 9.1). A highly variable PK/PD is found in people with critical illness, obese, and older adults [12]. Precision dosing of drugs through TDM is becoming important for these patient populations to improve outcomes and minimize toxicities [10, 13]. Currently, TDM is being used on antibiotics, antifungals, antivirals, antimicrobials, anticoagulants, sedative-analgesic, vasopressor-inotropic agents, and neuromuscular blocking agents [13]. The goal of this chapter is to discuss how TDM can be utilized to reduce toxicity in critically ill patients and improve patient outcomes from severe infections.

9.2 Antimicrobial Agents

9.2.1 General Pharmacokinetics (PK)/Pharmacodynamics (PD) Targets

Pharmacological drug modeling utilizes the characteristics, especially pharmacokinetics (PK) and pharmacodynamics (PD) parameters of the drug. PK involves studying the action of the body on the drug which involves its absorption (A), distribution (D), metabolism (M), and excretion (E). PD involves the study of the effect of the drug on the body which involves its interaction with different

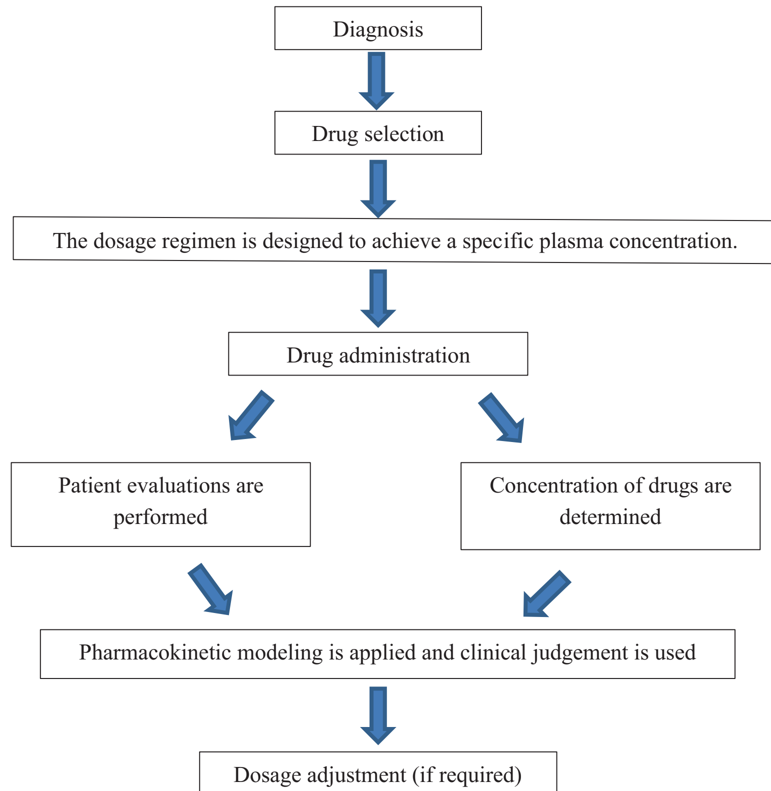

Fig. 9.1 Flowchart for reaching dosage decisions with therapeutic drug monitoring

receptors, enzymes, and signaling pathways to produce a desired effect in the body [14]. Many antimicrobials have shown benefits in terms of lower mortality, clinical efficacy, reduced half-life, and reduced toxicity. These are related to PK/PD target achievement, through modifications in dosing [15]. The concentration-time course of an antimicrobial agent is defined by PK parameters, based on the drug's ADME profile, adverse drug reactions, and protein binding. Furthermore, these parameters are strongly influenced by critical illness [16]. The absorption of the drug reflects on its bioavailability and is influenced by tissue or organ characteristics and physicochemical properties. In critical illness, problems regarding absorption often increase the need for intravenous administration, which ensures 100% bioavailability [17]. This chapter intends to discuss PK assessment in critically ill patients. The area under the curve (AUC), peak plasma concentration, and concentration before the next dosage(C_{min}) are some of the most important PK parameters which in combination with strategic dosing as per the drug's physicochemical properties are utilized for the determination of the concentration of drug in body fluids and tissues [18].

PD is the relationship between a drug's pharmacological influence and its PK which for antibiotics becomes minimum inhibitory concentration (MIC). The MIC of antibiotics is the concentration at which the drug effectively inhibits the growth of microorganisms or pathogens [16]. This interlinks PD; PK (blood concentration); the pharmacological activity of the drug, i.e., its inhibitory action on microorganisms or pathogens; and drug toxicity (Fig. 9.2) [15, 18, 19]. Theoretically, MIC should be directly proportional to PK for achieving the ideal PK/PD index [19].

Sepsis patients not admitted to ICU may experience altered exposure to medications [20]. The conventional antimicrobial dosages can have altered volume of distribution (Vd) and drug clearance (Cl) which may lead to subtherapeutic or toxic drug exposure [20]. At the very least, the human PK exposure target should be

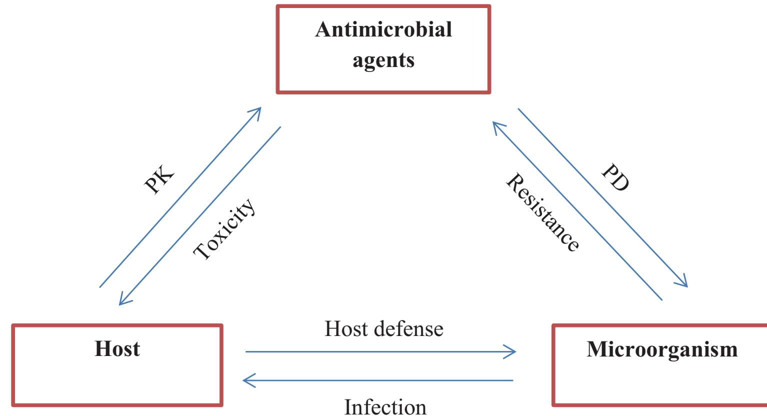

Fig. 9.2 Triangular relationship between antimicrobial drugs, host, and bacteria during treatment

determined using preclinical PK/PD relationships (e.g., fCmax/MIC, free maximal drug concentration to MIC ratio; fAUC/MIC, area under the free concentration-time curve to MIC ratio; fT > MIC, time the free concentration surpasses the MIC) [21].

Figure 9.3 lists frequent factors that can affect antimicrobial pharmacokinetics in critically ill patients. The Vd of hydrophilic antimicrobials like ß-lactams and aminoglycosides, which approximates the extracellular fluid volume, could be significantly increased by capillary leakage, fluid resuscitation, and third space losses [20]. Antimicrobials Cl are influenced by the patient organ function, drug clearance systems, and extracorporeal therapies. Antimicrobials Cl are reduced by renal hypoperfusion, acute kidney injury, and end-organ failure (Fig. 9.3). In critically ill patients, however, enhanced renal clearance (ARC) has been documented, in which accelerated antibiotic removal leads to subtherapeutic values [15, 22]. The effects of therapies like extracorporeal membrane oxygenation (ECMO) and renal replacement therapy (RRT) on antibiotic pharmacokinetics are multifaceted, varied, and complex, as previously addressed [15, 20]

All PK/PD attainments are expressed with respect to the pathogen's MIC, emphasizing that, in addition to antimicrobials concentration measurements, precise and timely MIC determination should be considered the base of antibiotic TDM. Disc method, E-test, microdilution broth method, EUCAST, adoption of local antibiograms and CLSI breakpoints, and automated microbiology system (e.g., Phoenix, Vitek 2) can all be used to define MICs for TDM [20, 22]. Clinicians, using TDM to treat serious infections, especially those involving resistant organisms, should be aware of each method's limitations.

The tissue distribution of hydrophilic antimicrobials is restricted to the extracellular space, with the majority of patients relying on renal clearance for survival [23]. However, lipophilic antimicrobials have an intracellular accumulation and depend on hepatic clearance [21]. As a result, hydrophilic medicines are more affected than lipophilic medications by critical illness PK changes, particularly in situations of sepsis, which leads to fluctuation in renal function and increase in Vd due to resuscitation of volume and leakage of capillaries which indicates whether or not hydrophilic antimicrobials loading and maintenance dosages in critically ill patients need to be adjusted [24]. The blood concentration of AB is also affected by dose, Vd, and bioavailability. Hydrophilic antimicrobials with an extracellular distribution have a low Vd, whereas lipophilic antimicrobials with a rapid cellular uptake have a high Vd [23]. Conventionally followed antimicrobial dosages will be inadequate to meet PK/PD parameters in ICU patients [20]. As a result, a tailored approach that takes into account specific MIC and regimens that are very likely to achieve PK/PD objectives can deliver viable answers [20, 24].

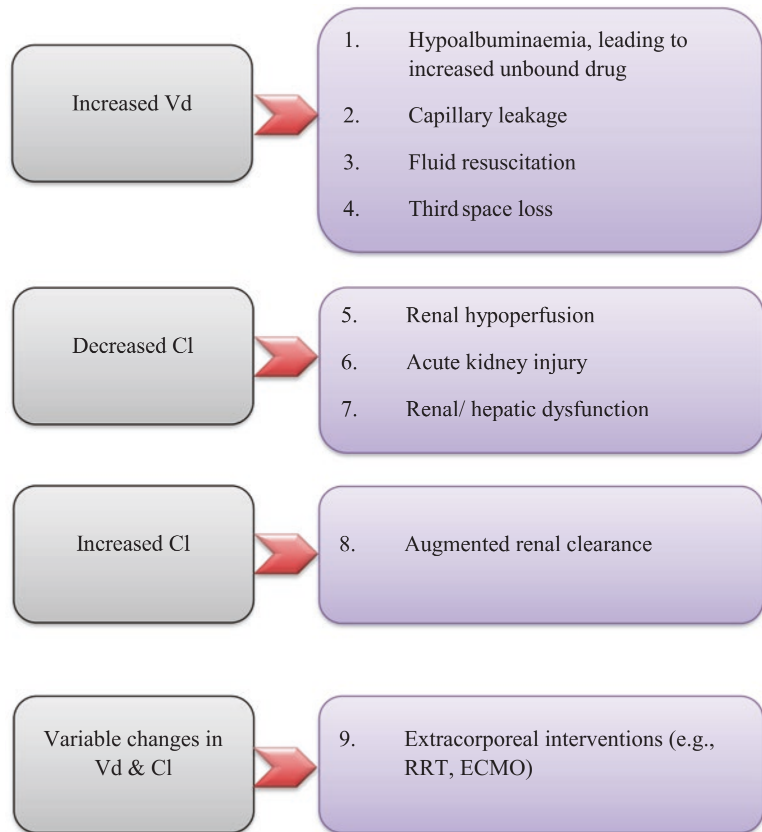

Fig. 9.3 Common factors affected with the pharmacokinetics of antimicrobials in critically ill patients

9.2.2 Antibacterial Agents/ Antibiotics

Antibiotics are one of the most regularly used medications in ICU patients. Critically ill and ICU patients who are at maximum risk of contracting community or hospital-acquired infections are administered antibiotics preemptively [16]. The most common bacterial infections which are acquired in the community or hospital settings are caused due to vancomycin-resistant enterococci (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA), extended-spectrum ß-lactamase-producing *Enterobacteriaceae* (ESBL-PE), MDR *Pseudomonas aeruginosa*, non-fermenter bacilli (NFB), and carbapenem-resistant *Acinetobacter baumannii* [25]. Statistics show that around 70% pool of critically ill patients in ICU are administered antibiotic treatment empirically or targeted. Antibiotic resistance in the local area must be closely monitored before empirical administration of antibiotics. The development of antibiotic-resistant infections in critically ill patients is the most serious adverse effect of the administration of antibacterial medications [25].

Antibiotics are divided into three PK/PD groups (concentration-dependent, time-dependent, and concentration-time dependent) based on their dose-response relationships [16, 24]. The efficacy of time-dependent antibiotics like ß-lactams is determined by the cumulative time percentage for which the concentration of the free antibiotics is more than MIC (percent fT > MIC) in the due course of 24 hours. Increased concentrations above MIC do not lead to an improvement in the killing rate [26]. The effect of concentration-dependent antibiotics, such as aminoglycosides, is determined by the ratio of peak concentration to MIC [17]. The spectrum and rate of bactericidal activity are directly pro-

portional to the concentration of antibiotics [18]. Concentration-time-dependent antibiotics like fluoroquinolones and glycopeptides utilize the ratio of AUC_{0-24} to MIC.

9.2.2.1 Aminoglycosides

PK/PD Targets

In clinical practice, it has become a standard practice for performing therapeutic drug monitoring of aminoglycosides like amikacin, gentamicin, and tobramycin to reduce drug toxicity while maintaining maximum efficacy [27]. The toxicity of aminoglycosides rendered it less popular for the treatment of critical illnesses in comparison to other available broad-spectrum antibiotics [28]. With the growth of multidrug-resistant (MDR) gram-negative infections, particularly hospital-acquired infections, there is a renewed interest in aminoglycoside therapy [28]. Characteristics of aminoglycosides include a high susceptibility rate for a variety of gram-negative bacteria commonly observed in critical illness [20]. Aminoglycosides have a post-antibiotic effect (PAE), in which even after the sinking of antibiotic concentration below MIC, the bacterial growth remains suppressed [29]. Aminoglycosides are small, hydrophilic compounds whose volume of distribution (Vd) is equivalent to the volume of extracellular fluid and their rate of clearance (Cl) is proportional to the glomerular filtration rate [30]. The studies have pointed out that fluctuations in Vd and Cl are observed in critically ill patients. Because of hypoalbuminemia, increased capillary permeability, or organ dysfunctions, PK (increased volume of distribution or changed drug clearance) of critically ill patients is severely affected [31]. Aminoglycosides exhibit concentration-dependent bactericidal actions, with maximum activity occurring at a peak concentration (C_{max}) of around eight to ten times the organism's minimum inhibitory concentration (MIC) ratio (C_{max} is $\geq 8-10 \times$ MIC) [32].

Current research study suggests ratio of AUC vs time curve during 24 hours period to MIC(AUC_{0-24}/MIC ratio) is potent indicator of response and can be effectively utilized to achieve target accomplishment in aminoglycoside extended interval dosing [29]. Ototoxicity and nephrotoxicity have been linked to the elevated C_{min} and continuous AUC exposures spanning across days [33].

Dose Adjustment Strategies

The commonly used PK/PD targets for aminoglycoside TDM are fT > MIC, AUC/MIC, and C_{max}/MIC; no other targets have been set specifically for the critical illness [34, 35]. As TDM data from the individual patient is provided, the software can check the expected parameters in an individual patient and hence provide precise dose recommendations that can achieve therapeutic PK/PD targets [30]. Bayesian dose adaptation software is the best predictor of aminoglycoside dosing requirements [27, 36]. Although no clinical benefits of software-based dosing methods have been demonstrated, they should be considered only in critically ill patients with severe infections. TDM by Bayesian dose adaptation resulted in decreased hospitalization duration and reduced instances of nephrotoxicity in patients receiving gentamicin [36, 37].

Bioanalytical Assay

Assay of aminoglycoside TDM is well established, with little, if any, disagreement in the scholarly literature [27]. For aminoglycosides, current immunoassay approaches like chromatographic assays are still very effective for amikacin, gentamicin, and tobramycin TDM [29, 30]. For regular clinical practice, validated, low-cost, and easy-to-use immunoassays are preferred in comparison to chromatography or capillary zone electrophoresis [20].

9.2.2.2 ß-Lactams

PK/PD Targets

Critically ill patients are frequently administered ß-lactam antibiotics for treatment of severe infections. ß-lactam antibiotics are generally hydrophilic, have a low volume of distribution (Vd), and are mostly eliminated from kidneys [38]. Protein binding of ß-lactams varies from moderate (30–70%) to low (30%) [39]. Early stages of infections witness significant alteration in Vd and Cl which can lead to altered PK and inadequate

concentrations of ß-lactam antibiotics [38]. Hypoalbuminemia leads to an increase in non-protein-bound drug concentrations of ß-lactams like ceftriaxone, semisynthetic penicillin (flucloxacillin, temocillin, and oxacillin), and ertapenem which are highly protein-bound, while it has been observed that toward the end of the dosing interval, they are found in lower concentrations in unbound form [38]. Piperacillin-tazobactam and meropenem are both ß-lactams, and as such, they are categorized as time-dependent antibiotics in terms of pharmacodynamics [40]. When the antibiotic's "free" or unbound plasma concentrations are above the minimum inhibitory concentration (MIC) during the dosing period (fT > MIC), these medicines have the greatest efficacy [40]. The % fT > (40–70%) MIC is the PK/PD measure linked with excellent ß-lactam action [41]. Despite the fact that myelosuppression is required for ß-lactam toxicity, no toxicity criteria have been established [29].

Dose Adjustment Strategies

In most TDM units, generalized but nonspecific dose modification strategies such as adjusting dose quantity or frequency, as well as the use of prolonged or continuous infusion, have been used regularly [39, 42]. However, monitoring concentrations for a clinician-selected target appears to be safe and more appropriate. If continual infusions are prescribed, a target of 100% fT > MIC might be a good choice [27]. C_{min} samples are commonly used at a steady state; however, dosing software could allow for much earlier sampling and dose optimization [20, 43]. Although Bayesian adaptive feedback software is the ideal technique for adjusting doses of ß-lactams, its availability at clinical sites may be limited [30].

Bioanalytical Assay

Liquid chromatography is a widely used assay method for ß-lactam TDM [20]. Several liquid chromatography-mass spectrometry (LC-MS/MS) and high-performance liquid chromatography with ultraviolet detection (HPLC-UV) assays have also been employed for therapeutic drug monitoring of ß-lactams [20, 27, 29].

9.2.2.3 Fluoroquinolones

PK/PD Targets

Fluoroquinolones are moderately lipophilic, having Vd affected by critical sickness, with excretion of one of the fluoroquinolones like levofloxacin [44]. The majority of fluoroquinolones bind to proteins in a moderate to low amount, and some are excreted by kidneys. The bactericidal efficacy of fluoroquinolones is concentration-dependent and is best predicted by the ratio of AUC_{0-24} to MIC. For optimal bactericidal pursuit, greater C_{max}/MIC ratios are necessary [45]. Gram-positive species with the AUC_{0-24}/MIC ratios of 25–30 also show efficacy, but gram-negative microorganisms require higher values [39, 46]. A ratio of >100–200 has also been indicated to limit the formation of resistance against gram-negative microbes in several investigations [47]. Despite an increase in reports of fluoroquinolone-related seizures, the criteria for the toxicity have not been established, and still causality is being contested [44, 46].

Dose Adjustment Strategies

In critical patients, a dosage plan for quinolones should be devised such that AUC0–24/MIC is higher than LD with higher maintenance doses [48]. TDM may prove beneficial in patients with quinolone resistance or to address inter-individual variability in PK. It will be useful in case bacteria exhibit MICs near the susceptibility breakpoint [16, 49].

Bioanalytical Assay

Literature suggests the use of sensitive techniques like LC-MS/MS and HPLC for fluoroquinolones TDM [29].

9.2.2.4 Vancomycin

PK/PD Targets

Vancomycin is the first-line therapy for methicillin-resistant *Staphylococcus aureus* (MRSA), and it is advised for therapeutic drug monitoring (TDM) to avoid nephrotoxicity and achieve optimal therapeutic outcomes [50]. While scientific community still debates the

efficacy of TDM in reducing toxicity and increasing the clinical effectiveness of vancomycin. The evidence for a link between high serum vancomycin concentrations and nephrotoxicity is mixed [51]. All plasma concentrations were assigned to one of three categories: therapeutic, subtherapeutic, or supratherapeutic. For common infections, a therapeutic vancomycin level was identified as 20–25 mg/L in adults, 25–35 mg/L in severe infections, and 10–15 mg/L in children and newborns [52]. Both CRRT and non-CRRT patients were included [53]. Vancomycin is hydrophilic in nature, has a lower Vd, and is mostly eliminated by the kidneys. The Vd and Cl of Vancomycin are altered by critical illness, resulting in fluctuating and low drug exposure [29, 54]. Based on PD of vancomycin, for *S. aureus* with MIC less than or equal to 2 mg/L, the ratio of AUC/MIC should be greater than or equal to 400 [51, 54].

Dose Adjustment Strategies

Dose adjustments can be accomplished by adjusting the dose according to the ratio between the measured and target concentrations. The target concentrations for intermittent (15–20 mg/L) and continuous (20–25 mg/L) administration are not the same, requiring a higher continuous infusion target to reach the same AUC as intermittent dosing [20]. When treating microorganisms with MICs >1 mg/L, safely achieving optimum AUC_{0-24}/MIC ratios is extremely difficult [55]. Dose individualization methods based on the calculation of individual pharmacokinetic parameters and PK/PD targets (AUC/MIC) are available, but they are not widely used in clinical practice [53, 55]. For dose adaptation, real-time Bayesian forecasting software combined with TDM is regarded to be the most accurate [52]. Despite the fact that vancomycin's current AUC_{0-24}/MIC attainments have consistently been around 400, the index came into existence from BMD MIC, and its E-test resemblance is 226 which is low [29].

Bioanalytical Assay

Vancomycin TDM immunoassays are widely available [29]. It is a homogeneous enzyme assay technique for quantitative analysis.

9.2.2.5 Linezolid

PK/PD Targets

Linezolid pharmacokinetic variability was formerly thought to be less significant than that of other antibiotics; therefore, dose modifications were avoided in patients with dysfunctional kidneys and liver [56]. The most common serious linezolid ADR is thrombocytopenia, which has been linked to linezolid plasma concentrations in numerous investigations [57]. Linezolid is a lipophilic molecule. Its Vd is equivalent to the body's total water, and its elimination is done by nonrenal clearance. Varied patient exposure to linezolid is due to the factors like intra- and inter-patient variability in PK [58]. Renal function serves as a substantial source of inter-patient variability in linezolid clearance because 30–40% of the linezolid dose is removed unaltered in the urine (Cl) [59]. Antibiotic efficacy of linezolid can be well characterized by the ratio of area under the curve of plasma concentration vs time graph to MIC (AUC_{0-24}/MIC) and the time period for which the antibiotic plasma concentration is higher than the minimum inhibitory concentration (MIC) for the given pathogen (T > MIC) [60]. To exert optimal antimicrobial activity, a T > MIC of 85 and an AUC_{0-24}/MIC >100 are necessary and are related to better clinical outcomes in seriously ill patients [60].

Dose Adjustment Strategies

Higher linezolid dosages and/or other administration procedures (e.g., continuous infusion and front-loaded dosing regimen) may assist critically ill patients. If TDM is available, these approaches should be backed up with it [61]. Obese patients, patients with acute respiratory distress syndrome and ARC, and patients infected with bacteria with MICs of less than 2 mg/L are

all subsets of patients who should be administered higher doses of linezolid [29, 62]. Table 9.1 summarizes the PK/PD index, efficacy, and toxicity threshold of various antibacterial drugs.

Bioanalytical Assay

Literature suggests the use of immunoassays, HPLC-UV, and LC-MS/MS but is not utilized in routine clinical TDM practice [27, 29, 63].

9.2.3 Antifungal Agents

The antifungal agents have a well-defined dose-response relationship for TDM essentially with respect to a narrow therapeutic index and substantial pharmacokinetic variability to impact clearance. Antifungals currently recommended for therapeutic drug monitoring include flucytosine, fluconazole, itraconazole, and voriconazole.

Table 9.1 PK/PD index, efficacy, and toxicity threshold of antibacterial drugs

Antibacterial class	PK/PD index	Clinical PK/PD threshold for efficacy	Clinical PK/PD threshold for toxicity
Aminoglycosides			
Gentamicin	AUC_{0-24}/MIC	AUC_{0-24}/MIC greater than or equal to 110 C_{max}/MIC greater than or equal to 8–10	C_{min} greater than 1 mg/L[a]
Amikacin	AUC_{0-24}/MIC	C_{max}/MIC greater than or equal to 8–10	C_{min} greater than 5 mg/L[a]
ß –lactams			
Penicillins	% fT greater than MIC	50–100% fT greater than MIC	C_{min} greater than 361 mg/L[d]
Carbapenems	% fT greater than MIC	50–100% fT greater than MIC	C_{min} greater than 44.5 mg/L[b]
Cephalosporins	% fT greater than MIC	45–100% fT greater than MIC	C_{min} greater than 20 mg/L[c]
Daptomycin	AUC_{0-24}/MIC	AUC_{0-24}/MIC greater than or equal to 666 mg/L	C_{min} greater than 24 mg/L[e]
Fluoroquinolones	AUC_{0-24}/MIC	AUC_{0-24}/MIC greater than or equal to 125–250 C_{max}/MIC greater than or equal to 12	Ambiguous
Glycopeptides			
Linezolid	AUC_{0-24}/MIC	AUC_{0-24}/MIC: 80–120 greater than or equal to 85% fT greater than MIC	AUC_{0-24} greater than 700 mg h/L[f] C_{min} greater than 20 mg/L[f]
Vancomycin	AUC_{0-24}/MIC	AUC_{0-24}/MIC greater than or equal to 400 C_{min} greater than 10–20 mg/L	AUC_{0-24} greater than 300[g] C_{min} greater than 7[g]
Teicoplanin	AUC_{0-24}/MIC	C_{min} greater than or equal to 10 mg/L	Ambiguous
Polymyxins			
Polymyxin B	AUC_{0-24}/MIC	Data unavailable	AUC_{0-24} greater than 100[f]
Colistin	AUC_{0-24}/MIC	Data unavailable	C_{min} greater than 2.4 mg/L[f]

AUC0–24/MIC the ratio of the area under the concentration-time curve during a 24-hour period to minimum inhibitory concentration, C_{max}/MIC the ratio of maximum drug concentration to minimum inhibitory concentration, C_{min} trough/minimum drug concentration, *fAUC0–24/MIC* the free (unbound drug concentration) ratio of the area under the concentration-time curve during a 24-hour period to minimum inhibitory concentration, *fT > MIC* the duration of time that the free drug concentration remains above the MIC during a dosing interval, *PK/PD* pharmacokinetic/pharmacodynamics

[a]Nephrotoxicity or ototoxicity

[b]Data only available for meropenem and related to nephrotoxicity or neurotoxicity

[c]Data only available for cefepime and related to neurotoxicity

[d]Data mostly on piperacillin and related to nephrotoxicity or neurotoxicity

[e]Myopathy indicated by creatine phosphokinase elevation

[f]Related to nephrotoxicity

[g]Related to hematological toxicity

9.2.3.1 Fluconazole

PK/PD Targets

Fluconazole drugs are available in oral and parenteral formulations, have a linear PK profile, and are highly absorbed from the gastrointestinal tract [64, 65]. It is somewhat lipophilic with Vd around 1 L/kg and is mostly eliminated by the kidneys. Substantial inter-individual PK variableness was observed [66]. The relationship between drug dose, exposure, in vitro susceptibility, and response to therapy has been demonstrated in preclinical and clinical research, implying that dosing may be crucial for outcomes in infections caused by bacteria with lower susceptibility [67, 68]. Values of 55.2–100 of the ratio of AUC_{0-24}/MIC have been defined as having a maximum therapeutic effect in patients with candidemia [69]. Since fluconazole AUC can be substituted by its dose, it has been utilized to calculate the dose/MIC ratio for evaluating its clinical outcomes [70]. Hepatotoxicity and convulsions are possible side effects of higher doses [71].

Dose Adjustment Strategies

This target was associated with a C_{min} of approximately 10e15 mg/L [69, 71]. TDM found that an FLC C_{min} > 11 mg/L was substantially linked with clinical outcomes in adult liver transplant recipients taking FLC for invasive candidiasis [65]. In critically ill patients with normal renal function, an intravenous low dose of 12 mg/kg followed by an adjusted intravenous dose of 6–12 mg/kg is recommended to achieve PK/PD outcomes – low (AUC_{0-24}/MIC ratio of 25) or high (AUC_{0-24}/MIC ratio of 100) [65, 66].

Bioanalytical Assay

For fluconazole TDM, several chromatographic tests have been documented [29, 64].

9.2.3.2 Flucytosine

PK/PD Targets

One of the first antifungal chemicals produced was flucytosine (5-FC), a pyrimidine derivative [27]. The medicine is effective against *Candida* species as well as *Cryptococcus neoformans*. Because of the rapid selection of resistant isolates when used as monotherapy, clinical usage of 5-FC is mostly limited to combination therapy with another antifungal like amphotericin B, for the treatment of cryptococcal meningitis [72]. Flucytosine has a Vd of 0.6–0.9 L/kg and is mostly eliminated by the kidneys [73]. There is a close link between 5-FC serum concentrations and both toxicity and efficacy, according to pharmacodynamic investigations [74]. The toxicodynamic link for two relatively common adverse effects, bone marrow suppression and hepatic dysfunction, has been the strongest of these correlations [47]. Variable flucytosine concentrations have been found due to significant inter-patient PK variability [75, 76]. In multiple clinical studies, a C_{max} of moreover 100 mg/L is associated with hepatotoxicity and myelosuppression. Resistant *C. albicans* mutants may be selectively amplified at concentrations of less than 25 mg/L [76]. Table 9.2 summarizes the PK/PD attainments and magnitudes associated with antifungal agents.

Dose Adjustment Strategies

There is inadequate clinical evidence to recommend changing flucytosine dose in critically ill patients for optimal outcomes. Dosing should be based on weight and suited to renal function [67]. In comparison to toxicity prevention, TDM commanded dosing to optimize flucytosine efficacy remains little documented. To reduce the toxicity and resistance of flucytosine, TDM can be utilized to achieve C_{max} = 100 mg/L and C_{min} = 25 mg/L [73, 76].

Bioanalytical Assay

For flucytosine TDM, several chromatographic assays have been documented [27, 67].

9.2.4 Antiviral Agents

Antiviral TDM is useful not only to minimize potential toxicities but also to increase the effectiveness of treatment. Acyclovir, ribavirin, fos-

Table 9.2 PK/PD attainments and magnitudes associated with antifungals

Antifungal drugs	PK/PD index	Clinical PK/PD efficacy threshold	Clinical PK/PD toxicity threshold
Itraconazole	AUC_{0-24}/MIC	C_{min} greater than or equal to 0.25–0.5 mg/L (Prop) C_{min} greater than or equal to 1 mg/L (Tx)	C_{ave} greater than or equal to 17.1 mg/L[d]
Fluconazole	AUC_{0-24}/MIC	AUC_{0-24}/MIC greater than or equal to 55–100	Ambiguous
Flucytosine	% fT > MIC	Data unavailable	C_{max} greater than 100 mg/L[b]
Posaconazole	AUC_{0-24}/MIC	C_{min} greater than 0.5 (Prop) C_{min} greater than 1 mg/L (Tx)	Data unavailable
Voriconazole	AUC_{0-24}/MIC	C_{min} greater than or equal to 1–2 mg/L	C_{min} greater than or equal to 4.5–6 mg/L[c]
Echinocandins	AUC_{0-24}/MIC	AUC_{0-24}/MIC greater than 3000[a]	Data unavailable
Isavuconazole	AUC_{0-24}/MIC	Data unavailable	Data unavailable

AUC0–24/MIC the ratio of the area under the concentration-time curve during a 24-hour period to minimum inhibitory concentration, C_{ave} average drug concentration, C_{min} = trough/minimum drug concentration, *fAUC0–24/MIC* the free (unbound drug concentration) ratio of the area under the concentration-time curve during a 24-hour period to minimum inhibitory concentration, *fT > MIC* the duration of time that the free drug concentration remains above the MIC during a dosing interval, *PK/PD* pharmacokinetic/pharmacodynamics, *prop* prophylaxis, *Tx* treatment

[a]In patients receiving micafungin for invasive candidiasis/candidemia

[b]Related to hematological toxicity and hepatotoxicity

[c]Mostly related to gastrointestinal toxicity

[d]Mostly related to hepatotoxicity and neurotoxicity

carnet, and ganciclovir are few examples of antivirals that essentially involve TDM.

9.2.4.1 Acyclovir/Valacyclovir

PK/PD Targets

Acyclovir and valacyclovir are moderately lipophilic entities with high Vd and are excreted by kidneys [77]. There is a scarcity of information relating acyclovir exposure to clinical efficacy and harm [78]. The efficacy of these drugs in the treatment of the herpes simplex virus (HSV) is connected to AUC and the time duration for which it is available above 50% inhibitory concentration (EC50; T > EC50). But these indices need to be investigated further [79]. Higher doses have been associated with an increased risk of gastrointestinal and neurological side effects, especially in patients with renal impairment [27, 79].

Dose Adjustment Strategies

In immunocompetent individuals with severe viral infections, a typical intravenous dose of 10–15 mg/kg every 8 hours is suggested [80]. Routine TDM in critical patients for acyclovir is neither favored nor opposed by the panel. A few reports on acyclovir TDM are published, and the majority of these feature individuals with encephalitis. If TDM is used, a C_{min} of 2–4 mg/L is advised [79].

Bioanalytical Assay

Multiple techniques like LC-MS/MS and HPLC-UV have been research for TDM of acyclovir [27, 81].

9.2.4.2 Ribavirin

PK/PD Targets

Ribavirin has a high Vd (about 18 L/kg) and is mostly eliminated by the kidneys [82]. This drug has been connected to a variety of PK differences between individuals, including bioavailability. Literature on the relationship between drug exposure, efficacy, and toxicity of ribavirin is conflicting [83]. A conflicting correlation was also found between the relationship of C_{min} and AUC to hepatitis C virus (HCV) eradication or long-term virological response [84]. A strong predictor of sustained virological response is the

area under the curve [AUC_{0-4} (1.76 mg h/L) and AUC_{0-12} (3.01 mg h/L)] after initial administration. Although a C_{min} of >2.3–3.5 has been connected to hemolytic anemia, some investigations have found no significant association [27, 85]. Other studies have found that other parameters (such as pegIFN-alpha-2a levels) may have a higher impact on the outcome than ribavirin levels [85]. Table 9.3 summarizes the PK/PD attainments and magnitudes associated with antiviral agents.

Dose Adjustment Strategies

Hepatitis C is not regarded as a pathogenic entity, and thus ribavirin TDM is not regarded as completely essential [83]. Additionally, data linking exposure to ribavirin to its efficacy and toxicity is contradictory [85]. While in chronic hepatitis C genotype 1, TDM dosing of ribavirin has shown better virological response in comparison to weight-based administration, and the resulting anemia in the TDM group although of severe grade could be adequately treated with erythropoietin beta [83]. TDM has recently been proposed as a technique for guiding ribavirin treatment in paramyxovirus-infected lung transplant patients [86].

Bioanalytical Assay

For ribavirin TDM, the literature suggests the use of LC-MS/MS and HPLC-UV techniques [27, 86].

9.3 Anticoagulant Agents

Prophylactic anticoagulation is required in high-risk patients because critically sick patients have a higher risk of venous thromboembolism (VTE) [87, 88]. VTE may also result in statistically significant increases in mortality and morbidity due to their reduced cardiopulmonary function. The most common symptom of VTE in ICU patients is deep vein thrombosis (DVT), which can lead to pulmonary embolism (PE). The rate of DVT in ICU patients varies, ranging from 30% in normal medical-surgical ICU patients to 70% in ICU patients with acute ischemic stroke [87]. ICU patients' clinical conditions, such as intubation, mechanical ventilation, sedation, delirium, and altered mental status, may mask the common presentations of VTE, resulting in a silent DVT in many ICU patients, highlighting the importance of prophylactic anticoagulant administration in complete bed rest patients. However, many of these people are in danger of serious bleeding if they are given full-strength anticoagulants [89]. Unfractionated heparin (UFH) and low molecular weight heparin (LMWH) are the most often used anticoagulants in the ICU. The most serious problem with UFH and, to a lesser extent, LMWH administration in critically ill patients is the development of heparin-induced thrombocytopenia (HIT), which can be caused by immunological or non-immune mechanisms and can result in thrombosis. In this case, calculating the "4Ts" score could aid in HIT diagnosis in ICU patients [89, 90]. The 4T score is determined by assigning points to each of the following parameters: thrombocytopenia, platelet count fall timing, thrombosis or other sequelae, and other causes of thrombocytopenia [90]. According to a recent study, the clinical and economic repercussions of VTE prophylaxis with UFH and LMWH are similar, with no statistically significant differences in critically ill patients. DOACs, or direct oral anticoagulants, are new anticoagulants that have been used to prevent VTE in critically ill patients. The simplicity of administration by oral route, shorter half-lives ($t_{1/2}$), and lack of HIT adverse effects are the major benefits of DOACs [91]. Partial hepatic metabolism and renal elimination are the most significant drawbacks, both of which can lead to possible drug-drug interactions (PDDIs) [91]. Clinical trials demonstrated that rivaroxaban reduced the incidence of VTE and the risk of significant bleeding when compared to LMWH, whereas apixaban was not superior to LMWH and was associated with increased bleeding risks [92].

Table 9.3 PK/PD attainments and magnitudes associated with antivirals

Antiviral drugs	PK/PD index	Clinical PK/PD efficacy threshold	Clinical PK/PD toxicity threshold
Acyclovir/valacyclovir	Ambiguous[a]	Ambiguous[a]	Ambiguous
Ganciclovir/valganciclovir	AUC	AUC: 40–60 mg h/L (Prop)	Ambiguous[a]
Ribavirin	AUC	Auc_{0-4} greater than 1755 mg h/L AUC_{0-12} greater than 3014 mg h/L C_{min} greater than or equal to 2 mg/L	C_{min} greater than 2.3 mg/L[b]
Foscarnet	Ambiguous[a]	Ambiguous[a]	No data[a]
Oseltamivir/oseltamivir carboxylate	Ambiguous[a]	Ambiguous[a]	Ambiguous[a]

AUC area under the concentration-time curve, *AUC0–4* the ratio of the area under the concentration-time curve during a 4-hour period, *AUC0–12* the ratio of the area under the concentration-time curve during a 12-hour period, C_{min} trough/minimum drug concentration, *PK/PD* pharmacokinetic/pharmacodynamics, *prop* prophylaxis

[a]While in vitro concentrations at which viral replication is inhibited by 50% (i.e., EC50 representing antiviral activity) have been widely determined, there are no/limited data which correlate these values with in vivo pharmacokinetic parameters (e.g., AUC) to describe magnitudes for preclinical efficacy

[b]Mostly related to anemia

9.4 Sedative and Analgesic Agents

Sedation and analgesia are frequently required in critically ill and mechanically ventilated ICU patients due to discomfort, anxiety, or delirium [25]. PK of sedatives and analgesics in critical patients is altered due to alteration of altered protein binding, fluid resuscitation, multi-organ failure, and unstable hemodynamics. Personalized pharmacotherapy can reduce the chances of adverse drug reactions and increase the chance of achieving optimal efficacy in critical patients admitted to ICU. Based on the patient's health parameters, accurate drug and dose selection is required to achieve optimum sedation in critical patients [93]. The use of opioids and/or benzodiazepines in the treatment of ICU delirium has been linked to a longer delirium episode in these critically ill patients [94]. They give consistent amnesic effects while also causing cardiovascular and respiratory depression [25]. Midazolam can be effectively utilized in intensive care for prolonged sedative effect without the occurrence of adrenal suppression as it is 94–98% protein-bound with a shorter half-life, steady-state Vd, shorter elimination half-life (1.5–5 hours), and intermediate clearance (Cl) [25]. Delayed ICU delirium management has been linked to a higher mortality rate [95].

9.5 Vasopressors and Inotropic Agents

Vasopressors are drugs that induce an increase in mean arterial pressure (MAP) by inducing vasoconstriction. Inotropes improve myocardial contractility [96]. Multiple molecules have both the characteristics of inotropes and vasopressors. The catecholamine neurotransmitters (dopamine, epinephrine, and norepinephrine) induce adrenergic and dopaminergic receptors and thus can be utilized as moieties for shock control [25]. In hemodynamically unstable patients, vasopressors improve vascular tone, and inotropes improve myocardial contractions [87]. These drugs are administered in patients who are hemorrhagic and cardiogenic, have heart failure, or are in septic shock and also trauma patients and patients undergoing surgery [96]. Alterations in metabolism, immune system, receptors, intracellular signaling pathways, and renal and hepatosplanchnic blood flow can later PK in critical patients [87, 97]. This necessitates careful medication selection and dosing in critical patients.

9.6 Neuromuscular Blocking Agents

Neuromuscular blockers are quaternary ammonium compounds that are ionized and water-soluble and can be utilized to facilitate mechanical

ventilation and lower respiratory muscle oxygen consumption. They are also employed in the treatment of tetanus and status epilepticus [25]. The trend of administering neuromuscular blockers in ICU is reducing with shorter-acting agents finding prominence [25]. Most commonly prescribed are nondepolarizing blockers (atracurium, pancuronium, and vecuronium) which are administered as an intravenous bolus or via continuous infusion [87]. Long-term use of neuromuscular blockers can lead to the buildup of drugs which can result in long-term weakness in critically ill patients, and thus twitch monitoring is practiced [98]. Pancuronium also builds up in patients with fulminant hepatic failure (FHF), and thus in the case of patients with renal or hepatic insufficiency, twitch monitoring is necessary [99].

9.7 Conclusion

TDM is an effective approach for dose individualization. TDM is becoming increasingly vital for critically ill patients to enhance their outcomes and reduce drug toxicity. Some patients may still receive sub-optimal antimicrobial exposure because of the long duration of medication and the PK/PD variability. TDM-guided dose adjustment strategy is safe and effective in assuring that all critically ill patients get therapeutic exposures. TDM of antibiotics and antifungals is highly recommended for achieving therapeutic index in critically ill and ICU patients. Antivirals will require additional clinical investigation to discover therapeutic targets for patient benefit. TDM of anticoagulants is restricted to specific clinical conditions and has recently gained importance for the treatment of critically ill patients. Understanding the therapeutic indices of sedatives, analgesics, and vasopressor-inotropic agents in critically patients will require extensive research involving TDM. Few studies of neuromuscular blocking agents employing TDM practices have shown beneficial effects in critical illness. From the clinical studies, it is recommended that TDM should be introduced as bedside practice for optimizing the therapeutic doses in critically ill patients so as to maintain therapeutic efficacy as well as decrease the instances of development of serious adverse events and drug toxicity.

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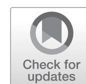

10 Quality Assurance of Samples for Therapeutic Drug Monitoring and Clinical Toxicology

Samuel O. Bekoe, Samuel Asare-Nkansah, and Kwabena F. M. Opuni

Abstract

The integrity of samples employed for therapeutic drug monitoring (TDM) and clinical toxicology (CT) has a significant impact on the quality of data obtained for clinical decision-making. Samples usually employed for TDM and/or CT are obtained either using invasive or non-invasive approaches, and these include blood, plasma, or serum (invasive approaches), urine, and saliva (non-invasive approaches). Lack of standard operating procedures for samples in the pre-analytical stage could lead to variabilities in analysis results because of factors such as haemolysis (blood samples), inappropriate cooling and storage (urine samples), and desiccation of saliva. Therefore, assuring the quality and integrity of samples obtained for TDM and CT studies is a requirement for reliable data suitable for informed clinical decisions for patients as well as medical emergencies. It is an irrefutable emphasis that the assurance of the quality of samples meant for TDM and CT assessments correlates strongly with the quality of data obtained for clinical interpretation and implementation of optimized care. This chapter thus addresses major concerns, regarding the quality assurance of samples for TDM and CT.

Keywords

Bioanalysis · Biological samples · Clinical toxicology · Quality assurance · Therapeutic drug monitoring

10.1 Introduction

Measurement uncertainties and variabilities are usually associated with TDM and CT studies. These challenges could result in the generation of unreliable data, which could affect the clinical decision-making required for optimal patient therapy ultimately. Several factors could account for such variabilities, but key amongst them is the integrity of the sample meant for the TDM and CT studies. The nature, integrity, and handling of TDM and CT samples strongly influence the quality of analytical data that can be generated from such samples for the purpose of critical healthcare decisions. Thus, certain key variables such as sample acquisition and handling (before, during, and after analysis) and sample prepara-

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tion and storage are to be considered carefully before any TDM and CT studies are carried out [1–6]. Other important factors include calibration of equipment, temperature monitoring of freezers, and data management. In a typical workflow, the factors that impact the quality of samples for TDM and CT are as indicated in Table 10.1 which will be respectively discussed in the ensuing sections.

10.2 Sample Acquisition, Handling, and Storage

Given the importance of the pre-analytical phase (sample acquisition, handling, and storage) of TDM and CT projects, a well-developed and tested plan/protocols, and standard operating procedures, that encompass sampling, handling, and storage of samples must be followed (optimized and standardized best practices). This ultimately ensures that the integrity of biological specimens is maintained in order to secure quality analytical data needed for optimum clinical decision for patients. Usually, laboratories responsible for the analytical phase of TDM or CT undergo proficiency testing and ultimately get certified for their purpose.

In TDM, for example, drug levels in adults are usually profiled in whole venous blood, serum, or plasma, while capillary blood specimens are collected for babies. In the collection of blood samples, drug levels are allowed to reach steady state, which can be predicted by at least five half-lives of the specific pharmacodynamic agent. However, in collecting samples for drugs with long half-lives (e.g. amiodarone), samples are taken before steady state is achieved in order to prevent toxicity in patients suffering from renal and metabolic impairments. It must also be noted that drugs such as cyclosporin A require whole blood to be sampled because the drug partitions itself between the red blood cells and plasma [7]. Therefore, the pharmacokinetic and pharmacodynamic properties of a drug are important to consider for a standard sample acquisition.

In essence, standard operating procedures for the collection of samples (blood, serum, plasma, urine, and saliva) for TDM or CT studies should require the availability of appropriate storage facilities, equipment, and qualified personnel, amongst others. Samples, be it blood, serum, plasma, urine, or saliva (in certain instances), should be obtained using well-established and approved techniques in appropriate containers. For instance, it is not acceptable to use lithium heparin as an anticoagulant for blood samples meant for lithium analysis. Again, in the monitoring of certain drugs such as phenytoin, some types of gel separator tubes are not suitable [8] because of the potential diffusion of drugs into the gel from serum. Guided by best practices, samples for TDM or CT should be collected to avoid unwanted contamination and losses which could affect the quality of analytical data generated. It is also important to have protocols and standard operating procedures (SOPs) followed, supervised, and approved by qualified personnel to ensure the quality of the various stages involved in specimen acquisition. In situations where specimens must be transported to another site for laboratory testing, appropriate and validated sample transport and handling systems must be ensured. Samples that are not analysed immediately must be stored in appropriate containers and environments (e.g. tem-

Table 10.1 Workflow for therapeutic drug monitoring and clinical toxicology cycle

	General sequence of technical activities for therapeutic drug monitoring and clinical toxicology
1	Study design
2	Informed consent
3	Sample collection protocol[a]
4	Sample collection[a]
5	Sample annotation[a]
6	Sample labelling[a]
7	Sample storage[a]
8	Data records[a]
9	Sample transportation[a]
10	Sample analysis (using analytical methods)[a]
11	Data analysis
12	Reporting

[a]Activities that impact the quality assurance of samples for therapeutic drug monitoring and clinical toxicology

perature and light) to ensure sample integrity is maintained [1, 9–14].

10.3 Sample Preparation and Validation Techniques

The stability of sample matrices and drug analytes is essential to be considered in TDM or CT, as these candidates can sometimes be susceptible to degradation. Sample degradation may be observed either at the pre-analytical stage or during the analytical phase through visual inspection or instrumental analysis. Therefore, comprehensive and validated procedures should be developed and be made accessible for easy identification of bio/chemical degradation of samples. Thus, separation methods or procedures should be designed to ensure that possible interferences from the matrix being studied or degraded or metabolic products from the analyte of interest are significantly avoided or minimized. For this purpose, the quality of solvents and reagents should be routinely checked, paying particular attention to storage conditions and expiry dates. The maintenance, calibration, and cleaning of equipment such as centrifuges should be routinely done, notwithstanding the availability of appropriate glassware, plasticware, and consumables. Where necessary, the use and application of preservatives should be well documented since compounds employed as preservatives could negatively influence analytical data generated. Internal standards could also be considered to help minimize random errors associated with both extraction procedures, sample dilution, and instrumental effects [3, 10–14].

10.4 Sample Testing or Analysis

It must be ensured that appropriate sensitive analytical techniques that have been well validated are used for the testing of samples. The calibration of equipment used for the method development should be regularly checked to avoid any form of instrumental effects that could result in measurement uncertainties and significant variabilities in data [3, 11, 13].

10.5 Automation of Testing Laboratory

The use and application of advanced technology involving appropriate statistical tools will be an essential tool required for the assurance of quality in TDM and CT. Such tools help to provide valuable information and also monitor the reliability and reproducibility of analytical data. The replicate determinations on samples are a key requirement that should be considered for TDM and CT, and having such automation could provide the needed data. Moreover, batch-to-batch in-process controls are ensured to establish sound scientific patterns amongst samples analysed. Such tools also do help in preventing analysts or laboratory/clinical scientists from making any form of alteration to data obtained [1, 3, 11, 13].

10.6 Conclusion and Outlook

The integrity of samples for TDM and CT are assured when appropriate sample quality management approaches are used [15]. More importantly, invasive approaches to sampling of blood, plasma, or serum can be minimized using microsampling techniques which are less invasive. Emerging microsampling techniques such as dried blood spot, plasma microsampling, volumetric absorptive microsampling, and microneedle have been implemented successfully for sample collection [16]. Also, dried blood and serum spots have proven an important technique for sample storage and transportation especially in resource constraint countries [17]. Although the suggested strategies have some challenges, optimization and improvement in these techniques can improve sample integrity prior to analysis.

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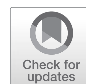

11 Therapeutic Drug Monitoring and Toxicology of Anticancer Drugs

Seema Kohli and Lavakesh Kumar Omray

Abstract

The successful management of disease is completely dependent on drug dosage regimen strategies. Appropriate dosage regimen helps to achieve optimum level of a drug at the receptor site in order to obtain optimum therapeutic response with minimal adverse reactions/events. But there always occurs inter-individual variation toward drug response, which can be attributed to individual's pharmacokinetics/pharmacodynamics (PK/PD) characteristics. Therefore, there is a need for clinical evaluation and therapeutic monitoring of drug. Drugs with narrow therapeutic indices such as digoxin, aminoglycosides, antiarrhythmics, anticonvulsants, and anti-asthmatic agents need strict individualization of drug regimen. The dose of these drugs is carefully individualized to avoid fluctuations in plasma drug concentration. Often, the individualization of dose and adjustment depends on the toxicity of the drug and the patient's ability to tolerate the drug. Therapeutic drug monitoring (TDM) is not commonly employed for chemotherapeutic agents. However, a number of antineoplastic agents may have narrow therapeutic indices. As such, it is very difficult to provide right individualized treatment for cancer chemotherapy. Despite several limitations of TDM for cytotoxic drugs, several clinical trials of anticancer drugs have shown the benefits of maximum chemotherapy using TDM protocols. The chapter details TDM applications for the therapeutic efficacy and toxicological implications of various anticancer drugs.

Keywords

Therapeutic index · Individualized drug therapy · Drug monitoring · Anticancer drugs

11.1 Introduction

The successful management of disease is completely dependent on the drug dosage regimen strategies. Additionally, appropriate dosage regimen helps to achieve optimum level of drug at the receptor site in order to obtain optimum therapeutic response with minimal adverse reactions/events. There always occurs inter-individual variation toward drug response, which can be attributed to individual's pharmacokinetics/pharmacodynamics (PK/PD). Therefore, there is a substantial need for clinical evaluation and

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therapeutic monitoring of drugs. It is important to remember some basic aspects of treating patients and using medicines before identifying drugs that require monitoring. For most medicines, the benefits could be easily observed and measured, so it is possible to know if the administered dose is appropriate for that patient or if some dose adjustments are required either to avoid toxic effects or to obtain the desired benefit.

For most medicines, routine monitoring is not recommended. Only clinically relevant testing should be carried out. Due to the fact that drug assays are expensive, it's important to think about why you're monitoring and what new information you'll get (if any). As a result, in a clinically stable patient, routine monitoring of many medicines is not required [1].

11.1.1 Individualization of Drug Therapy

In the past, drugs were used in similar doses for almost every patient. This theory is known as flat dosing. But the pharmacokinetic/pharmacodynamic parameters and individual's variation, viz., body weight and surface area, result in variations in drug responses. Individualizing of dosage regimen is not required for all drugs. Drugs having wide margin of safety with wide therapeutic window do not need strict individualization of therapy.

Drugs with narrow therapeutic window such as digoxin, aminoglycosides, antiarrhythmics, anticonvulsants, and anti-asthmatics need strict individualization of drug regimen. The objective of dosage design for the abovementioned category of drugs is to produce a maximum therapeutic response with minimal adversities or both. The dose of these drugs is carefully individualized to avoid fluctuations in plasma drug concentration. Pharmacokinetic and pharmacodynamic monitoring is advocated for drugs having fluctuations in plasma concentration levels. In concern therapy, the individualization of dose and adjustment depends on the toxicity of the drug and the patient's ability to tolerate the drug.

11.2 Therapeutic Drug Monitoring (TDM)

The course of measurement of the amount of drug or its metabolism product in blood at a defined period is expressed as therapeutic drug monitoring. Some of the drugs are being evaluated to have a low "therapeutic index." Therapeutic index is described as the proportion of the toxic (lethal) and therapeutic (effective) doses of medication.

$$\text{Therapeutic Index} = \frac{\text{LD50}}{\text{TD50}}$$

In order to maintain the drug's effective concentration in the body, a patient needs to be administered a dose of drug at consistent interludes. Maintaining a constant state for some medications is more difficult than just administering a normal dose. Drugs are absorbed, metabolized, utilized, and eliminated at varied rates depending on the person's age, general health, and genetic composition. The concentration in circulation eventually yields a pharmacological response (Fig. 11.1).

The TDM approach involves monitoring the incidence and intensity of both the desired therapeutic and adverse drug effects by measuring the average plasma drug concentration. The therapeutic range of a drug is a probability concept, and care should be taken toward prescribing/administering drugs to a patient in that range. While administering drugs to the patient, the drug plasma level must be maintained within a narrow range of the therapeutic window. The dose of a drug is frequently accustomed as per the body weight/body surface of the individual and the PK/PD of the drug [2].

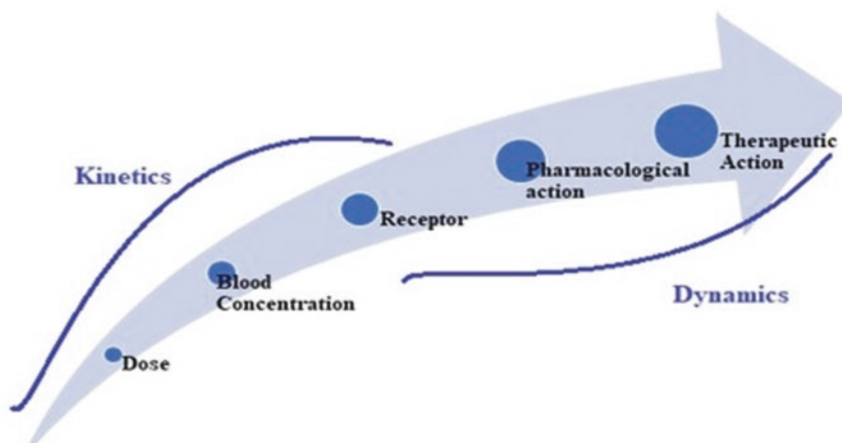

Fig. 11.1 Drug dose and response

11.2.1 Criteria for TDM

- Drugs having low therapeutic index.
- Direct correlation between drug and its metabolite's plasma level and the pharmacological or toxic effects.
- Individual variability in drug plasma concentration profile on single dosing.
- Assessment of therapeutic effect cannot be made clinically.
- Suitable analytical techniques not available for estimation of drug and its metabolite.

11.2.2 Features of Drug for Apt Candidature for TDM

- Drugs with significant pharmacokinetic variations.
- Drugs having dose-associated healing and adverse effects.
- Drugs with low or narrow therapeutic index.
- Drugs having distinct targeted therapeutic range.
- Drug monitoring is problematic.

If the clinical efficacy of a drug can be assessed by simply as the heart rate/ blood pressure, the dose may be adjusted based on response. If this is not feasible, then therapeutic drug monitoring is used in the following situation [3]:

- Drugs taken as a preventative measure to avert convulsions, arrhythmias related to the heart, CNS depression, events of mania, asthma reversions, or refusal of organs
- Avoidance of drug's major toxic effects. For example, aminoglycoside antibiotics, which have a narrow therapeutic range as compared to other antibiotics

The apposite suggestions for TDM are as follows [1]:

- Toxicity: When the clinical pattern is not simple to identify, it's difficult to diagnose toxicity as the case of inexplicable nausea in a person on digoxin therapy.
- Dosing: Adjustment of dose after attaining steady levels; evaluation of loading dose, e.g., on commencement of phenytoin therapy; prognosis of drug dosing to estimate patient dose requirement, e.g., aminoglycosides.
- Monitoring: Evaluating acquiescence; diagnosing under treatment; and futile treatment.

The fact that TDM is the measurement of medication concentrations in plasma or blood to aid patient management is an important component of TDM that should be addressed. This means that the laboratory-assisted medication concentration levels are evaluated to personalize and optimize dose schedule of the patient and

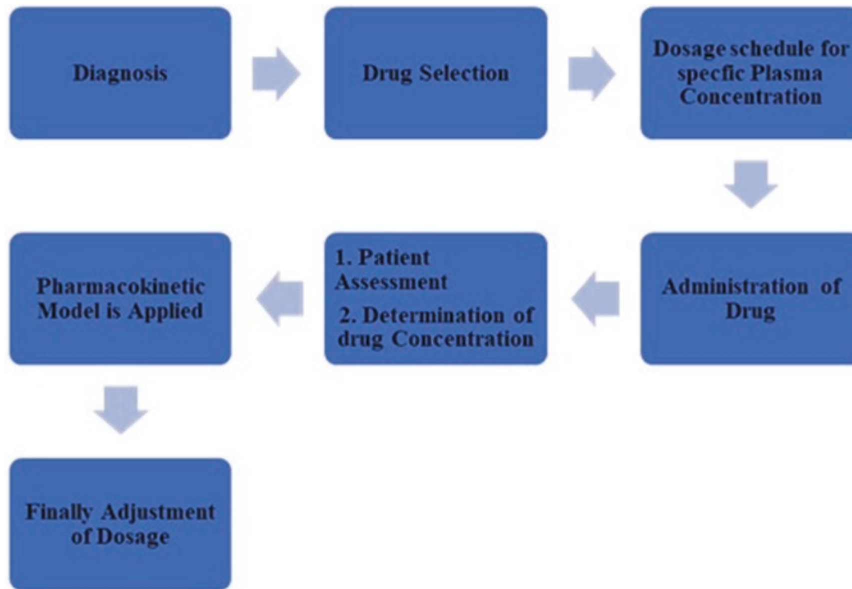

Fig. 11.2 Process of TDM

treatment results using drug dose in a precise therapeutic window [4]. Figure 11.2 shows the process of dosage regimen adjustment using TDM approach.

11.3 Therapeutic Drug Monitoring for Various Drugs

It is imperative to express that various drugs which need intense observations during treatment have been used chronically. These monitoring must be well maintained at steady concentrations even for years. Pregnancies, infections, transient diseases, physical and mental stressors, accidents, and surgeries are just a few examples of life events that can change a person's therapeutic level. Over time, the patient may develop other chronic disorders that necessitate lifelong medicine, which could interfere with the monitoring of drugs. Kidney disorder, heart problems, liver disease, thyroid disorders, and HIV are just a few examples. These fluctuations are traced by therapeutic drug monitoring and allow them to be accommodated. This would help in individualizing the drug dosing. The evaluation of liver and kidney functions, as well as the monitoring of therapeutic drugs, may assist in determination of body's ability to metabolize and eliminate therapeutic drugs. Several categories of drugs which need monitoring are shown in Table 11.1.

11.4 Importance of Clinical Pharmacokinetic Parameters in Context to Anticancer Agents

The investigation of the correlation between drug dosage regimens and drug concentration-time profiles is known as clinical pharmacokinetics that is the study of absorption, distribution, metabolism, and elimination (ADME) status of drugs. The parameters usually investigated are as follows:

- First-order elimination rate constant (k_e)
- Clearance (C)
- Volume of distribution (V_d)
- Elimination half-life ($t_{1/2}$)

Table 11.1 Details of drugs that need monitoring

Drug category	Drugs	Treatment used
Immunosuppressant	Tacrolimus, cyclosporine, azathioprine, sirolimus, mycophenolate mofetil	Inhibit refusal of transplanted organs and autoimmune ailments
Anticancer drug	Methotrexate and all cytotoxic agents	Psoriasis, rheumatoid arthritis, various cancers, non-Hodgkin lymphoma, osteosarcoma
Psychiatric drugs	Lithium, valproic acid, some antidepressants (imipramine, amitriptyline, nortriptyline, doxepin, desipramine)	Bipolar disorder (manic depression), depression
Cardiac drugs	Digoxin, digitoxin, amiodarone, lidocaine, quinidine, procainamide, N-acetylprocainamide (a metabolite of procainamide)	Congestive heart failure, angina, arrhythmias
Antibiotics	Aminoglycosides (gentamicin, tobramycin, amikacin), vancomycin, chloramphenicol	Resistant cases of bacterial infections
Antiepileptics	Phenobarbital, phenytoin, valproic acid, carbamazepine, ethosuximide, and other antiepileptic drugs	Protection from epilepsy and seizures and also as stabilizer of moods
Bronchodilators	Theophylline, caffeine	Asthma, chronic obstructive pulmonary disease (COPD), neonatal apnea

Precision dosing or personalized dosing in a patient is made easier using these criteria. Anticancer drug dosing has traditionally been based on body surface area (BSA), with the assumption that there is a link between BSA and clearance (CL) or volume of distribution (Vd). However, in many case, this relationship is not accurate and does not present factual reasons for variation in drug responses in population [4, 5]. This happens when there is drug-to-drug interaction, hepatic/renal impairment and in the case of geriatric/pediatric patients. These factors lead to a wide range of therapeutic practices, making it difficult for doctors to make decisions based on their prior experience. Patients with several comorbidities/co-medications may receive insufficient pharmacotherapy which could result in undesirable intensities of toxicity or effectiveness. There are several sources for inter-individual/intra-individual variations in PKs of anticancer drugs, viz.:

- Age and age-related alterations
- Obesity
- Concomitant medications
- Pharmacogenomics
- Renal/hepatic impairment
- Alcohol consumption
- Racial groups

The utility of clinical pharmacokinetics must be assessed in terms of patient results, such as whether or not to include dose-related toxicity, and how long it takes to reach a conclusion, taking into consideration the most essential variables and deciding on the most favorable concentration-effect connection [5].

11.5 Methods for Individualization of Drug Therapy

The methods employed for individualization of drug therapy are as follows:

- A priori method: A priori approaches determine the dose required to achieve a desired exposure in a given patient based on morphological, biological, and physiological factors such as body weight, age, gender, serum creatinine level, and glomerular filtration rate (GFR).
- A test dose method: This method comprises following:
  - In the first step, specific pharmacokinetic characteristics are assessed after a bolus injection of a moderate dose is administered using numerous blood samples.

  - This step comprises the use of PK parameters to estimate the complete dose required to accomplish the target revelation.
- A posteriori method: In this nomogram, multiple linear regression (MLR) and maximum a posteriori (MAP) Bayesian method are applied [6].

11.6 Therapeutic Drug Monitoring Approach of Anticancer Drug

High intersubject variance, a narrow therapeutic index, minimal inter-occasion variation, and a robust relationship between concentrations of drug in plasma and clinical effects of the treatment are all characteristics of successful therapeutic drug monitoring. Process, methodological, funding, logistical, social, and religion barriers also contribute in the challenges for the successful implementation of right methods to individualized drug treatment.

11.6.1 Importance of TDM in the Management of Cancer

The international association of TDM and clinical toxicology defines that TDM is a multidisciplinary approach in the treatment of cancer, which is aimed at improving patient treatment and care. On the basis of clinical experience, the dose of the medicine is adjusted individually. Clinical studies have proven that treatments based on clinical trials improved outcomes in the target populations suffering from cancer disease. It can be based on a priori, posteriori and/or biomarkers effective measurement and measurement of concentrations of drugs in plasma [7].

Targeting of anticancer drugs is intended for action on explicit recognized tumor substrate in cells in a biopsy from an individual patient which is generally protein. These proteins are tremendous substances for TDM. The anticancer drugs are administered by patient through suitable route, i.e., generally parenteral or oral administration. This approach offers appropriate inhibitory concentrations of drug for their target sites only. It is useful for treatment-limiting side effects also [8].

The therapeutic index of cytotoxic anticancer medicines is limited, and they have a highly toxic property and a significant level of interpatient pharmacokinetic changeability. These are the most important characteristics that support TDM's usefulness [9]. However, these features are often not sufficient in some case where TDM has been employed in the treatment of cancer patients in normal practice. Thus, currently the role and importance of TDM for the patient care in cancer treatment are limited [10].

11.6.2 Limitations of TDM for Anticancer Drugs

Therapeutic drug monitoring approach is not commonly employed for the administration of chemotherapeutic drugs. Antineoplastic agents have narrow therapeutic indices. As such, it is very difficult to provide precise individualized treatment for cancer chemotherapy. The clinical value of TDM for chemotherapeutic agents is presently constrained by several factors [11–13]. Limitations for the use of TDM are given below:

1. An incomplete knowledge of the pharmacokinetics and pharmacodynamics of most antineoplastic therapeutic agents.
2. Blood concentration of an anticancer drug is an incidental measurement of the amount of drug present in the objected tissue, as the site of action of the drug may be distant from intravascular spaces. Therefore, it is difficult to correlate and quantify the drug at the site of action.
3. In cancer chemotherapy, there is a large lag period between the assessment of drug quantity in biological tissues and variation in efficacy.
4. Cancer is summarized as a group of heterogeneous complex disease. It is having inherent features between the concentration and effect relationship for antineoplastic drugs. This affects the activity of drugs. Anticancer

medicines frequently exhibit variability in blood supply and cellular characteristics, resulting in varying levels of susceptibility, tolerance, and resistance [14]. In this condition, only few antineoplastic drugs fulfill the entire criterion required for the implementation of TDM, since there aren't any well-defined correlations between therapeutic efficacy and systemic exposure. Such relationships have been demonstrated by few drugs in particular cancer disease only. For example, methotrexate is used as a TDM approach in acute lymphoblastic leukemia (B lineage), and 5-fluorouracil (5-FU) is used as the right TDM approach in metastasis colorectal [12].

5. Solid tumors present in any part of the body may have their own distinctive source of blood and affect the concentration of drugs. This results in poor relationship between plasma and tumor concentration of the drug.
6. There is a natural holdup period between the measurement of drug concentration in plasma and the evaluation of the drug's final pharmacodynamic effect, and hence it is difficult to establish a correlation between them.
7. If the outcome variable in TDM therapy is an increase in cancer cure rates, then at least 5 years of therapy follow-up is usually required to accurately analyze the outcome and establish the correct link. As a result, these trials take longer to complete and have a more complicated treatment approach. This appears to be in contrast to research conducted on medicines with faster effects, such as antibiotics exemplified by clarithromycin, erythromycin, etc.
8. There is a difficulty in establishing a relationship between concentration and effect because anticancer drugs are given in combination. Therefore, it is very difficult and problematic to precisely define the pharmacokinetics and pharmacodynamics of each individual drug for TDM approach.
9. With combination therapy, it is also difficult to determine pharmacodynamics of drug toxicity [15].
10. TDM is costly in comparison with conventional therapy, is time consuming, and required technical field force which is difficult to manage all the time.

11.7 Beneficial Aspects of TDM for Cancer Therapy

Many drugs need special attention during the therapy due to its narrow therapeutic window. Among these, the anticancer drugs have major concerns for successful treatment [15]. TDM refers to the need to treat cancer patients with the smallest dose possible in order to achieve the required concentrations in the upper end of the therapeutic window's nontoxic range, increasing the likelihood of drug response.

Many anticancer drug agents satisfy the criteria for the selection of TDM in the treatment of cancer. Variability of pharmacokinetics and narrow therapeutic indices of antineoplastic agents makes it a suitable candidate for TDM. In addition to this, while treating patients of cancer disease, seeking the highest efficacy of drug is an important principle because of the magnitude of the consequences for cancer patients. Physician cannot take the risk of sub-optimal therapy. It can greatly reduce the probability of treatment for curable cancer diseases. On the other hand, side effects, such as myelosuppression, can be life-threatening and may be related to higher than optimal therapy. It can also be addressed using TDM. Despite the various limitations facing the TDM approach for the treatment of antineoplastic agents, the possibilities for development of cancer treatment are also available, provided TDM is properly accomplished. Some important benefits of anticancer TDM are outlined below:

1. TDM is useful in identifying the efficacy of anticancer agents that are being given either in overdose or underdose in any given condition.
2. TDM in the treatment of cancer chemotherapy provides added advantages apart from individualizing drug therapy to improve patient

compliance and effectiveness and avoid toxicity of drugs.

3. It is easy to correlate and treat the efficacy and toxicity of combination antineoplastic drug therapy.
4. TDM provides the possibility of dose modification in patients with liver and kidney impairment [14].
5. There are chances of minimization of patient to patient pharmacokinetic variability [14].
6. TDM is useful in the detection of drug interactions and drug-food interaction [16].
7. TDM enhances patient compliance.
8. Subsequently, therapeutic range of drugs in the plasma expresses efficacious as well as toxicity concentrations having massive clinical usefulness.

TDM approach for some anticancer drugs is discussed underneath.

11.7.1 Carboplatin

Carboplatin, an antimetabolite, is mainly used in ovarian cancer. It is also used in pediatric cancers and other cancers including neuroblastoma and retinoblastoma [17]. Carboplatin shows dose dependent response up to a certain level, and after that an increase in dose, the toxic effect of the drug starts. In other words, it has a clear relation between plasma concentration and the pharmacological effect of drugs on the patient [18]. Mechanism of action and TDM approach of carboplatin and other anticancer drugs are given in Table 11.2. Small increments of drugs in AUC result in increased antineoplastic activity of drugs. Due to narrow therapeutic index, further augmentations in carboplatin dose result in augmented toxic effect of drug. The foremost toxic effect of carboplatin in small increased dose is myelosuppression. Successful TDM concentration of carboplatin AUC is about 4–7 mg/min/ml recommended in cancer of the ovary. As a result, even minor modifications in carboplatin dose and exposure can have a significant clinical impact. Similarly, a reduction in the dose of carboplatin up to 10%, results in double the reversion rate of cancer for 5 years. The clearance (CL) of carboplatin quantity in plasma is diligently interrelated with kidney functioning. It is employed to monitor the carboplatin dose to accomplish an anticipated target of AUC by using Calvert’s equation as given below [19].

$$\text{Calvert's equation : Dose}(\text{mg}) = \text{Area under the curve of drug}(\text{AUC})(\text{mg/ml/min}) \times (\text{Glomerular filtration rate}(\text{GFR}) + 25\ \text{ml/min})$$

The constant of 25 ml/min is used to account for non-renal clearance. TDM of carboplatin is mainly proposed for high-dose protocols [20].

11.7.2 Methotrexate

Methotrexate is known as antimetabolites immune suppressant and used as an anticancer drug. It is employed in the management of certain cancerous diseases, adult and childhood acute lymphoblastic leukemia, severe psoriasis, and autoimmune diseases like rheumatoid arthritis [11–13]. Methotrexate exhibits its anticancer effect by competitively suppressing dihydrofolate reductase enzyme present inside the cell. This type of enzyme is accountable for conversion of folates to tetrahydrofolate in the cell. The reduced folate helps in the transfer of carbon units.

Purine synthesis and the methylation of uracil to thymine in DNA synthesis both utilize these carbon units [21]. Leucovorin is an antidote (a folate analogue) and used to avoid excessive destruction of host normal cell. Leucovorin is a man-made material for dihydrofolate reductase, and it allows the renewal of thymine development and the re-initiation of DNA production. Mechanism of action of methotrexate is shown in Fig. 11.3.

In effective doses, methotrexate has the potential for significant toxic effects. Patients being treated with methotrexate should be continuously observed for their pharmacokinetic and pharmacodynamic effects so that toxic effects are detected promptly to give antidote or rescue therapy. Methotrexate is the only anticancer drug whose dose and concentration are

Table 11.2 List of anticancer drugs and their TDM approach

Drug	Indication	Mechanism of action	TDM approach	References
Carboplatin	Ovarian cancer, pediatric cancers, neuroblastoma, and retinoblastoma	Carboplatin activates and generates reactive complexes inside the cell that cause intra- and inter-strand cross-linking of DNA molecules	Concentration of carboplatin AUC kept between 4 and 7 mg/min/ml recommended in ovarian cancer for optimum effect	[18, 19]
Methotrexate	Childhood acute lymphoblastic leukemia, non-Hodgkin lymphoma, and osteosarcoma	Competitively inhibiting dihydrofolate reductase	High-dose methotrexate and initiation of treatment with fixed time interval and tailored dose of folinic acid rescue approach	[22, 23]
13-cis-retinoic acid (Isotretinoin)	Neuroblastoma, skin and ovarian cancer by increasing cell apoptosis and cell growth inhibition	It inhibits ornithine decarboxylase, thereby decreasing polyamine synthesis	Dose intensity, duration, and plasma drug concentration	[26, 28]
Busulfan	Bone marrow stem cell transplantation and treatment of hematologic malignancies such as acute and chronic myeloid leukemia, etc.	Bifunctional alkylating agent of DNA. Alkylation leads to breaks in the DNA molecule as well as cross-linking of the DNA strands	The target concentration range for optimum effect of busulfan AUC between 78 and 101 mg•h/L	[29]
5-Fluorouracil	Used for the treatment of colorectal cancer, esophageal cancer, stomach cancer, pancreatic cancer, breast cancer, cervical cancer, etc.	5-FU acts in several ways as an anticancer drug but principally acts as a thymidylate synthase inhibitor	Administered as a continuous infusion via an implanted port system using a body surface area (BSA)-based dose calculation	[30]
Mitotane	Adrenocortical carcinoma	Interfere with the peripheral metabolism of steroids and suppresses the adrenal cortex	Concentration-dependent effect and achievement of steady-state concentration	[31]
Tamoxifen	Used to treat breast cancer	Selective estrogen receptor modulator	Monitoring endoxifen plasma concentration	[32]
Imatinib	Philadelphia chromosome-positive chronic myelogenous leukemia and gastrointestinal stromal tumors	Specific tyrosine kinase receptor inhibitor	Establishing relationship between dose and plasma concentration	[34]
Pazopanib	Inhibition of spread of cancer cells	Kinase inhibitor inhibits the vascular endothelial growth factor receptor, platelet-derived growth factor receptor, etc.	Exposure-response correlation specifies that pazopanib would benefit from pharmacokinetically guided dosing	[35]

regularly individualized based on TDM. Plasma concentration of methotrexate can be used to monitor the toxic effect. Higher concentration of methotrexate therapy can be neutralized with leucovorin or folinic acid as rescue treatment. Therefore, in the treatment of methotrexate, initially higher concentration is given and normal function of cell restored by using leucovorin treatment. The timing of leucovorin introduction and dose is guided by the routine use of TDM for high-dose methotrexate therapy. Leukemia, osteogenic sarcoma, non-Hodgkin lymphoma, and breast and lung cancer have all benefited by leucovorin rescue. After adminis-

tration of methotrexate infusion, the patients having plasma concentrations above 10 μM after 24 h, 1 μM after 48 h, and 0.1 μM after 72 h are known to have threat of bone marrow and gastric organs [22, 23].

Methotrexate is metabolized via two pathways, 7-hydroxylation and polyglutamation as given in Fig. 11.3. Methotrexate and its metabolites are active. Aldehyde oxidase catalyzed 7-hydroxylation and produced a less effective inhibitor of dihydrofolate reductase. This metabolite is responsible for nephrotoxicity which occurs during high dose of methotrexate treatment. Methotrexate and its metabolite 7-hydroxy methotrexate convert to intracellular polyglutamate formation. These polyglutamate metabolites are as potent as methotrexate and dihydrofolate reductase inhibitors. In both methotrexate metabolic pathways, restoration of DNA synthesis is required [24].

11.7.3 13-Cis-Retinoic Acid

Among the retinoids, mainly 13-cis-retinoic acid exhibits pronounced potential in numerous cancer diseases; however, severe skin toxicity may occur. Whether a cancer or a tumor is caused by chemical, physical, or viral source, 13-cis-retinoic acid is an effective anti-promoter. Antiproliferative activity of 13-cis-retinoic acid has been validated in in vitro investigation. The mechanism of action of retinoic acid is presented in Fig. 11.4. The results of various preneoplastic and neoplastic lesions of tissue histology have been demonstrated and are being explored further for cancer prevention. The ADME of 13-cis-retinoic acid has been explored in the number of cancer patients. 13-cis-retinoic acid is employed for the management of cancer in children with diseases such as neuroblastoma having great risk factor and increases a 3-year survival benefit up

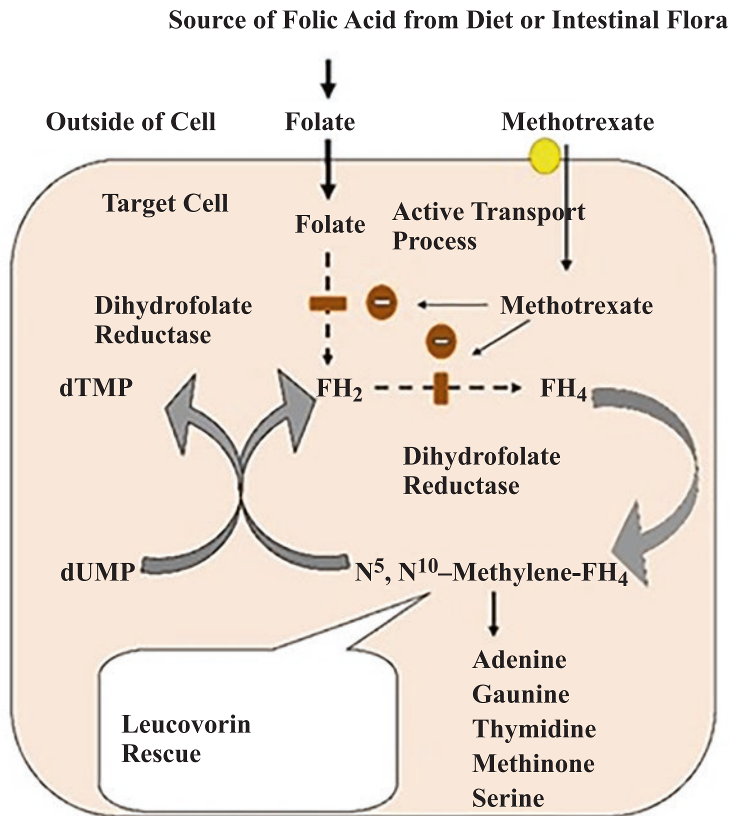

Fig. 11.3 Mechanism of action of methotrexate and leucovorin rescue. FH_2, dihydrofolate; FH_4, tetrahydrofolate; dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophosphate

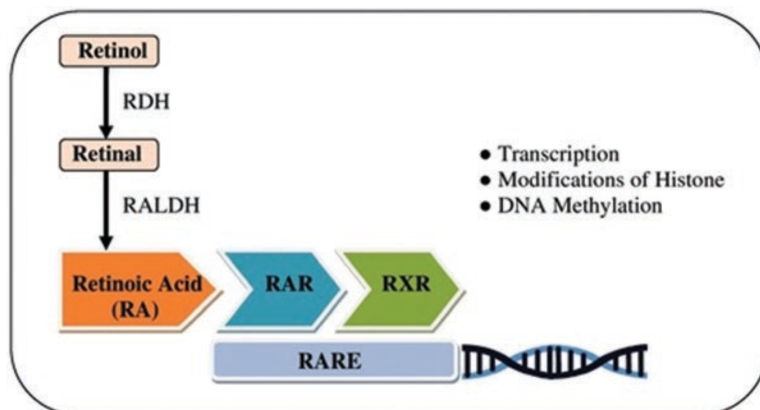

Fig. 11.4 Mechanism of action of retinoic acid

to 46% improvement as compared to 29% in other conditions [25]. The standard dosing protocol of drug about 160 mg/m2/day for 2 weeks treatment study, observed to have up to 20 times-variability in Cmax and AUC. The lack of clinical benefit with an alternate drug regimen of low and continuous dosing suggests that dose intensity and plasma drug concentration are critical for pharmacological therapeutic efficacy. Dose-dependent toxicity is also related with the amount of drug in plasma having more than 10 μM quantity in the blood [26].

By stimulating cell differentiation, retinoic acid as a medication aids in the transformation of cell types from proliferative to maturation phage. Retinol converts to retinal in the presence of retinol dehydrogenase (RDH), which changes to retinoic acid in the presence of retinaldehyde dehydrogenase (RALDH). The cellular retinoic acid-binding protein (CRABP) site binds retinoic acid in the cytoplasm, and in the cell nucleus, the receptors retinoic acid receptor (RAR) and retinoid X receptor (RXR) are involved in its binding. Retinoic acid comes in a variety of biochemically active forms, each of which isomerizes under different physiological conditions. These isomeric forms of retinoic acid act on diverse receptors present in the cell for the expression of gene response. In the nucleus, it is having mainly three steps, i.e., transcription, histone modification, and DNA methylation through retinoic acid response element (RARE) [27].

In a controlled dosing study of 13-cis-retinoic acid for TDM, it was found that the target of minimum Cmax of 2 μM was attained in 90% of patients in the given protocol. The pharmacokinetic variability in patient was also considerably lowered particularly in the pediatric patients suffering from neuroblastoma (<12 kg) who were given dose of 5.33 mg/kg. The TDM protocol of 13-cis-retinoic acid is currently suggested in the high-risk neuroblastoma of European patients. However, further intensive studies for proper implementation of TDM are required to get confirmed clinical benefit of the drug [28].

11.7.4 Busulfan

Busulfan is an anticancer drug having bifunctional alkylating property of DNA in the target cell. Alkylation leads to breakdown of the DNA molecule along with cross-linking of the strands of DNA. It consequently leads to interloping of DNA replication and RNA transcription. It is used in high doses as preparative chemotherapeutic cures in patients experiencing hematopoietic stem cell transplantation (HSCT) for patients with numerous cancerous conditions. The oral dose of busulfan in cancer patients is about 1 mg/kg body weight given four times daily or 3.2 mg/kg IV in continuous mode once every day. Pharmacokinetic profile of busulfan follows single compartment model which is difficult to correlate concentration of drug in the target site. Oral absorption of busulfan is rapid and maximum concentration achieved within 1 h of administration of the drug. Oral bioavail-

ability of busulfan is approximately 70–90% due to erratic absorption of the drug. This absorption is variable and associated with very wide inter- and intra-individual unpredictable intestinal absorption. These problems are resolved by developing an IV formulation of busulfan, which has a more predictable pharmacokinetic profile with less interpatient variability. TDM of busulfan is based on the right dose selection which involves the administration and gathering of some [4–7] blood samples at known predetermined period. These are later employed to estimate busulfan clearance of an individual and study pharmacodynamic effects. The effective management of busulfan pharmacokinetic dose-based targeting reveals reduced rejection, relapse, discontinuity of drug, and death in patients of HSCT receivers. However, pharmacokinetic sampling of drugs has been a barrier to common acceptance of busulfan based on dose targeting [29].

11.7.5 5-Fluorouracil (5-FU)

5-Fluorouracil is an antimetabolite given as a parenteral dosage form to treat breast, colon, rectum, stomach, and pancreatic cancer and as a cream to treat actinic keratosis (it is a skin condition that may convert into cancer) and certain other types of basal cell skin cancer. 5-FU interface with the combination of fluorouridine triphosphate (FUTP) into RNA strand, combination of fluorodeoxyuridine triphosphate (FdUTP) into DNA strand and inhibition of thymidylate synthase (TS) enzyme by fluorodeoxyuridine monophosphate (FdUMP), leads to consequential DNA damage which finally results in apoptosis. Figure 11.5 shows mechanism of action of 5-fluorouracil.

Therapy is based on measurement of body surface area (BSA) and calculated administration of a dose of 5-FU. This produces extensive deviation of 5-FU systemic exposure in different patients that can be linked with the efficacy of the drug. The TDM of 5-FU administration is personalized for particular patients to the extent of BSA-based dosing. However, results of BSA do not link with any PK parameters in adult patients. A strong correlation has been established among concentration, efficacy, and toxicity. The dose modification of 5-FU is a viable and suggestively important technique for positive clinical results by reducing toxicities and boosting efficacy of TDM therapy [30].

11.7.6 Mitotane

Mitotane is used as an anticancer drug in some cases. It serves as a standard of drug to care for the patient after completion of surgical resection of adrenocortical carcinoma. It is useful in the treatment of cancer of the adrenal gland that cannot be treated with surgical procedure. It reduces the growth or size of the adrenal tumor. Mitotane interferes with the steroidal metabolism of peripheral parts and suppresses adrenal cortex activity. It reduces 17-hydroxycorticosteroids concentration in the absence of decreased corticosteroid level and increases formation of 6β-hydroxycortisol. The FDA recommends a starting dose of mitotane from 2 to 6 mg daily in divided doses, with a gradual increase in dose from 9 to 10 grams per day. This increment is dependent on the patient tolerance. The long half-life of mitotane occurs after the achievement of steady-state concentrations which is possible after some weeks of treatment. The recommended therapeutic dose of mitotane is from 14 to 20 mg/L. This is based on the recommendation of the European Medicines Agency in the treatment of adrenal cancer [31].

11.7.7 Tamoxifen

Tamoxifen is an antiestrogen medication that works by blocking estrogen's effects in breast cells and tissues. It is the most commonly prescribed selective estrogen receptor modulator (SERM) for breast cancer treatment, and it has been approved by the US Food and Drug

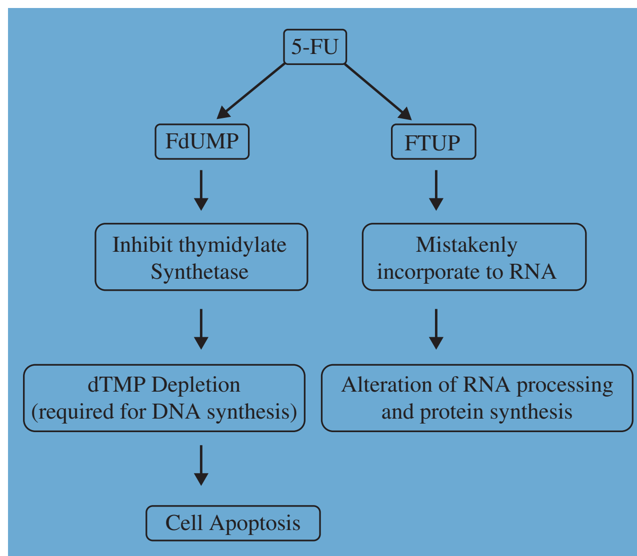

Fig. 11.5 Mechanism of action of 5-fluorouracil

Administration. It can help with estrogen receptor-positive breast cancer and its complications. Another agent mediates the activity of the selective estrogen receptor modulator (tamoxifen); it is primarily influenced by significant and more potent metabolites such as endoxifen. Endoxifen is largely processed by the hepatic microsomal enzyme CYP2D6, which is an aberrant enzyme. This is the problem of common genetic polymorphism in cancer patient.

Enzyme CYP2D6 is an indirect measure to predict the endoxifen plasma concentration. Therefore, TDM of tamoxifen is based on the monitoring of endoxifen plasma concentrations, and it has been advocated for individualizing tamoxifen therapy. The plasma concentration of the metabolite endoxifen can be increased by raising the tamoxifen dose without causing substantial adverse effects such as hot flushes or severe gastrointestinal discomfort and regardless of the patient's CYP2D6 genetic problem [32].

11.7.8 Imatinib

Imatinib is a monoclonal antibody. It serves as a specific tyrosine kinase receptor inhibitor employed in the treatment of different types of leukemia, myelodysplastic disease, systemic mastocytosis, dermatofibrosarcoma protuberans, hypereosinophilic syndrome, and other tumor conditions. The above conditions are noticeable by an unusual, constitutively articulated tyrosine kinase, and it results in nonregulated cell growth. Imatinib displays inter-individual variability in their pharmacokinetic. Greater body weight and increased apparent clearance of imatinib have both been linked to chronic myeloid leukemia. Decreased apparent clearance is associated with renal impairment and patients on concomitant medications with potent inhibition of cytochrome P450 3A4 enzyme. Patients with Philadelphia chromosome-positive chronic myelogenous leukemia and gastrointestinal stromal tumors are treated for a long period of time. As per available data, TDM for imatinib may give more information on efficacy, compliance, and safety than clinical evaluation alone [33]. The substantial variability in the relationship between medication dose and concentration is due to variations in CYP3A4 enzyme activity, which mediates the major metabolic route and controls both efflux from the gut wall and active secretion in the bile [34].

11.7.9 Pazopanib

Pazopanib is another monoclonal antibody antineoplastic agent; it is a kinase inhibitor type of anticancer drug. Pazopanib is used for stopping or

slowing the spread of cancer cells in the affected organ. Pazopanib is used in the treatment of advanced renal cell carcinoma (RCC, a type of cancer that begins in the cells of the kidneys) in adults' cancer patient. The vascular endothelial growth factor receptor (VEGFR) is inhibited by pazopanib. It also inhibits other receptors like platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and stem cell (c-Kit). Pazopanib similar to other tyrosine kinase inhibitors also has substantiation of interpatient variability in pharmacokinetic profile resulting in increased risk of suboptimal dosing when given in empirical fixed dose schedules of drugs in conventional form.

A study assessing the efficacy of pazopanib in renal cell carcinoma found a clear link between plasma concentration and medication outcome, with patients who achieved a steady-state trough concentration of pazopanib above 20.5 mg/L having a longer response and better tumor reduction. However, greater pazopanib plasma concentrations were linked to several side events, including diarrhea, hypertension, an increase in hepatic enzymes, and stomatitis. These issues can be managed if supportive medicine is administered concurrently. Pazopanib would benefit from a pharmacokinetically guided dosage approach due to its low safety margin, variable PK, and indications of an exposure-response link [35, 36].

11.8 Final Remark

Cancer is a major concern worldwide. The use of various techniques of TDM in oncology is a debatable issue as evident from the above discussions. The use of anticancer drugs is associated with severe side effects (mainly because some have narrow therapeutic indices), and often depending upon the patient's condition and state of cancer, drugs are given in combination. Consequently, the application of TDM may be required to assess the efficacy of anticancer drugs. However, the estimation of drug levels in cancer is a challenge in TDM. Finally, the TDM approach in cancer needs more research and studies for effective implementation of individualized therapy.

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12 Therapeutic Drug Monitoring and Toxicology of Immunosuppressant

Anshul Shakya, Rajdeep Sarma, Neha Ghimire, Surajit Kumar Ghosh, Hans Raj Bhat, and Obaidur Rahman

Abstract

Immunosuppressive medications have reached a great milestone in the treatment of numerous autoimmune diseases and the maintenance of immune response after organ transplantation. Antibodies, glucocorticoids, calcineurin inhibitors, and antiproliferative are the four main immunosuppressive drug classes now being utilized successfully in the current situation. It has been suggested for the treatment of severe autoimmune disorders and acute immunological rejection of organ transplants, but these medicines necessitate long-term usage and non-specifically inhibit all the immune system, resulting in patients suffering from higher risks of unwanted effects. Since, the pharmacokinetics of immunosuppressive medications is complicated and unpredictable; drug parameters such as therapeutic index, absorption, distribution, and elimination are unique and changeable to each individual. Every physician's goal is to personalize a patient's drug treatment to achieve the best feasible balance between therapeutic efficacy and the risk of side effects. Due to complicated inter- or intra-patient variability, getting the desired outcome isn't always as simple as it appears. Monitoring drug levels is critical, to ensure that immunosuppressants are maintained within their therapeutic ranges in the blood, thus minimizing the risk of rejection or toxicity. Some immunosuppressant drugs that have been shown to be effective (azathioprine, mycophenolic acid, tacrolimus, cyclosporine, sirolimus, everolimus, etc.) are briefly reviewed in this chapter, with a summary of their mechanisms of action, adverse effects, and toxicities followed by a general discussion of the monitoring of these immunosuppressive agents. Finally, a brief discussion of future trends in immunosuppression therapy is provided, as well as information on monitoring individual immunosuppressive medications.

Keywords

Immunosuppressants · Therapeutic drug monitoring · Immunosuppressants toxicity · Drug interactions · Cyclosporine

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12.1 Introduction

Immunosuppressants, also known as antirejection drugs, are those medicines that suppress the body's immune system and also lower the ability

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to reject a transplanted organ. For a long time, organ failure resulted in the patient's immediate death. Human organ transplantation has rescued a large portion of the population, during the last three decades. Unfortunately, there aren't enough organs to go around to save every patient's life. The available organs must be used as efficiently as possible, and the function must be maintained continuously, and for that organ rejection must be minimized. To avoid rejection of the transplanted organ, immunosuppressive medication must be administered after transplantation. Immunosuppressive medicines or immunosuppressants play a key role in preserving transplanted organ function and immunogenicity of the receiver's physiological system [1]. Earlier, allograft survival rates for organ transplants are very less because of their powerful cellular and humoral immunological responses. The implementation of novel immunosuppressive drugs has improved allogeneic transplantation survival rates dramatically over the past quarter century. Now, allogeneic bone marrow transplants are being performed more than 100,000 times a year throughout the world. In contrast to the early immunosuppressive drugs, which are mostly glucocorticoids and antimetabolites, newer immunosuppressive drugs have been launched, including belatacept (Nulojix, a CTLA4-Ig fusion protein), the first biologic drug, which disrupts a vital stage in the beginning of T-cell-mediated immune responses [2].

The therapeutic range of the immunosuppressive drugs currently in use is extremely low. As a result, for the patients who are in immunosuppressant therapy, their blood plasma concentration levels for immunosuppressant must be monitored continuously [3]. In immunosuppressant therapy, it is hard to predict an individual's reaction to a certain dose of immunosuppressive drugs, as immunosuppressants have notable pharmacokinetic variations between peoples; due to this, administration of an immunosuppressive drug is difficult. Therapeutic drug monitoring is an assay that determines a drug's concentration in blood plasma. So, the dosage of medication that has been taken is both safe and effective. In order to optimize individual dosing regimens, therapeutic drug monitoring measures certain medications at predetermined intervals. Medical professionals use therapeutic drug monitoring to keep track of a patient's blood levels of immunosuppressant and adjust their dosage accordingly. Therapeutic drug monitoring is only used to monitor drugs with limited therapeutic ranges, drugs with substantial pharmacokinetic variability, medications with difficult target concentrations to monitor, and drugs with known therapeutic toxicity and adverse effects. Due to their limited therapeutic index and considerable inter-individual variability in blood levels, immunosuppressants necessitate therapeutic drug monitoring. There are many factors that play a key role in this blood level variability, viz., sex, age, drug-nutrient interactions, inflammation, liver mass, infection, polymorphism, drug-disease interactions, and renal clearance impairments [4]. Some of the most frequently used immunosuppressants in several organ transplants are everolimus, cyclosporine A, sirolimus, and tacrolimus. In which, lymphocyte proliferation and cytokine productions are inhibited by cyclosporine A and tacrolimus, which act as calcineurin inhibitors, and by preventing the synthesis of interleukin-2. Sirolimus and everolimus impede the progression of the T-cell cycle. Serious adverse effects may appear if plasma blood level concentration of immunosuppressants is too high (including neurological side effects, nephrotoxicity, cardiotoxicity, and increased risk of infections) [5].

12.2 Therapeutic Drug Monitoring of Immunosuppressants

Numerous studies have indicated that therapeutic drug monitoring of immunosuppressants improves the clinical outcomes of transplanted patients. A summary of classification of the various immunosuppressants is shown in Fig. 12.1. These medications need constant monitoring due to the limited therapeutic index as well as the various drug-dietary interactions and drug-disease interactions that they have. When used in combination therapy, immunosuppressive medications have an additive effect because of

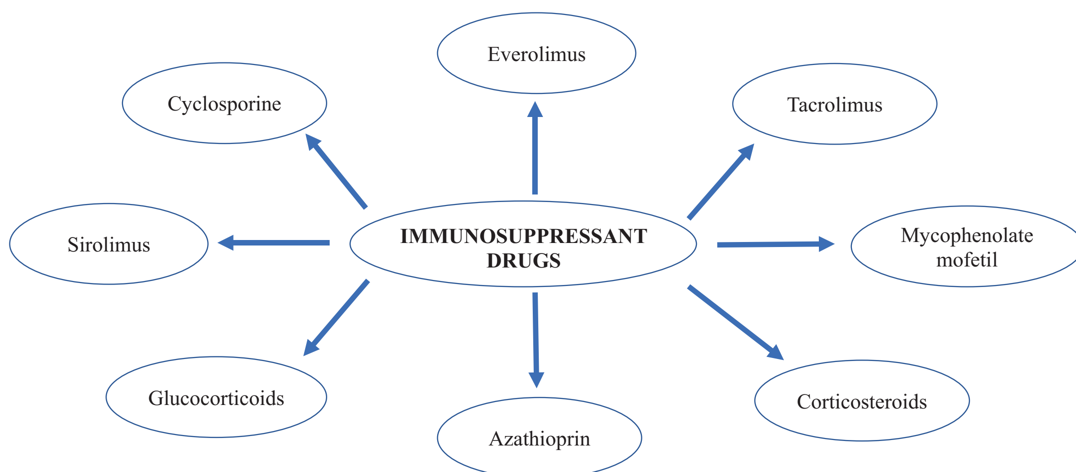

Fig. 12.1 Immunosuppressant drugs

their complementary modes of action. In therapeutic practice, they are commonly combined in order to minimize negative effects. This combination therapy of medications necessitates a simultaneous determination of immunosuppressive medicines. Drug monitoring is common for drugs like cyclosporine, tacrolimus, sirolimus, and mycophenolic acid [6, 7].

For several reasons, immunosuppressant drug treatment needs constant drug concentration monitoring.

1. Immunosuppressive medicines have narrow therapeutic ranges. Immunosuppressants such as cyclosporine, tacrolimus, sirolimus, and mycophenolate have a narrow therapeutic index and are critical-dose medicaments that show the intended therapeutic potential with acceptable tolerability only within a narrow blood concentration range [8].
2. Immunosuppressive medicines have severe consequences if the required therapeutic range is not met during therapy. There is an increased risk of infection and malignancies when over-immunosuppression or drug toxicity has occurred, and also there is a possibility of graft dysfunction and graft loss [9].
3. In between clinical disease stage, toxicodynamic effects of immunosuppressants can be difficult to diagnose, for example, distinguishing the source of nephrotoxicity is difficult when it is caused by either BK virus nephropathy or calcineurin inhibitors (cyclosporine and tacrolimus) which occurs during the impairment of kidney graft function [10].
4. In immunosuppressants therapy, the dose/exposure relationship is highly varied between intra- and inter-patient. When it comes to adjusting the dosage and target ranges for immunosuppressive drugs, it is all patient-specific. The dose/concentration relationship depends on a number of factors, such as genetic polymorphisms and drug-drug interactions, as well as interactions with food, and the environment might complicate [11].
5. Immunosuppressant drugs treatment cause drug dependency which can lead to critical conditions, especially in adolescents and young patients, and requires regular monitoring [12].

12.3 Classification of Immunosuppressant Drugs

12.3.1 Calcineurin Inhibitors: (Cyclosporine and Tacrolimus)

Cyclosporine Cyclosporine is a fungal cyclic polypeptide which contains of 11 amino acids, which has drastically altered the field of organ

transplant in the past 30 years. It acts as an immunosuppressive agent by disrupting the initiation and escalation of the cytotoxic T cells [13]. Cyclosporine acts by blocking the production of a T-lymphocyte lymphokine, i.e., interleukin-2. Lymphokines are responsible for the regulation of the immune response to a transplanted organ. Cytochrome P450 is the enzyme that breaks down cyclosporine and oxidized to at least 12 metabolites in the liver [14]. In kidney transplant patients, poorly absorbed cyclosporine may lead to graft rejection. The ethno-pharmacokinetics of racial groups haven't been noted, but it has been observed that the black patients absorb less than that of the whites [15].

Cyclosporine is used as a combination therapy with corticosteroids, azathioprine, and mycophenolic acid for kidney, liver, heart, skin, and bone marrow transplantation. Cyclosporine is administered orally at a dose of 7–9 mg/kg/day [16]. The inhibitors of CYP3A4 (viz., erythromycin, fluvoxamine, nefazodone, losartan, grapefruit juice) increase the level of cyclosporine by inhibiting CYP3A4. The inducers of CYP3A4 or P-glycoproteins (like phenobarbital, phenytoin, rifampin, modafinil, St. John's wort) decrease the level of cyclosporine [13]. Higher than normal levels of cyclosporine may lead to neurotoxicity or nephrotoxicity but below the therapeutic range of drugs may lead to transplant rejection [13].

The pharmacokinetic study of cyclosporine has a low oral bioavailability of about 30% [17]. Distribution of cyclosporine in the blood is 41–58% approximately in erythrocytes, 5–12% in granulocytes, 33–47% in plasma, and 4–9% in lymphocytes [18]. It is metabolized by the cytochrome P450 3A (CYP3A). 90% of the drug is excreted via bile [19]. Absorption of cyclosporine is affected by P-glycoproteins after oral administration, as it clears the drug from the cells to the intestinal lumen and is metabolized by 3A4 intestinal activity [13].

Tacrolimus Tacrolimus is procured from *Streptomyces tsukubensis* and is used for the preventive treatment of liver and kidney transplant exclusion and T-cell-mediated autoimmune diseases [16]. The presence of clinical findings along with fever, decrease in urine output, rise in blood pressure, weight gain, and increase in the level of serum creatinine leads to the determination of acute renal transplant refusal. Percutaneous renal transplant biopsy confirms the suspected cases of rejection (acute) before induction of antirejection treatment [20]. Tacrolimus inhibits T-cell activation by attaching to the FK-binding protein. The resultant complex binds to calcineurins and hinders dephosphorylation of the transcription factor [21], and signal transduction pathways in T cells are interrupted by the tacrolimus-FKBP12 complex [22]. Blockade of interleukin-2 gene transcription leads to failure of T-cell clonal expansion and differentiation of precursor to mature cytotoxic T cells [21]. The oral dose of tacrolimus is *bid* 0.15–0.30 mg/kg/day and by *i.v.* route is 0.05–0.20 mg/kg/day [22]. Tacrolimus has a low oral bioavailability, with considerable intra- and inter-individual variability ranging from 4% to 89% [21]. The plasma concentrations are reached after about 0.5–1 h. Further, tacrolimus is eliminated by hepatic metabolism in the bile [22]. Inhibitor of CYP3A4, which enhances the tacrolimus activity, includes diltiazem, fluconazole, erythromycin, clarithromycin, itraconazole, and indinavir. Rifampin, phenobarbital, and phenytoin are the inducers of CYP3A4 which decreases the level of tacrolimus. The adverse effect includes, hyperkalemia, hypomagnesemia, tremors, insomnia, paresthesias, and irritability [23].

12.3.2 Antiproliferative Agents: (Mycophenolate Mofetil, Azathioprine, Methotrexate, Cyclophosphamide, and Chlorambucil)

Mycophenolate Mofetil Mycophenolate mofetil is a prodrug of the mycophenolic acid that inhibits the proliferation of T and B lymphocytes through reversible and non-competitive inhibition of the inosine monophosphate dehydrogenase. Mycophenolate mofetil is basically administered at a fixed dose. The recommended

starting dose is 720 mg/kg/day [24]. It may be active against both acute and chronic refusal [25]. The mechanism of action of mycophenolic acid is based on blocking of purine synthesis [26] and mesangial proliferation [27]. Purine synthesis occurs by two major pathways: the salvage pathway and the de novo pathway [28]. In the de novo pathway, ribose 5-phosphate which is a product of the pentose phosphate pathway is phosphorylated to 5-phosphoribosyl-l-pyrophosphate by adenosine triphosphate. The glycosylation and expression of adhesion molecules, as well as lymphocyte and monocyte migration to inflammatory sites, are all inhibited by mycophenolic acid [29]. Mycophenolic acid reversibly inhibits inosine 50-monophosphate dehydrogenase, which is a key enzyme in the immune system. The de novo synthesis of guanosine nucleotides is blocked by the inhibition of inosine 50-monophosphate dehydrogenase, which are necessary substrates for RNA and DNA synthesis [30]. Oral administration of mycophenolate mofetil is rapid and quickly gets converted to its active metabolite, i.e., mycophenolic acid [31]. The glucuronide metabolites are eliminated through the kidney, and 95% of a given dose of mycophenolate mofetil is excreted in the urine as glucuronide metabolites [32]. The adverse effects related to mycophenolate mofetil are abdominal pain, nausea, diarrhea, decreased appetite accompanying weight loss, vomiting, hematological problems such as leukopenia, and symptomless anemia due to bone marrow toxicity [33].

Azathioprine Azathioprine is considered the first immunosuppressive agent that was acceptable for successful renal transplantation in humans [34]. The initial dose of azathioprine is 3–5 mg/kg orally or i.v. once a day. The medical efficacy of azathioprine is reliant on its metabolism to 6-thioguanine nucleotides and the amalgamation of 6-thioguanine into cellular DNA [35]. Azathioprine interferes with the synthesis of RNA and DNA and exerts its action by preventing the differentiation and escalation of B and T lymphocytes. Azathioprine is considered an inhibitor of nucleotide synthesis. In maintenance drug protocol, azathioprine is used in combination with cyclosporine/tacrolimus and corticosteroids [36]. Azathioprine and allopurinol when given in combined form cause leukopenia. The adverse effect of azathioprine is gastrointestinal disturbances, hepatic toxicity, and bone marrow suppression [37].

12.3.3 mTOR Inhibitors: (Sirolimus and Everolimus)

Sirolimus Sirolimus obtained from *Streptomyces hygroscopicus* consists of a macrocyclic lactone, which is characterized by its potent immunosuppressant activity. Interestingly, sirolimus is used in organ transplantation because of its distinctive mechanism of action, low side effect, and ability to synergize with other immunosuppressants. At the later stage of the cell cycle, sirolimus reduces the activation of T lymphocyte by preventing the post-interleukin-2 receptor mTOR signal transduction pathway. Cytochrome P450 and 3A4 isoenzyme metabolize the drug [38]. The mechanism of sirolimus is that it operates in the cytosol by binding to the immunophilin FK-binding protein-12. This complex prevents the activation of the mammalian target of sirolimus (mTOR), which is a key regulatory kinase. Hence, the inhibition will suppress the cytokine-mediated T-cell proliferation inhibiting the progression from the G_1 to the S phase of the cell cycle. Thus, at the later stage, sirolimus will inhibit the progression of the cell cycle. The oral dose of sirolimus is 3–5 mg/kg [39]. Sirolimus is absorbed rapidly; the mean peak whole-blood sirolimus concentrations (C_{max}) occur 1 h after administration of a single dose and 2 h after multiple doses in renal transplant patients. The systemic availability of sirolimus is 14% [40]. In human whole blood, sirolimus is distributed among red blood cells (94.5%), plasma (3.1%), lymphocytes (1.01%), and granulocytes (1.0%) [41]. Sirolimus is primarily metabolized not only by CYP3A4 but also by CYP3A5 and CYP2C8 [42]. The area under the curve of sirolimus is increased by diltiazem and ketoconazole by 60% and 90%; however, rifamy-

cin decreases the area under the curve by 82% [41]. The concentration of sirolimus is increased by diltiazem, ketoconazole, and cyclosporine, whereas rifampin reduces sirolimus exposure [42]. Inhibitors like diltiazem, verapamil, itraconazole, ketoconazole, voriconazole, fluconazole, erythromycin, and clarithromycin increase the concentration of sirolimus. Enzyme inducers, viz., phenytoin, carbamazepine, and rifampin, decrease the sirolimus exposure [43]. The adverse effects related to sirolimus are bone marrow suppression, hyperlipidemia [44], anemia, post-transplant diabetes mellitus, proteinuria, edema, mouth ulcer, and joint pain [45].

Everolimus The hydroxyethyl derivative of sirolimus is everolimus [46]. A comparable immunosuppressive mechanism to that of sirolimus, namely, the suppression of the mammalian target for rapamycin, is found in everolimus [47]. It also lowers angiogenesis while reducing cytokine-mediated T-cell proliferation. In comparison with sirolimus, the half-life of everolimus is shorter, and it quickly achieves steady-state trough concentrations. It does, however, have a small therapeutic window of 3–8 mg/mL [48]. When given in doses of 2.5 mg/kg, everolimus is rapidly absorbed following oral administration, with an average *C*-max of 45 mg/L. After 24–78 minutes, the maximal concentration of the drug is reached. Additionally, CYP3A4, 3A5, and 2C8 are involved in the metabolism of everolimus, and it is a substrate for P-glycoprotein. Everolimus used as 1 mg/kh of body weight twice a day for renal transplants and 0.75 mg/kg of body weight for liver transtplants recipients [49].

Corticosteroids Corticosteroids largely affect T-cell activation by decreasing the synthesis of T-cell cytokines including IL-2, IL-6, and interferon gamma, which are necessary for lymphocytes and macrophages to respond to allograft antigens. They also promote T-cell migration from the intravascular compartment to lymphoid tissue by inhibiting antibody and complement binding [50].

Glucocorticoids (Prednisolone) Immunosuppressive medications such as glucocorticoids were first introduced to the market in the 1960s and played a key role in making organ transplantation possible. Peripheral blood lymphocyte counts are reduced rapidly due to the lysis and redistribution of lymphocytes induced by glucocorticoids. In the long run, receptors, glucocorticoid-induced proteins, or other interacting proteins regulate the transcription of numerous genes in response to hormones [51]. IkB expression is enhanced by glucocorticoid receptor complexes, which suppresses NF-kB activation and induces apoptosis in activated cells. The downregulation of key pro-inflammatory cytokines including IL-1 and IL-6 is critical. There is a block on the production and proliferation of IL-2 by T cells. Cytotoxic T-lymphocyte activation is suppressed. Chemotaxis is poor in neutrophils and monocytes, and also lysosomal enzyme release is reduced. As a result, glucocorticoids exert anti-inflammatory effects on a variety of immune system components [52]. An oral dose of prednisolone is 5–60 mg/kg/day. Diltiazem and ketoconazole both being the inhibitors of CYP3A4 increase the levels of prednisolone, thus enhancing the toxic activity of the drug. The inducers of CYP3A4 such as phenytoin, rifampin, and phenobarbital increase the metabolism of glucocorticoids and thereby lower the level of prednisolone. Side effects related to glucocorticoids are osteoporosis, predisposition to infection, hyperglycemia, weight gain, hypertension, glucose intolerance, adrenal suppression, psychosis, and depression [53].

12.4 Adverse Effects and Toxicity of Newer Immunosuppressant Drugs

Leflunomide and Malononitriloamides The newest class of immunosuppressant medicines, leflunomide and the malononitriloamides, are currently being studied for use in transplantation. Leflunomide's anti-inflammatory and immunomodulating capabilities were discovered in 1985, which set it apart from other anti-inflammatory

and immunosuppressive medications. Leflunomide's immunosuppressive effects have been studied extensively in animal transplantation models. Leflunomide's clinical development has been limited to usage in patients with autoimmune illnesses such as rheumatoid arthritis due to its long half-life (11–16 days) in humans [54, 55]. The most important side effect of leflunomide and the malononitriloamides in cynomolgus monkeys was anemia. GI problems, rashes, allergic reactions, weight loss, and reversible baldness were among the side events documented in the leflunomide research. Infections were not more common in the leflunomide group, but hematocrit and hemoglobin levels were lower in all groups [56].

Mycophenolic Acid *Penicillium* spp. cultures were used by Gosio in 1896 to produce mycophenolic acid, which was refined by Alsberg and Black in 1913. In the 1940s, antibacterial and antifungal properties were discovered. Mycophenolic acid was researched for psoriasis but did not acquire clinical use when its antitumor action was discovered in 1968 [57]. Its immunosuppressive characteristics have been demonstrated by Mitsui and Suzuki [58]. Mycophenolic acid was approved by the FDA in 1995 for the treatment of acute renal allograft rejection. For heart transplant recipients, it was approved for usage in 1998 [59]. Diarrhea, vomiting, opportunistic infections, and leukopenia are the most prevalent side effects of mycophenolic acid in humans. Mycophenolic acid's myelotoxic mechanism is still a mystery. Only proliferating lymphocytes will be affected by mycophenolic acid's specific suppression of de novo purine production. Patients with psoriasis treated with mycophenolic acid rarely experience leukopenia, unlike transplant recipients [60].

Sirolimus Sirolimus was discovered initially as an antifungal agent in the mid-1970, which is a microbial product derived from the actinomycete *Streptomyces hygroscopicus* [61]. Because of its immunosuppressive effects, it was not further developed for clinical use as an antibiotic [62]. Preclinical investigations and Phase I and II studies in stable kidney recipients have largely predicted the current profile of adverse events in humans. Short-term sirolimus administration has been linked to headache, nausea, dizziness, changes in blood glucose levels, epistaxis, infection, and a decrease in platelets and white blood cells [63, 64]. The long-term usage of rapamycin has been linked to an increased risk of hypertriglyceridemia. In multiple trials in rats and pigs, sirolimus was found to be free of the nephrotoxicity seen with tacrolimus and cyclosporine A, probably due to the lack of calcineurin inhibition. However, in normal rats receiving sirolimus, hypomagnesemia and tubular damage were observed, and in spontaneously hypertensive rats, the progression of kidney failure has been documented. In rats, excessive doses of sirolimus have caused heart and retinal infractions [65–67].

Tacrolimus Tacrolimus is a metabolite of *Streptomyces tsukubaensis*, an actinomycete that was initially shown to be immunologically effective in rat heart allograft recipients in 1987 [68]. In various experimental models, it was quickly discovered to be a strong alternative to cyclosporine A. Patients using tacrolimus have experienced significant nephrotoxicity and neurotoxicity. The suppression of calcineurin phosphatase is one plausible mechanism for neurotoxicity, but the etiology of its renal vasculopathic consequences is unknown. Increased thromboxane A2, endothelin production, or enhanced intrarenal renin production are all linked to decreased renal glomerular and cortical blood flow and increased renal vascular resistance [69, 70]. Cardiomyopathy, anemia, persistent diarrhea, diabetes, and allergies have all been documented in tacrolimus patients. Hypercholesterolemia and hypertension are less common when compared with cyclosporine A, while gingival hyperplasia and hirsutism are almost non-existent in patients using chronic tacrolimus [71]. Tacrolimus-based immunosuppressive treatments have been linked to lymphoproliferative illness and infections [7].

12.5 Immunosuppressant Drug Interactions

Immunosuppressants can have a number of interactions, some of which are enumerated in Fig. 12.2.

Drug-Nutrient Interactions The importance of drug-nutrient interactions is increasingly becoming recognized. Depending on the patient's diet, the metabolism of some medicines might be affected. Many medication's metabolisms are altered when eaten in large quantities with grapefruit juice, for example. Studies have shown that the CYP3A-mediated metabolism of cyclosporine is inhibited by grapefruit juice and enhancing medication absorption by blocking P-glycoprotein efflux transporters. As a result, if a patient is taking cyclosporine and grapefruit juice at the same time, the medication concentration should be checked [72, 73].

Drug-Disease Interactions Drug-disease interactions may possibly have a role in the diversity of plasma immunosuppressive concentrations across individuals. For example, due to a decrease in protein binding, renal insufficiency can cause a change in the free fraction of mycophenolic acid. Furthermore, a decrease in cytochrome P450 enzyme activity is commonly related with inflammation and infection. Cytochrome P450 enzyme enhances the amount of cyclosporine A metabolism [74].

Gender Gender also has an impact on drug concentration. Variances in drug responses between men and women can be caused by differences in their biology. Both pharmacokinetic and pharmacodynamic parameters vary in men and women, but more evidences have been reported on variance of pharmacokinetic parameters. For example, women may have slightly better bioavailability following oral medication administration than men, particularly in CYP3A substrates. It is known that mycophenolic acid is primarily metabolized in the liver [75]. Velickovic et al. studied renal transplant recipients taking the immunosuppressive drugs such as tacrolimus, mycophenolate mofetil, methylprednisolone, and basiliximab were showed substantial gender-related differences after the first oral dose [76].

Polymorphism Polymorphism has demonstrated functional consequences of many drug-metabolizing enzymes. CYP3A4/5 and P-glycoprotein are recognized substrates for cyclosporine A. One of the most important CYP3A enzymes, CYP3A4/5, has a polymorphic expression pattern and is ethnically dependent. Cytochrome P450 (CYP)3A enzymes in the intestines and liver are primarily responsible for the metabolism of tacrolimus. It also serves as a substrate for P-glycoprotein, which carries diffused tacrolimus back into the gut lumen after it has left intestinal cells. Tacrolimus is predicted to have an effect on the elderly because of age-related variations in CYP3A and P-glycoprotein expression as well as changes in liver mass and body composition [19, 77].

12.6 Toxicity Related to Immunosuppressant Agents

As new immunosuppressive drugs with novel mechanisms of action, new formulations, and improved means for routine therapeutic drug monitoring have become available to transplant recipients, the efficacy and tolerance of therapy have improved. Life expectancy and quality of life for organ transplant recipients have improved dramatically in recent years. Neurotoxicity, nephrotoxicity, hepatotoxicity, and bone marrow depression (Fig. 12.3) are all the serious side effects that come along with the use of most immunosuppressive drugs that have not yet been adequately studied. Hematological toxicity, such as leukopenia, thrombocytopenia, and anemia due to bone marrow suppression or hemolysis, may occur as a result of immunosuppressive drug delivery. Allogeneic bone marrow suppression is more common with the use of azathioprine and

Fig. 12.2 Immunosuppressant drug interactions

mycophenolate mofetil, but hemolytic uremic syndrome is more common with the use of cyclosporine, tacrolimus, or muromonab. Long-term usage of immunosuppressant drugs such as cyclosporine can lead to side effects like hirsutism and gingival hyperplasia as well as alopecia or cushingoid facies and an increase in blood creatinine [45].

12.7 The Toxicities Related to Various Immunosuppressants Are as Follows

Nephrotoxicity Nephrotoxicity is defined as the functional abnormalities of the kidney, which are often caused by drugs. Nephrotoxicity is basically dose-dependent and related to the patient's bioavailability. Increased renal vascular resistance leads to acute nephrotoxicity. Acute nephrotoxicity is categorized by the decrease in glomerular filtration rate and reduction of renal blood flow with an increase of filtration fraction. However, nephrotoxicity is quite difficult to distinguish from long-term renal transplant rejection. Though nephrotoxicity has been reported in both cyclosporine and tacrolimus, it has also been reported that cyclosporine is more nephrotoxic than tacrolimus [46]. Cyclosporine, when prescribed for a longer period of time, may cause a rise in serum creatinine levels. On the other hand, cyclosporine prescribed in lower doses or when the patient is prescribed with other immunosuppressants such as azathioprine leads to a decrease in serum creatinine levels [14].

Hepatotoxicity Hepatotoxicity indicates chemical-driven liver damage. It is the leading cause of liver failure. Cyclosporine and azathioprine have been reported to cause infrequent but significant hepatotoxicity. Conjugated hyperbilirubinemia is a common symptom of cholestasis caused by cyclosporine. Cyclosporine-induced cholestasis appears to be a result of bile acid transport impairment. In several studies, cyclosporine has been associated with the development of bile duct calculi. Hyperbilirubinemia may occur in patients receiving large oral doses of cyclosporine to an extent of 30–50% [78]. High doses of glucocorticoids may cause hepatomegaly, and macrovesicular steatosis may result from a low dose of prednisolone. Azathioprine has been related to a wide range of hepatotoxic responses, all of which are believed to be caused by azathioprine-induced destruction to the endothelial cells that line the hepatic terminal venules and sinusoids. Azathioprine elevates liver damage when used for a longer period of time. Some research suggests that liver transplant recipients who have viral hepatitis or are experiencing continuous rejection are more susceptible to developing these hepatotoxic responses [79].

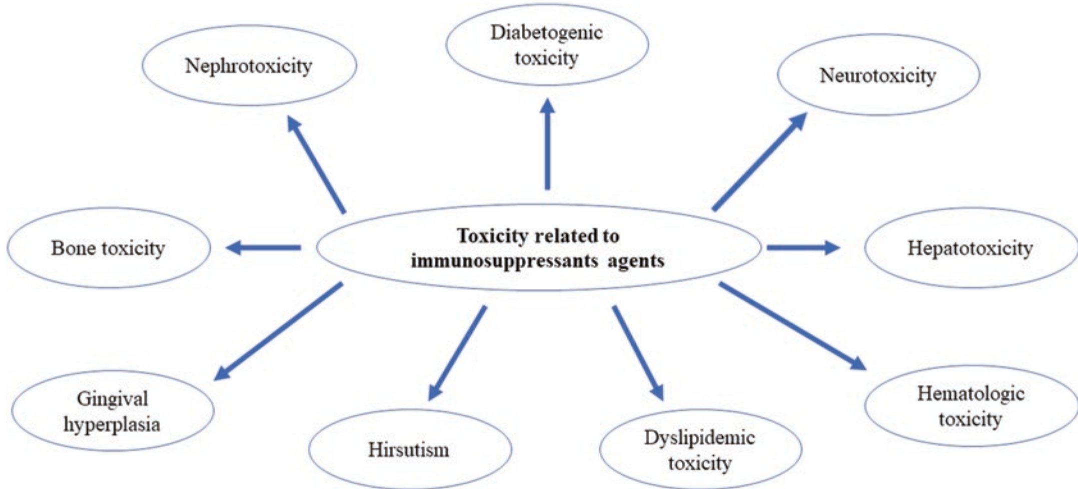

Fig. 12.3 Toxicity related to immunosuppressant agents

Neurotoxicity The frequency of neurotoxicity varies according to the organ transplanted. Fine tremor, generalized seizures, paresthesia, and encephalopathy have been related to cyclosporine therapy [80]. The symptoms of mild neurotoxicity using tacrolimus include tremor, headache, nightmares, vertigo, insomnia, photophobia, mood disturbance, or dysesthesia, and the symptoms of severe neurotoxicity include akinetic mutism, focal deficits, cortical blindness, seizures, psychosis, or encephalopathy [81].

Hirsutism Hirsutism is a well-known, dose-dependent pharmacological effect of cyclosporine therapy, occurring in approximately 40% of the patients. The combination of minoxidil and cyclosporine leads to dramatic hair growth [82]. In renal transplantation allograft patients, the use of cyclosporine for immunosuppression has been associated to hypertrichosis. Patients who are switched to tacrolimus may have an improvement in these effects with little risk of rejection or allograft failure [83]. Eighty percent of individuals assessed had hirsutism, alopecia, acne, or cushingoid facies as a result of immunosuppression. In cyclosporine-treated patients, 94% had hirsutism, compared to non-cyclosporine-treated patients. Hypertrichosis, with hair growth concentrating on the upper body and face, appeared by the third month in over half of 402 kidney transplant recipients. The greatest number of people who were affected were children. Cyclosporine may cause hypertrichosis by raising the activity of 5-reductase in peripheral tissues, but the exact mechanism is unknown. Tacrolimus stimulated hair growth in animal experiments [14].

Gingival Hyperplasia Dose-related gingival hyperplasia is more common in children, and it can be severe enough to necessitate gingivectomy. Another well-known adverse effect of cyclosporine is gingival hyperplasia. McGaw et al. [84] found fibrous hyperplasia of the gums in up to 33% of patients, and King et al. [85] documented it in 22% of kidney transplant recipients. Gingival hyperplasia is especially dangerous and stressful for pediatric transplant recipients since it might cause teeth to erupt later and speech development to be hampered. Recently, a link between gingival overgrowth and alterations in renal function in juvenile renal transplant recipients has been discovered. This shows a link between the fibrosis seen in gingival overgrowth and cyclosporine-induced nephrotoxicity, and it's tempting to think that transforming growth factor beta is involved in the mechanism [86].

Hematologic Toxicity Immunosuppressants can cause hematologic damage, such as bone marrow suppression or hemolysis, thrombocytopenia, and leukopenia, when taken for a long time. Hemolytic uremic syndrome can be caused by the use of tacrolimus, cyclosporine, or muromonab. The most prevalent and dangerous side effect of azathioprine therapy is myelosuppression, particularly leukopenia [87]. In addition, platelet and leucocyte counts were reduced, resulting in a myelosuppression. Immunosuppressive medications may be reduced or discontinued, blood transfusions may be required, or cytokines, such as erythropoietin and colony-stimulating factors, may be used to treat bone marrow failure [88].

Bone Toxicity Risk factors for bone disease, such as limited mobility, menopause, cirrhosis, diabetes, hyperparathyroidism, and renal osteodystrophy, are common among transplant patients. Heart, kidney, liver, lung, and bone marrow transplantation have all been linked to the development of post-transplantation osteopenia. Kidney transplant recipients, on the other hand, appear to be less vulnerable than heart and liver transplant recipients [89]. Immunosuppressant-induced or immunosuppressant-exacerbated bone loss can occur after transplantation and is usually identified as a decrease in bone mineral density. The immunosuppressants most strongly linked to osteopenia and osteoporosis are steroids [90]. More than half of people who had long-term glucocorticoid medication developed osteoporosis, according to a retrospective evaluation of 160 research published since 1970. Many studies have linked steroids to post-transplantation osteopenia and osteoporosis in the context of transplantation. The incidence of demineralization is linked to both cumulative and daily steroid dosage levels. Experiments on rats have demonstrated that cyclosporine and tacrolimus alter the equilibrium between bone formation and bone resorption, resulting in considerable bone loss [91, 92].

Diabetogenic Toxicity Some immunosuppressants have been found to be diabetogenic, meaning they can cause diabetes in people who aren't diabetic. This makes pre-transplant and post-transplant care even more difficult. Furthermore, those with diabetes mellitus after a transplant are more prone to get serious infections and have lower patient and graft survival rates. The development of glucose intolerance and post-transplant diabetes mellitus has long been regarded to be one of the main side effects of high-dose steroid regimens [93]. In a recent study, glucose intolerance was evaluated in 173 kidney transplant patients utilizing oral glucose tolerance tests or diabetes mellitus diagnosis 10 weeks after donation. It has been found that 0.01 mg/kg/day of prednisolone increases the likelihood of developing diabetes mellitus following a transplant by 5%. Higher steroid dosage and older age are both connected to the development of post-transplant glucose intolerance. Steroids' diabetogenic side effects are likely to be more severe in black people, who have been proven to metabolize steroid dosages more slowly, resulting in increased drug exposure. Steroids are still implicated in the development of post-transplant diabetes mellitus, according to new research [94].

Dyslipidemic Toxicity One of the most common side effects of immunosuppressant therapy is dyslipidemia, which affects 80% of heart transplant recipients, 60–70% of kidney transplant recipients, and 45% of liver transplant recipients. Allograft survival is reduced when cholesterol and triglyceride levels are increased [94]. It was shown that cyclosporine has a detrimental influence on the serum lipid levels of people with amyotrophic lateral sclerosis (ALS). These patients exhibited elevated levels of serum low-density lipoprotein cholesterol. In a study of liver transplant recipients, tacrolimus and cyclosporine were found to dramatically raise serum levels of cholesterol and triglycerides [95].

Table 12.1 Classification of the immunosuppressive agents with their possible mechanism of action, indication(s), toxicity, and dose

Sl No.	Classification	Sub class	Mechanism of action	Indication(s)	Toxicity	Dose	Citation
1.	Calcineurin inhibitors	Cyclosporine	Cyclosporine suppresses the generation of the T-cell lymphokines y-interferon, macrophage inhibitory factor, and macrophage chemotactic factor, which decrease monocyte activity indirectly	Immunosuppressive agents used in organ transplantation	Nephrotoxicity, hirsutism and gingival hyperplasia, hepatotoxicity, neurotoxicity, lymphoproliferative neoplasms	7–9 mg/kg	[14]
2.	Calcineurin inhibitors	Tacrolimus	Tacrolimus works by attaching to FKBP-12, an intracellular protein that inhibits T-lymphocyte activation	Prevent the rejection of liver and kidney transplants. According to the pediatric liver transplantation studies registry, it is the most commonly utilized maintenance therapy for pediatric transplant recipients	Nephrotoxic, diabetogenic, neurological, and cardiovascular effects	i.v. dose is 0.05–0.20 mg/kg/day and oral dose bid 0.15–0.30 mg/kg/day 12 h apart	[96]
3.	Antiproliferative agents	Mycophenolate mofetil	Guanosine monophosphate nucleotide synthesis is inhibited, and purine synthesis is blocked, preventing T- and B-cell proliferation	Add on drug to glucocorticoid and cyclosporine in renal transplantation	Gastrointestinal toxicity, occasional leukopenia	720 mg/kg/day	[32]
4.	Antiproliferative agents	Azathioprine	It inhibits purine synthesis, affects T cells, and inhibits cytolytic lymphocytes	Azathioprine has a long history of use in the treatment of inflammatory bowel disease	Bone marrow depression hepatotoxicity, post-transplant lymphoproliferative disorder (PTLD)	3–5 mg/kg orally	[97, 98]
5.	m-TOR inhibitors	Sirolimus	Target of rapamycin and interleukin-2 inhibited by the complex which is formed by binding to FKBP12	For therapy and prophylaxis of graft rejection reaction	Edema, diabetes, elevation in lipids, anemia, thrombocytopenia, proteinuria, impaired wound healing, interstitial lung disease, lymphoma, and various cancers are only a few examples	3–5 mg/kg daily	[44, 99]
6.	IL 2 receptor antagonist	Daclizumab and basiliximab	Activated T cells are depleted, and interleukin-2-induced T-cell activation is inhibited by binding and blocking the interleukin-2 receptor A chain (CD25 antigen)	It prevents renal and other transplant rejection reaction		5 mg/mL; 150 mg/mL	[100]

12.8 Conclusion and Future Perspective

Based on the above information, it can be concluded that the therapeutic drug monitoring of immunosuppressants has undergone an evolutionary change to minimize drug toxicity. Therapeutic drug monitoring is still evolving rapidly in the field of organ transplantation with continuous practices and currently has become a significant standard for most of the immunosuppressive drugs (compiled in the Table 12.1) such as tacrolimus, sirolimus, cyclosporine, mycophenolic acid, etc. It is necessary to perform therapeutic drug monitoring for all of these drugs, despite the fact that their pharmacokinetics are complex and variable, because of the small therapeutic ranges, large inter-individual variability in blood concentrations, gender differences in drug metabolism, and interactions between drugs, nutrients, and other drugs. Nearly two decades, cyclosporine has been the most widely investigated immunosuppressant medication in transplantation, leading to its advanced role in combination therapy that has brought a major improvement in clinical outcomes in transplant recipients. Therefore, combination therapy is becoming more common. Physicians strive to personalize a patient's drug therapy in order to establish the best possible balance between therapeutic efficacy and the risk of adverse effects, but because of complicated inter- or intra-patient variability, getting the desired outcome isn't easy, which concludes that understanding the pharmacokinetic precepts of immunosuppressant drugs is critical for transplant success.

Most given immunosuppressants attach to red blood cells and plasma proteins; the unbound drug is the only one that can interact with the targets. As a result, determining the free drug concentration rather than the total drug concentration in whole blood may offer more information on immunosuppressive effects. The adaptation of appropriate extraction techniques and the accomplishment of extremely low quantification limits are two of the key drawbacks of this methodology. New immunosuppressants, such as sotrastaurin, are now being investigated and show pharmacokinetic variability equivalent to already used immunosuppressants, which may be useful in monitoring therapy. Along with the development of new immunosuppressive medications, generic formulations of existing potent immunosuppressant drugs, such as cyclosporine, will be available in the coming age, and the possibility of xenotransplantation will be a future challenge that will require a significant amount of therapeutic drug monitoring. It is clear from the book chapter that therapeutic drug monitoring has significantly improved treatment outcomes, but understanding the limitations of therapeutic drug monitoring is critical for the appropriate development of immunosuppressive drug therapy in the organ transplantation.

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13 Therapeutic Drug Monitoring and Toxicology: Relevance of Measuring Metabolites

James Akingbasote, Sandra Szlapinski, Elora Hilmas, Patrik Miller, and Natalie Rine

Abstract

Therapeutic drug monitoring (TDM) is a valuable tool that healthcare providers have employed to optimize therapeutic efficacy of drugs while also maintaining drug concentrations below toxic levels. Although TDM has predominantly focused on determining the level of the parent drug compound in order to guide the dosing regimen, it is important to consider that some drugs are inactive in their parental forms and only their metabolites have therapeutic applications. Moreover, some drug metabolites possess distinct toxicity profiles from the parental compounds which are important to consider when evaluating the toxicity of a drug as a whole. This chapter investigates the role of metabolites in the safety assessment of drugs that are subject to TDM, such as those with narrow therapeutic windows. Examples of TDM of metabolites and mechanisms involved in metabolite-induced drug toxicity are discussed, drawing from several examples of drugs that are used in the clinical setting. This chapter emphasizes the utility of TDM of metabolites, demonstrating how select drugs can be converted to reactive metabolites with toxicity potential, and these should be considered when evaluating the efficacy and toxicity of a drug. The impact of pharmacogenetics in drug metabolism and the potential impact on efficacy and safety of drugs subject to TDM are also discussed. Finally, a cost-benefit analysis is made to support the inclusion of metabolite determination in routine TDM practice toward optimization of drug therapy.

Keywords

Bioactivation · Cytochrome P450 · Metabolites · Polymorphism · Pro-drugs · Uridine diphosphate glucuronosyltransferase

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13.1 Brief Overview of Therapeutic Drug Monitoring (TDM)

As described in previous chapters in this book, TDM involves the determination of drug concentrations in the plasma, serum, or blood during treatment or for diagnostic purposes. In the clini-

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cal setting, TDM is aimed at guiding drug dosing regimens to ensure that drug concentrations remain within their target range in the blood [1]. This is especially important to guide dosing for drugs with variable or unpredictable pharmacokinetics and for drugs with narrow therapeutic indices, to guide withdrawal of drug therapy, to monitor and detect drug interactions, to monitor efficacy failure, to monitor unexpected treatment failures or adverse effects, and to monitor drug compliance particularly for drugs with non-readily observable therapeutic responses and for drugs with long-term therapy (e.g., anticonvulsants) and life-threatening situations (e.g., epilepsy or sepsis) [2–6]. While TDM may help account for pharmacokinetic differences among drugs, pharmacokinetics of these drugs can also be further complicated by intra- or inter-individual variability due to body size and composition, age, organ function, disease states and differences in expression and activity of drug-metabolizing enzymes (e.g., cytochrome P450 (CYP450) and uridine diphosphate glucuronosyltransferase (UGT), among others), enzyme induction/inhibition, and drug-drug interaction [4]. These differences are especially important for drugs that have a narrow target range or those with concentration-dependent pharmacokinetics [1]. Faced with these factors, TDM is a very important tool in the hand of healthcare providers in order to optimize therapeutic efficacy while also maintaining drug concentrations below toxic levels.

In TDM, drug concentrations are quantified using techniques such as radioimmunoassay, high-performance liquid chromatography (HPLC), fluorescence polarization immunoassay (FPIA), enzyme-multiplied immunoassay technique (EMIT), enzyme-linked immunosorbent assay (ELISA), and chemiluminescence immunoassay (CLIA), among others. The method chosen for TDM must be sensitive, must be specific for the detection of the compound of interest, must be feasible for use as a routine assay, and must be able to distinguish the drug of interest from other co-administered drugs [7].

For robust TDM, a multidisciplinary team of scientists, clinicians, nurses, and pharmacists is essential to ensure that clinically meaningful drug concentrations are attained since it relies on a rapid and accurate determination of drug levels [1, 3]. The accurate interpretation of analytical readouts of drug concentrations by the multidisciplinary team requires information on the time of blood sample collection, dose administered, dosage regimen, patient demographics, comedications, indication for monitoring and pharmacokinetics, and therapeutic range of the drug [8], with an ultimate goal of ensuring accurate drug dosing to bring about an optimal therapeutic effect while reducing toxicity.

13.1.1 Brief History of TDM

While TDM presents as a powerful tool for ensuring an optimal benefit in drug dosing, its clinical benefits have been underappreciated in the present-day clinical setting [9]. Nonetheless, the critical influence of dosage in a drug regimen has long been acknowledged in toxicology and can be attributed to the Renaissance physician Paracelsus (1493–1541) credited to be the "Father of Toxicology," who famously stated:

> What is there that is not poison? All things are poison and nothing is without poison. Solely the dose determines that a thing is not a poison [10].

Therefore, the scientific community has known for centuries that the dosage of a drug is important for optimal patient outcomes. However, it was not until 1932 that the Swedish Scientist, Erik Widmark, demonstrated the ability to monitor the blood concentration of a drug after he developed two formulae commonly used today for the determination of blood alcohol levels [11]. A classic historical example that demonstrates the importance of TDM is lithium – originally used as used as a sodium-free table salt for individuals with hypertension in the 1940s before being withdrawn due to reports of severe toxicity and death [2]. However, there were also concurrent reports of improvement in symptoms in hypertensive patients with manic-depressive disorders who used lithium. Despite its potential therapeutic benefits, the inability to accurately

monitor plasma levels of lithium so as to ensure that drug concentrations were not in the toxic range, led to its withdrawal [2], and it would be a few more years before TDM was finally introduced, thereby enabling the establishment of the therapeutic dose range of lithium [12, 13].

By the 1960s, landmark papers were published that introduced TDM as a new aspect of clinical practice based on a publication of pharmacokinetic studies that associated mathematical theories to patient outcomes; there was also a review published outlining the importance of drug monitoring [14, 15]. In the late 1960s to early 1970s, a new discipline known as "clinical pharmacokinetics" emerged, which enabled scientists to study adverse drug reactions and establish therapeutic ranges for drugs so as to reduce the incidence of their toxicity [3].

Improvements in analytical technology and high-throughput computerization subsequently resulted in significant advancements in the field of clinical pharmacokinetic monitoring [16]. For instance, the development of chromatographic techniques such as gas chromatography, high-performance liquid chromatography (HPLC), and mass spectrometry enabled the monitoring of the concentration of drugs, while the use of immunoassays for saliva samples facilitated the ease of performing TDM [9, 17]. In 1978, the applicability of TDM was further demonstrated as simultaneous quantification of drugs was reported, demonstrating the powerful potential of TDM on patient outcomes in a clinical setting where patients often co-administered drugs [18]. The clinical utility of TDM was demonstrated as early as 1981, wherein TDM of the beta-lactam antibiotic, cefoperazone, was reported in patients [19]. Cefoperazone displays differing pharmacokinetics relative to other first-generation cephalosporins (e.g., cephalothin, cephapirin), as it is not readily metabolized and has a much longer serum half-life, necessitating the use of TDM in patients taking the drug.

From the 1990s onward, the advancement of much more sophisticated techniques further advanced the field of TDM as pharmacogenetics and pharmacogenomics research found its application in TDM; this was made possible by the large dataset generated by the Human Genome Project between 1990 and 2001 [20]. Considering that there are numerous reported genetic polymorphisms that affect the disposition of drugs, TDM can be combined with pharmacogenetics in order to optimize pharmacotherapy for patients [2]. In these situations, a patient receives a dose of the drug based on a pharmacogenetic assessment, after which conventional TDM can be used to ensure optimal patient outcomes [2]. Scientific advances of TDM were also supported by the introduction of noninvasive and minimally invasive methods to be used for monitoring, including wearable sensors. For example, the first wearable sensor that consists of an ingestible sensor for TDM was introduced in 2013 for monitoring adherence to anti-tubercular therapy [21]. The system was able to correctly identify the ingestible sensors with high accuracy and confirm medication compliance while also posing a low risk to users. On the other hand, another minimally invasive technique was introduced in 2016 which relied on painless hollow microneedles for drug quantification rather than requiring a blood draw [22]. The needles were suitable to extract small volumes of interstitial fluid and drug analytes which were rapidly quantified with high sensitivity using appropriate detection techniques. A fast and versatile multianalyte (up to eight enzyme-linked assays) single-use biosensor assay was also introduced using human plasma for simultaneous detection of antibiotics in less than 15 minutes [23]. In 2019, the clinical relevance of TDM was further demonstrated as the World Health Organization included TDM on the list of essential in vitro diagnostic tests for a list of drugs to be monitored on a priority basis [24].

Despite the plethora of advancements in TDM, the first in-human study for continuous drug monitoring in healthy volunteers was only published in 2019. In this study, TDM was used for monitoring phenoxymethylpenicillin using a minimally invasive microneedle-based beta-lactam biosensor [25]. While chromatographic techniques have largely been used in the field, they are limited due to the lack of standardization, high turnaround time and instrumentation cost, and laborious sample preparation process

[9]. Furthermore, sample preparations requiring blood draws pose a hindrance as a potentially painful process to many individuals. As such, the scientific advancements in recent years using noninvasive or minimally invasive methods for TDM will be revolutionary for the feasibility and applicability in the field.

13.1.2 Importance of TDM in Clinical Settings

As shown above, therapeutic drug monitoring (TDM) is an important clinical tool that ensures maximum therapeutic effectiveness and avoids toxicity for medications with narrow therapeutic indices [3]. While most drugs in clinical practice have a wide therapeutic index [3], there are several agents with narrow therapeutic windows for which TDM is required to determine dosing and dosing frequency and to prevent toxicity. Medications are often developed to provide a therapeutic effect at a standard dose; however, due to patient variability, there can be quite a wide range of drug exposure leading to differences in efficacy and toxicity that results from the manufacturer-recommended standard dose [26]. Use of TDM aligns with precision medicine as it allows individualized dosing to assist clinicians in determining the right dose to give to a specific patient [26]. In the age of tremendous advancements in pharmacogenomics, where the genotyping of an individual patient's drug-metabolizing enzymes or transporters is becoming more commonplace, it may be assumed that the practice of TDM may eventually be retired. However, due to the fact that TDM can achieve target drug concentrations in patients more accurately than pharmacogenomics, perhaps the better approach is to use TDM and pharmacogenomics together to achieve the highest level of precision medicine [26]. Examples of drug classes that may require TDM include antibiotics, antiepileptics, and antineoplastics [27]. While TDM has primarily focused on measuring levels of the parent compound, metabolite monitoring is more of an academic exercise [28, 29]. A wide variety of approaches have been taken for determining blood levels of the parent compound. In clinical practice, the use of drug trough levels (drug level that is drawn at the point where the amount of drug in the body would be at its lowest amount when the drug level is at a steady state in the blood) is commonplace [30]. An example of the use of drug troughs is the monitoring of vancomycin. If the vancomycin is given every 8 hours, a trough is usually drawn after three doses, and the blood is obtained right before the 4th dose is given [31]. Since a few doses have been given, it would be expected that a measurable amount of the drug be detected in the body when a trough is drawn due to drug accumulation [31]. Troughs can be used in ensuring that the medications do not accumulate; achieving a certain trough has been shown to correlate with treatment success [30]. On the other end of the spectrum of the parameters measured in TDM are drug peaks, which correlate with the highest detectable drug concentration. Drug peaks are important in ensuring that target ranges are not exceeded, in mitigating unwanted toxicities, and in ensuring therapeutic success [3]. The importance of drug peak measurement is illustrated in the case of amikacin where the use of an extended interval dosing strategy helped achieve a peak drug concentration, which directly correlated with a successful antimicrobial effect [32]. Since a higher plasma concentration of amikacin is required for the management of sepsis [33], the measurement of its peak concentration is an important step to ensuring efficacy [34]. Finally, another approach in TDM is the measurement of several sequential levels such as through the estimation of area under the curve (AUC). In this approach, therapeutic success is determined by the exposure of the drug in the body over time, such as in the cases of vancomycin and mycophenolate [30, 34, 35]. Beyond measuring blood levels of the parent compound is the determination of the level of surrogate efficacy markers which are measurable indicators of the intended clinical outcome [36]. In the course of therapy with the anticoagulant drug, enoxaparin, the surrogate marker of efficacy is a measurement of the inhibition of clotting factor Xa [37]. In the case of

Table 13.1 List of drug classes requiring TDM

Drug class	Example medications	References
Antineoplastics	Methotrexate, mercaptopurine, carboplatin, busulfan, thioguanine, azathioprine	[1–5]
Immunomodulators	Tacrolimus, sirolimus, mycophenolate, cyclosporine	[6–9]
Antiepileptics	Valproic acid, phenytoin, phenobarbital, levetiracetam, carbamazepine	[2, 10–12]
Anticoagulants	Enoxaparin, heparin, bivalirudin, argatroban, fondaparinux	[13]
Antifungals	Posaconazole, voriconazole, itraconazole	[14, 15]
Antibiotics	Amikacin, tobramycin, gentamicin, vancomycin	[2, 16, 17]
Antivirals	Valganciclovir	[18]
Antipsychotic	Lithium	[19]
Antiarrhythmics	Digoxin, lidocaine, procainamide, quinidine	[19, 20]
Respiratory agents	Theophylline, caffeine	[19]

warfarin, prothrombin time serves as the surrogate efficacy marker which is employed in the calculation of international normalized ratio (INR), a measurement used to determine efficacy and safety of medications that affect clotting time [38, 39].

In clinical practice there are a wide variety of drug classes where dose individualization based on TDM has been a part of medical management [3, 27, 34, 37, 40, 41]. In Table 13.1, a listing of drug classes where TDM is commonly used along with specific examples is shown.

13.1.3 Current Clinical Practice Gaps Associated with Measuring Parent Compounds

Current clinical practice often focuses on the measurement of blood levels of parent compound administered. However, drugs are often biotransformed within the biological system with the active entities being the metabolites thereof as is the case with pro-drugs. A pro-drug is a compound which after having been administered is metabolized within a body compartment into a pharmacologically active drug [42, 43]. Some examples of pro-drugs include codeine, tramadol, clopidogrel, and mercaptopurine [41, 44, 45]. However, because not all patients can biotransform the parent compound into the active compound to the same extent, a field of research known as pharmacogenetics (or pharmacogenomics) has been developed to study the effects of gene and gene expression on the response of individual patients to drugs to which they are exposed [46].

The impact of pharmacogenomics on TDM is aptly illustrated in the case of tacrolimus, a commonly used immunosuppressant with a narrow therapeutic window [47]. Tacrolimus, in complex with immunophilin, binds to calcineurin and inhibits calcineurin phosphatase activity [48] which results in an inhibition of calcium-dependent activities including nitric oxide synthase activation, cell degranulation, and cytokine production (proinflammatory IL-2, which results in suppressed T-cell proliferation) [45]. Hence, tacrolimus is effective in preventing xenograft attack [49]. TDM is an important part of tacrolimus therapy, and regular blood draws of whole blood tacrolimus levels are critical to guide therapeutic management. Elevated tacrolimus levels are associated with increased risk of nephrotoxicity, hypertension, and hyperglycemia [50], while a subtherapeutic blood level of tacrolimus results in rejection of the implanted organ thereby causing organ dysfunction or failure [47]. One key factor involved in this varied response to tacrolimus therapy that is observed among various patient populations is the polymorphism in the expression and activity of the enzymes principally responsible for its metabolism – cytochromes P450 (CYP) 3A4 and 3A5 [51]. In 2015, guidelines were published by the Clinical Pharmacogenetics Implementation Consortium (CPIC) to provide initial dosing guidance based on an individual patient's genotype [51]. They

suggested that extensive and intermediate metabolizers tend to have lower tacrolimus trough concentrations and a decreased chance of achieving target tacrolimus concentrations. Therefore, their initial doses were recommended to be 1.5–2 times the recommended starting dose of normal metabolizers. Although poor metabolizers may have higher trough concentrations, the recommendation for this population is to start with standard recommended doses [51, 52]. In most cases, therapeutic drug monitoring is recommended to guide any dose adjustments after initiation [45]. Although tacrolimus is not a pro-drug and may not have an active metabolite, this example demonstrates the impact of metabolism and its variability within a patient population. The remainder of this chapter discusses the importance of metabolites in TDM and drug safety, the mechanism of metabolite-induced drug toxicity, and the impact of pharmacogenetics in drug metabolism and the potential impact on efficacy and safety of drugs subject to TDM.

13.2 Metabolites and Drug Monitoring

Although TDM is principally concerned with the measurement of parent compounds with direct clinical use, its use can also be applied to the measurement of metabolites of the parental compounds. Metabolite measurement is important in TDM because some drugs including those with narrow therapeutic windows are converted to reactive metabolites and these need to be analyzed. Furthermore, some drugs are inactive in their parental forms, and only their metabolites have therapeutic applications. Since some of these metabolites have a narrow therapeutic window, they require the application of TDM.

13.2.1 Importance of Metabolite Measurements in TDM

As discussed in a review by Kang and Lee [3], one of the difficulties inherent in TDM is the possible presence of metabolites which may have distinct therapeutic and toxicity profiles that differ from the parental compound. In such a case, there is need for the determination of the concentration of the parental compound and its metabolite in biological fluids in order to show the relationship between the amount detected and their pharmacological effects. While this may not be part of what is typically done on a day-to-day basis in clinical practice, it is key to recognize the overall impact of measurement of metabolites in such a scenario. As an example, N-acetylprocainamide (acecainide), a metabolite of procainamide, has been shown to possess anti-arrhythmic potency, similar to the parental compound [53–56]. Given the fact that this metabolite of procainamide possesses similar (and possibly equal) pharmacological activity as the parental compound, a knowledge of the metabolite plasma concentration would be key in determining dosing so as to modulate efficacy and reduce any potential toxicity. Other drugs with active metabolites include losartan [57], whose metabolite E-3174 has 10- to 40-fold the potency of the parent compound [58] and is implicated in "on-target" effects which are directly related to the primary mechanism of action of the parent compound. Alternatively, there are drugs, metabolites of which are involved in secondary effects or those characterized as "off-target." In these cases, the metabolites result in pharmacological effects different from those of the parent compound. Such is the case with fenfluramine, an anorectic drug [59] whose metabolite(s) (+)- and (−)-norfenfluramine have diverse pharmacological effects including substrate activity at norepinephrine transporter (NET) to release norepinephrine (NE) and direct agonist activation of 5-hydroxytryptamine, 5-HT2B and 5-HT2C, receptor subtypes [60]. Carbamazepine, a drug with a narrow therapeutic window, is also metabolized to carbamazepine-10,11-epoxide, which is an active metabolite of this anti-convulsant drug [61]. It is imperative to measure levels of this metabolite as carbamazepine overdose has been reported not only with the parent compound but in association with this metabolite as well [62]. A case was reported by Russell et al. [63] of an overdose of carbamazepine wherein the level of

carbamazepine-10,11-epoxide was shown to be 450% higher than the parent compound. The authors suggested that this metabolite played a role in the carbamazepine toxicity. Doxorubicin is an anthracycline antineoplastic drug with a low therapeutic index that induces cardiovascular toxicity [64–66]. This toxicity is attributed to its metabolite, doxorubicinol [67], which upon accumulation following long-term use causes myocardial toxicity in humans [65]. Since cardiotoxicity results from the accumulation of doxorubicin and doxorubicinol in cardiovascular tissues, monitoring plasma levels of this drug and its metabolite in breast cancer patients is key to minimizing toxicity [68].

Other drugs like acetaminophen, when taken at a high dose, form the highly reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI), with hepatotoxic potential [69]. Further, other drugs such as troglitazone [70], felbamate [71], and diclofenac [72] also generate metabolites which induce idiosyncratic drug toxicity. In the example of procainamide above, it has been shown that the procainamide-induced agranulocytosis was due to the formation of protein free radicals [73]. Since there is potential of formation of pharmacologically active metabolites and metabolites with toxicity potentials, metabolite measurement can help optimize therapy and abrogate possible toxicity in the clinical setting.

The importance of metabolite measurement has been highlighted in FDA's Center for Drug Evaluation and Research Guidance for Industry on Safety Testing of Metabolites and the International Conference on Harmonization (ICH) which provided guidance on the amount of metabolite relative to the parent compound which is of safety concern. The threshold of human metabolites which can raise a safety concern can be as little as 10% of the parent drug concentration during systemic exposure at steady state [74], and the recommendation is that a series of tests be conducted to evaluate the safety of metabolites in a manner similar to the parent compound [75]. A similar stance was adopted by the Pharmaceutical Research and Manufacturers Association (PhRMA) which highlighted the need for more rigorous studies to investigate the role of metabolites in the safety assessment of drugs with narrow therapeutic windows, such as those which are primarily measured in TDM [76]. They also determined that if metabolites constitute a minimum of 25% of the circulating drug-related material in the study system, such a metabolite should be considered as being of a safety concern. However, in the review by Robison and Jacobs [74], there are other cases in which further safety study of metabolites might not be required such as in the case of metabolites with no structural alerts for reactive products. The nature of the metabolite is also an important factor to consider. Therefore, while oxidative metabolites (commonly called phase I metabolites) are readily linked to reactive species with potential for toxicity, conjugative metabolites (commonly called phase II metabolites) are mostly water soluble and easily excreted. The general assumption is that most oxidative metabolites would require further safety evaluation provided other criteria are met, while conjugative metabolites do not require further evaluation [77]. An exception to this assumption lies in the fact that other products of conjugation like acyl glucuronides and acyl-CoA thioesters are unstable and have been shown to interact covalently with cellular macromolecules to cause organ-system toxicity [78]. This is particularly true for drug molecules that contain a carboxylic acid moiety, 14% of which were withdrawn from the global pharmaceutical market in the past century due to toxicity [79]. This would be a concern for carboxylic acid-containing drugs such as levothyroxine, valproic acid, argatroban, mycophenolic acid, methotrexate, and vincristine which have been shown to have a narrow therapeutic window [80–86]. Although the metabolism of these drugs may not be primarily through the glucuronidation pathway, the mere presence of the carboxylic acid group is a potential structural alert for the generation of a reactive metabolite [78, 87]. The

relationship between drug metabolism and toxicity will be discussed in Sect. 13.2.3.

13.2.2 Metabolite Measurement in TDM

In the context of TDM and metabolite determination following oral administration, highly reactive metabolites may form other unstable metabolites that are difficult to detect in biological fluids like plasma due to their relatively short half-lives [74]. However, the potential they have to form stable conjugates with endogenous molecules like glutathione and cysteine which can be readily measured can provide insight into their formation and presence in the plasma [88]. This has been reported in the case of acetaminophen (APAP) which is primarily metabolized by CYP2E1 to form N-acetyl-*p*-benzoquinone imine (NAPQI) [89], and NAPQI readily reacts with hepatic glutathione (GSH) to form the detoxified product APAP-GSH [89]. This concept is further discussed below. Other examples of drugs that are metabolized to toxic metabolites include valproic acid which is metabolized to 4-ene-valproic acid [90] (Fig. 13.1), mycophenolic acid which is glucuronidated to an acyl glucuronide [91], phenytoin which is metabolized to 5-(4′-hydroxyphenyl)-5-phenylhydantoin which is produced via the formation of a reactive arene oxide intermediate [92], and carbamazepine which is converted to carbamazepine-10,11-epoxide [62]. Although carbamazepine-10,11-epoxide is an active metabolite with a similar pharmacology to the parent compound, the fact that it is an epoxide poses a structural alert for toxicity. Epoxides are highly reactive electrophilic moieties that have been shown to induce genetic toxicity [93, 94]. Little wonder authors have suggested that the toxicity seen in carbamazepine might be associated with the formation of its epoxide metabolite [63, 95], and other authors have shown a positive correlation between the blood level of carbamazepine-10,11-epoxide and adverse effects in carbamazepine therapy [96]. Another layer of complexity associated with carbamazepine therapy lies in the fact that it is a potent inducer of CYP3A4, other oxidative enzymes involved in its own metabolism as well as the conjugative enzyme family of glucuronosyltransferases [97]. CYP3A4 is polymorphic [98] as are other enzymes involved in the metabolism of carbamazepine, making for a wide variation in the metabolism of carbamazepine with a potential for toxicity in the case extensive metabolizers via the oxidative pathway [99] (Fig. 13.2).

The examples cited above – valproic acid, phenytoin, carbamazepine, and mycophenolic acid – are all drugs in clinical use and which have a narrow therapeutic window. While the mainstay of clinical practice is to determine the levels of the administered drug compound, the fact that some of the metabolites of these drugs have toxicity potential is a reason that metabolite measurement is important in TDM. This will help mitigate the overall risk posed by exposure to the parent compound and the active substance, in addition to possibly toxic metabolites [3]. As a consequence, metabolite measurement is warranted in TDM for:

1. Drugs with metabolites possessing pharmacological actions, e.g., procainamide and carbamazepine [100, 101]
2. Pro-drugs, e.g., tenofovir disoproxil [102]
3. Drugs which produce metabolites with established toxicity, e.g., doxorubicin, procainamide, daunorubicin, and valproic acid [103]
4. Drugs with high variability in their pharmacokinetics (e.g., absorption, distribution, metabolism, and elimination), e.g., carbamazepine, thiopurines, clopidogrel, and codeine [103–105]
5. Drugs known to demonstrate a nonlinear relationship between the administered dose and blood/serum/plasma concentrations, e.g., phenytoin and theophylline [104, 105]

Because some drugs with narrow therapeutic windows have been shown to produce reactive metab-

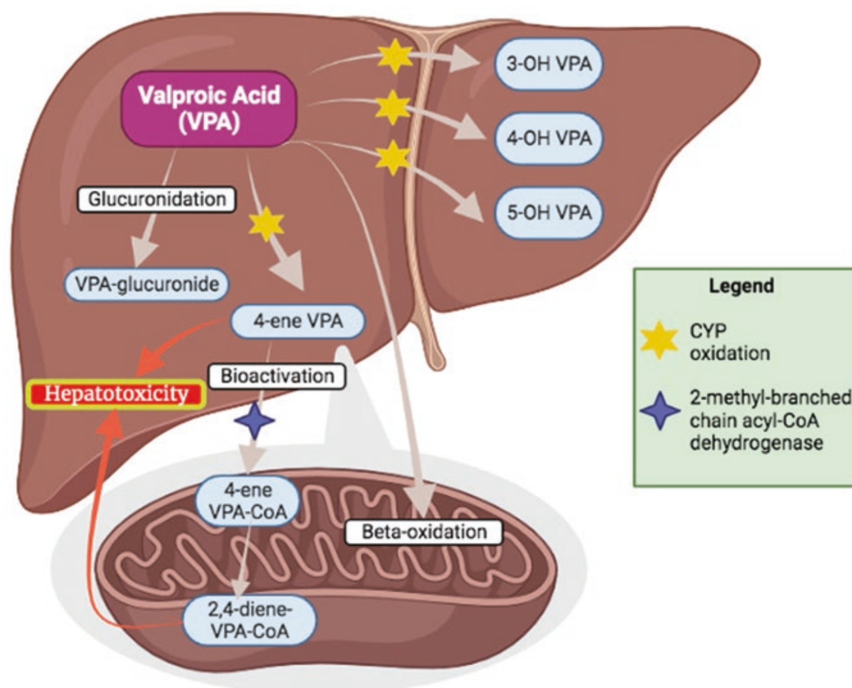

Fig. 13.1 Valproic acid metabolism and bioactivation. There are three routes of VPA metabolism in humans, including glucuronidation (major route, 50%), beta-oxidation in the mitochondria (major route, 40%), and cytochrome P450 (CYP)-mediated oxidation (minor route, 10%). It has been shown that CYP2C9 and CYP2A6 are the key enzymes involved in CYP-mediated oxidation of VPA, while CYP2B6 is a less predominant route. Also, glucuronidation of VPA is mediated by several UGTs including UGT1A4, UGT1A10, UGT1A3, UGT1A9, UGT1A8, UGT1A6, UGT2B7, and UGT2B15. In the mitochondria, mitochondrial beta-oxidation generates 3-oxo-VPA, CoA-SH, and propionyl-CoA and pentanoyl-CoA. Bioactivation of VPA involves the entry of 4-ene-VPA into the mitochondria, followed by conversion to 4-ene-VPA-CoA ester via 2-methyl-branched chain acyl-CoA dehydrogenase. Subsequently, beta-oxidation forms the reactive 2,4-diene-VOA-CoA ester. This putative cytotoxic metabolite gets further conjugated with glutathione and together with 2,4-diene-VPA-CoA ester can deplete mitochondrial glutathione pools and form conjugates with CoA, ultimately inhibiting enzymes involved in the beta-oxidation pathway. (Figure created with BioRender.com)

olites which need to be measured in biological fluids, the following section gives an overview of the mechanisms by which these metabolites induce toxicity in the biological system.

13.2.3 Drug Metabolism and Toxicity

As described in the sections above, in the majority of situations, the parent drug is the active compound, and alteration in its metabolism can greatly impact its efficacy, as was observed with tacrolimus [47]. However, there are cases in which the metabolites of a drug are toxic and have been shown to result in severe organ/system damage, as was observed with acetaminophen (APAP) [106]. In the example below, the drug acetaminophen is not a drug requiring TDM. It is, however, a classical example of a drug that produces toxic metabolites which may need to be measured and monitored as a determinant of its safety in cases of overdose.

Reviewing the metabolism of acetaminophen in an overdose scenario provides some insight on the impact of metabolism on drug toxicity. Although acetaminophen is one of the most commonly used analgesic and antipyretic medica-

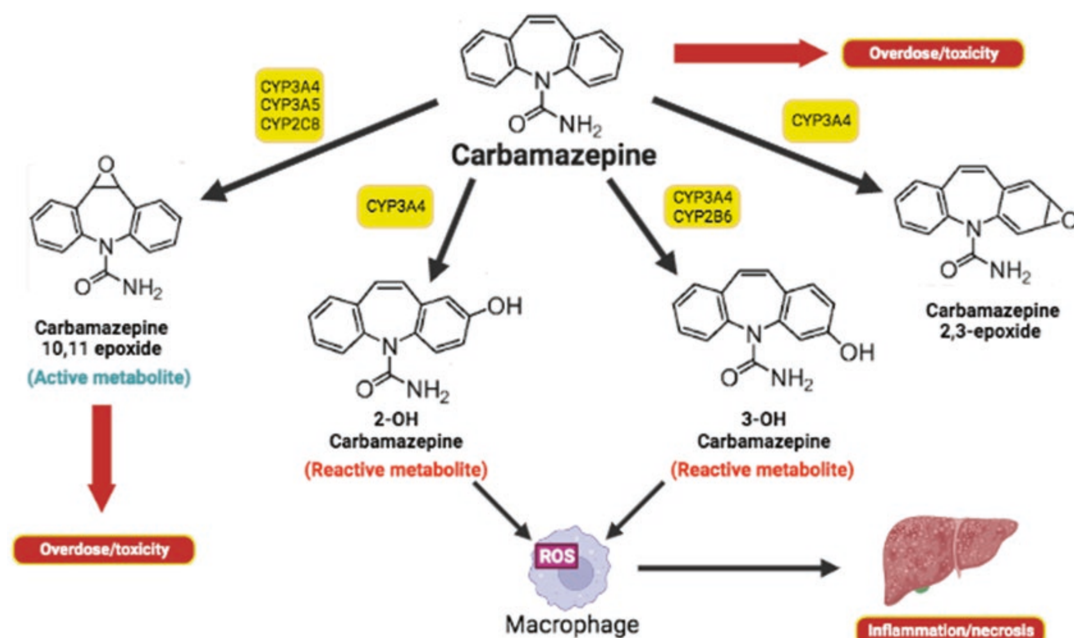

Fig. 13.2 Metabolism and reactive metabolites of carbamazepine. Carbamazepine undergoes hepatic metabolism mediated by several CYP enzymes. Select metabolites of carbamazepine, including 2-OH carbamazepine and 3-OH carbamazepine, have been shown to induce the production of reactive oxygen species in macrophages. The macrophages subsequently release signals that lead to the secretion of proinflammatory cytokines and chemokines, resulting in liver inflammation. In excess, the active metabolite carbamazepine 10,11 epoxide has also been associated with adverse events including toxicity, overdose, and genetic toxicity. Similarly, excessive intake of carbamazepine itself can lead to toxicity and overdose. (Figure created with BioRender.com)

tions, it is one of the principal causes of acute liver failure in industrialized nations [107].

Acetaminophen is metabolized by CYP2E1 to yield N-acetyl-*p*-benzoquinone imine (NAPQI), a highly reactive compound that forms adducts with cellular macromolecules, including glutathione. Conjugation of NAPQI with the cysteine residue of glutathione molecule results in its depletion and removal from the body [108]. Additionally, the interaction between NAPQI and glutathione has been shown to be involved in the inactivation of NAPQI leading to APAP-CYS which can be measured in the plasma as a biomarker of impending liver toxicity [109]. Studies in the past decades have demonstrated novel methods of efficiently quantifying the formation of toxic metabolites via the measurement of acetaminophen-protein adduct levels in human serum [106, 110, 111]. Measurement of acetaminophen adducts can be more accurate than acetaminophen levels because the adducts persist much longer in serum with a half-life of 1–2 days compared to the half-life of acetaminophen which is typically around 5 hours [111]. A high proportion of ingested acetaminophen is metabolized via glucuronidation and catalyzed by uridine 5′-diphospho-glucuronosyltransferase to yield APAP-glucuronide or by sulfotransferases thereby enhancing the renal elimination of the drug [112, 113]. Another reaction that occurs is glutathionylation, but this usually occurs after phase I metabolism [114].

Although APAP is not a drug that typically requires TDM, a knowledge of the levels of circulating metabolites can better inform clinical decision-making for specific patients and creates an opportunity to implement precision medicine [115, 116].

13.2.3.1 An Overview of Mechanisms Involved in the Bioactivation of Drugs to Toxic Metabolites

Metabolism plays a key role in the conversion of drugs, which are foreign substances to the body, to various forms including less toxic, water soluble, and excretable forms [117, 118]. While in many cases, metabolism is a detoxification process, it can produce pharmacologically active forms of the compounds with therapeutic applications, as is observed with pro-drugs [119] (further discussed in Sect. 13.4). In other cases, drugs are metabolized to forms that are reactive with the potential to induce organ-system damage. This organ-system damage can arise from the interaction between these metabolites – many of which are electrophiles in nature – and cellular macromolecules like proteins, nucleic acids, or unsaturated lipids, ultimately causing degradation of these macromolecules, cellular dysfunction, and cell death [120–122].

This section gives an overview of the mechanisms of toxicity induced by metabolites using specific examples of, wherever applicable, drugs with narrow therapeutic windows. Briefly, drug metabolism can take place in different ways depending on the enzymes involved and the chemical structure of the compound in question. In what has been typically called a phase I reaction,[1] drugs undergo different reactions including oxidation, reduction, substitution, hydrolysis, and elimination under the action of different enzyme systems including the cytochrome P450 system, most of which is resident in the liver [123–125]. In regard to conjugative metabolism of xenobiotics (also called phase II metabolism), reactions may involve a series of enzymes including uridine 5′-diphospho-glucuronosyltransferase (UDP-glucuronosyltransferase, UGT) which is a microsomal enzyme belonging to the glycosyltransferase family; they are involved in the transfer of the glucuronic acid component of UDP-glucuronic acid to a small hydrophobic molecule [126]. Other conjugative reactions include sulfation, acetylation, glutathionylation, and methylation [89].

The *cytochrome P450* system catalyzes a series of reactions that can bioactivate drugs and other xenobiotics into toxic forms. Located predominantly in liver microsomes, this enzyme system catalyzes oxidative reactions such as C-, S-, and N-oxidations; O-, S-, and N-dealkylations; dehalogenation; and deamination [127]. Other enzyme systems involved in drug metabolism include *FAD-containing monooxygenase* which oxidizes nucleophilic sulfur, nitrogen, and organophosphorus compounds [128]; *xanthine oxidases* which oxidize purine derivatives like theophylline and doxorubicin, among others [129]; and *alcohol dehydrogenase* and *aldehyde dehydrogenase* which oxidize a vast array of alcohol and aldehydes into aldehyde and acids, respectively [130]. There are also *peroxidases* which catalyze the oxidation of hydrogen peroxide and organic peroxides [131]. Some of the reactions above are known to generate reactive metabolites in drugs as in the case of chloramphenicol. Chloramphenicol is metabolized by the CYP monooxygenases to chloramphenicol oxamyl chloride, which is produced via *oxidative dechlorination* of the dichloromethyl moiety of the compound and then the elimination of the hydrochloric acid portion of the ensuing product [132]. Chloramphenicol oxamyl chloride is a reactive metabolite that reacts with the epsilon-amino group of the lysine portion of the CYP enzyme, which subsequently results in the inhibition or inactivation of the enzyme [133, 134]. CYP enzymes have also been shown to catalyze *oxidation of unsaturated bonds* into epoxides

[1] While it is common to refer to this as phase one reaction, this is not an accurate description of the reactions that take place. Referring to this as phase I only suggests that they take place first and other reactions follow. However, it has been shown that the other kind of reaction in the liver, which is generally referred to as phase II, may actually take place before "phase I," simultaneously with "phase I," or may even be the only major reaction that the xenobiotic undergoes. Therefore, phase I reactions are more accurately and broadly referred to as oxidative metabolism because a vast majority of reactions that take place via this route are oxidative in nature, while phase II reactions are more accurately referred to as conjugative metabolism as the other chemical moieties are added (conjugated) to the parent compound or its oxidative metabolite to create a new form.

which are reactive metabolites involved in the toxicity of the unsaturated compounds. This is the case with aflatoxin B_1 which is oxidized by the CYP enzyme system to aflatoxin B_1 epoxide. Aflatoxin B_1 epoxide subsequently adducts with the guanine portion of DNA, thereby resulting in hepatic carcinogenicity [135, 136]. A clinically relevant epoxide metabolite of a drug with a narrow therapeutic window is that of carbamazepine, as was discussed previously. Carbamazepine is oxidized by CYP 3A4 to carbamazepine-10,11-epoxide which, although possesses a similar efficacy profile to the parent compound [137], has been shown to induce adverse drug reactions and has been implicated in carbamazepine toxicity [63]. In other cases, unsaturated compounds with aromatic rings have also been shown to be oxidized to benzoquinone and hydroquinone which are highly reactive compounds [138] as is the case with acetaminophen. Acetaminophen is converted by CYP2E1 to N-acetyl-p-benzoquinone imine (NAPQI); this metabolic product is involved in its hepatotoxicity as discussed in details above [139]. Other oxidation reactions involve *N-oxidation* of primary and secondary amines to hydroxylamines which have been shown to be hepatotoxic and mutagenic as is the case with acetamino-2-fluorene [140, 141]. Examples of CYP-mediated toxicity metabolism giving rise to reactive metabolites include the *oxidation of heteroatoms* like sulfur or nitrogen and the *reduction of polyhalogenated, nitro-, keto-, and azo derivatives* in such compounds as daunorubicin. The oxidation of heteroatoms occurs in the metabolism of thiophene, which becomes oxidized to sulfoxide, thiophene S-oxide, or epoxides and subsequently adducts with glutathione or proteins [142–145]. *The reduction of polyhalogenated, nitro-, keto-, and azo derivatives* occurs in compounds such as daunorubicin, which forms a hydroxyl radical causing oxidative stress and cardiac toxicity; it also forms another free radical derivative, the 7-deoxydaunorubicin radical, which alkylates DNA resulting in genotoxicity [146, 147].

While conjugative metabolism mainly results in the formation of water-soluble derivatives, facilitating their excretion, some of these derivatives have been implicated in the toxicity of these compounds. *UGT-mediated conjugative metabolism* is involved in the formation of acyl glucuronides of carboxylic acid containing-drugs like diclofenac [148, 149]. The aromatic amines in these drugs can also be converted to hydroxylamine o-glucuronide which after being broken down in the bladder forms a carcinogenic proximate hydroxylamine [150].

Since drugs can be bioactivated to toxic metabolites, an essential aspect of TDM should include the measurement of metabolites especially in cases where metabolites have been implicated in the toxicity of drugs with a narrow therapeutic window. The next section examines the effect of genetic polymorphisms in drug metabolism and how this can impact drugs subject to TDM.

13.3 Polymorphism in Drug Metabolism

Since TDM involves monitoring for an optimal plasma concentration of drugs to achieve the desired therapeutic effect at the right dose while also avoiding the toxic effects, the plasma concentration of the substance of interest can be affected by their metabolism, resulting in an alteration of the effective plasma concentration, or generation of toxic metabolites (as shown in the last section) with potential organ-system dysfunction. Genetic polymorphism in the expression and activity of drug-metabolizing enzymes has been shown to have a significant effect on the efficacy and safety of drugs, including those with narrow therapeutic windows [151, 152]. Genetic polymorphism describes the simultaneous occurrence of two or more discontinuous alleles or genotypes in a population and results in an alteration in gene expression; an alteration in gene expression could also result in a change in the structure and function of the resulting gene products [153, 154]. Cytochrome P450 enzymes (CYPs) are prone to polymorphism, which may account for inter-individual differences in drug pharmacokinetics and phar-

macodynamics [155, 156]. Several factors may play a role in polymorphism, including transcriptional regulation, transcription errors, single-nucleotide polymorphisms (SNPs), and copy number variants [157–160]. Polymorphisms which result in a gain of function may lead to either decreased response to a therapeutic medication or increased metabolism and toxic metabolite formation in some cases [161]. On the other hand, polymorphisms which lead to loss of function could result in overexposure to a medication and increased undesirable off-target and on-target effects [162].

13.3.1 Polymorphism in Expression of CYPs

The cytochrome P450 enzymes (CYPs) are primarily expressed in hepatic tissue where they metabolize both endogenous compounds and xenobiotics [163]. CYPs are also expressed in the small intestinal mucosa, lung, kidney, brain, placenta, olfactory mucosa, and skin, with the intestinal mucosa being the main extrahepatic site of drug biotransformation [164, 165]. Inter-individual variation in the expression of CYPs may be partially explained by differential transcriptional regulation via nuclear receptors [166]. Thus, the extent to which each person metabolizes a certain drug is a combination of gene expression which is influenced by environmental factors and individual variations in amino acid sequences as a result of transcription errors [167]. CYP polymorphisms are denoted as * (star) alleles, each corresponding to particular sequence variations within the coding sequence for the enzymes [168]. The subsection below explores the differences in metabolism as a result of CYP enzyme polymorphisms. In humans, there are 57 genes considered to be functional and 58 pseudogenes [169]. Based on sequence similarity, these genes are divided into 18 families and 44 subfamilies. Most of the CYP's roles relate to metabolizing endogenous substances (bile acids, eicosanoids, and steroids) [170]. Of the 18 families, CYP1, CYP2, and CYP3 are the main CYPs that contribute to the oxidative metabolism of most drugs used clinically [171]. We will focus our discussion on members of these three families.

13.3.1.1 CYP1A1

CYP1A1 is thought to be expressed in the liver [172, 173], but with a significant variability [174]. It is also expressed in human extrahepatic tissues including intestine [175]. While CYP1A1 is involved in the metabolism of several common drugs, including caffeine [176], amiodarone [162, 177], R-warfarin [178, 179], ondansetron [180], haloperidol [181], cyclobenzaprine [182], and propranolol [183], there have been no studies to date showing clinically relevant polymorphisms in the expression of CYP1A1 enzymes. To date, 15 allelic variants and subvariants (*2A to *13) have been described in the CYP1A1 gene [184].

13.3.1.2 CYP1A2

CYP1A2 accounts for about 10% of the total CYP content in human liver [185]. Several clinically relevant drugs are metabolized by CYP1A2 including caffeine [186], clozapine [187], ropivacaine [188], olanzapine [189], lidocaine [190], imipramine [191], propranolol [183], verapamil [192], propafenone [193], and bortezomib [194]. There are notable inter-individual differences (40- to 130-fold) in CYP1A2 expression and activity [195]. CYP1A2 is also influenced by environmental factors such as smoking and food components [196]. There have been at least 40 variant alleles and a series of subvariants (*1B to *21) identified [197], with CYP1A2*1A being the wild type.

CYP1A2 accounts for about 30% of the metabolism of clozapine, an atypical antipsychotic, which is subject to TDM [198, 199]. In smokers with the CYP1A2*1F genotype, a variant that results in increased enzyme activity, low plasma levels have been reported to result in resistance to therapy [200]. Likewise, patients in whom two CYP1A2 variants with decreased enzyme activity (CYP1A2*1C and *1D) are present have been found to have higher clozapine levels [201], resulting in hyperthermia, alterations in consciousness, seizures, cardiac arrhyth-

mias, excessive mucus production in bronchi, hepatitis, and cardiac arrhythmias [202, 203].

13.3.1.3 CYP2C9

CYP2C8, 2C9, 2C18, and 2C19 make up the CYP2C subfamily and are primarily located in the liver; these enzymes account for approximately 26% of total CYP contents [185]. Together, the CYP2C subfamily is responsible for the metabolism of about 20% of clinically relevant drugs [204]. In particular, CYP2C9 is among the most abundant CYP enzymes in the liver (~24% of total CYP content), where it metabolizes approximately 15% of clinically used drugs, including S-warfarin and phenytoin – drugs with narrow therapeutic indices [205, 206].

There are more than 60 variants and subvariants of CYP2C9 [207]. Two of the most common allelic polymorphisms in the CYP2C9 gene, *2 and *3, result in the "poor metabolizer" (PM) phenotype and thus have a marked effect on S-warfarin metabolism [208]. CYP2C9 PMs clear S-warfarin up to 85% less efficiently than normal metabolizers, which leads to longer warfarin half-life, longer time to achieve goal INR, and increased bleeding risk [209]. Likewise, CYP2C9 PMs are at risk for phenytoin toxicity as they require 30–50% dose reduction compared to normal metabolizers [210].

13.3.1.4 CYP2C19

Located on chromosome 10, CYP2C19 is found primarily in hepatic tissue, but the intestinal wall also contains a significant amount of the enzyme. CYP2C19 is responsible for the metabolism of approximately 10% of commonly used drugs [211, 212], including proton pump inhibitors (PPIs), tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), benzodiazepines, phenobarbital, phenytoin, bortezomib, and voriconazole [213, 214]. CYP2C19 is also involved in the activation of clopidogrel, along with several other CYP enzymes [215]. Thus far, over 40 variants and subvariants of CYP2C19 (*1B to *39) have been described, and the *1A allele has been reported to be the wild type [216].

Clopidogrel is an antiplatelet agent used to treat several different indications including secondary prevention in recent stroke or myocardial infarction and primary prevention of thromboembolism atrial fibrillation, to name a few [217]. Clopidogrel is an irreversible inhibitor of platelet P2Y12 adenosine diphosphate receptor which leads to decreased platelet aggregation. It is a pro-drug which is activated in a two-step pathway that involves CYP2C19. Several studies have shown that PMs do not activate clopidogrel to the same extent as normal metabolizers (NMs) [218]. The CYP2C19 genotype has also been linked with clinical outcomes for several indications [219–223]. For example, the CYP2C19*17 allele leads to increased function and has been associated with an increased risk of bleeding [224]. Because of the strength of evidence, a guideline published by CPIC suggests alternative antiplatelet therapy for CYP2C19 intermediate metabolizers (IMs) and PMs [218].

13.3.1.5 CYP2D6

Though it is one of the less abundant CYPs in the liver (~5%) [185], CYP2D6 plays a role in the metabolism of about 25% of drugs used clinically [225]. Drugs that are extensively metabolized by CYP2D6 include tricyclic antidepressants, SSRIs, other nontricyclic antidepressants, beta-blockers, opioids, antiemetics, and antihistamines. CYP2D6 is highly polymorphic and leads to significant inter-individual variation in enzyme expression and activity. In fact, it has been reported that there are nearly 150 variants of CYP2D6 (*1B to *149) [226]. CYP2D6 also exhibits gene duplication, which results in multiple functional copies of the enzyme and increased activity. Hence, as a result of the variability in the CYP2D6 phenotype, individuals can be classified as poor, intermediate, normal, or ultrarapid metabolizers [227], and these can vary by racial backgrounds [228]. The example of codeine aptly illustrates the variability in the expression and catalytic activity of CYP2D6 [229]. Codeine is inactive but is bioactivated to morphine by CYP2D6, the extent of which depends on the variant of CYP2D6 present in the

patient. While poor or intermediate metabolizers show decreased conversion to morphine and a reduced analgesic effect, ultrarapid metabolizers may show life-threatening adverse effects after codeine administration [229, 230]. This example is further discussed in Sect. 13.4.1.

13.3.1.6 CYP3A4/5

CYP3A4 and CYP3A5 are expressed in the liver, and these enzymes are responsible for metabolizing more than 50% of medications used clinically [231, 232]. While CYP3A4 activity is highly variable, genetic differences do not account for all of the variability [233]. CYP3A5 is structurally similar to 3A4 and thus results in substrate overlap. Tacrolimus, a CYP3A5 substrate, is subject to therapeutic drug monitoring in clinical practice; however, evidence is scant when assessing clinical outcomes associated with genotyping. There are no genotype-based guidelines available for CYP3A4 or CYP3A5, but the CYP3A5 genotype has been associated with varying tacrolimus concentrations [51]. CYP3A4 and CYP3A5 also play a role in carbamazepine (CBZ) metabolism [234]. Particular variants such as CYP3A5*3, the most common nonfunctional variant, results in higher levels of CBZ plasma concentration [235]. Patients who harbor this variant may require lower doses of CBZ, though the clinical impact of this is disputed. Furthermore, CYP3A4*22 results in a lower CBZ-diol/CBZ epoxide ratio, the clinical significance of which has yet to be fully elucidated [99].

13.3.2 Uridine Diphosphate-Glucuronosyltransferases (UGTs)

Mostly complementary to oxidative metabolism, mediated by the CYP enzyme family, uridine diphosphate-glucuronosyltransferases (UGTs) are a superfamily of enzymes and probably the most important conjugative enzyme [236]. UGTs catalyze the conjugation of a glucuronic acid molecule unto the substrate to enhance its water solubility thereby enhancing its renal excretion [237]. Like the CYP enzymes, UGTs are primarily located in the liver [238–240] but can be found in a number of other tissues including the kidneys, gastrointestinal tract, lungs, epithelium, ovaries, testis, mammary glands, and prostate [238, 240–242]. While functional genetic variations have been found in UGTs, including those which are important in drug metabolism [243–254], less is known about which variants lead to altered enzyme function.

13.3.3 Other Polymorphisms in Drug Metabolism

13.3.3.1 TPMT and NUDT15

Thiopurine methyltransferase (TPMT) and nudix (nucleoside diphosphate-linked moiety X)-type motif 15 (NUDT15) play an important role in the metabolism of thiopurines (azathioprine, thioguanine, and mercaptopurine) [255]. TPMT inactivates mercaptopurine via catabolism, and individuals who inherit two nonfunctional TPMT alleles are at greatly increased risk of severe myelosuppression with standard doses of mercaptopurine or azathioprine [256]. A guideline developed and published by CPIC suggests dose adjustments for TPMT phenotypes based on genotype [257].

NUDT15 catalyzes the conversion of thioguanine triphosphate metabolites to thioguanine monophosphates which are less toxic [257]. Patients with defective or less active NUDT15 can show severe myelosuppression [258]. Several variants have been assigned star alleles, and a guideline exists in tandem with TPMT to assign metabolizer status to individuals based on genotype and accordingly dose-adjust the thiopurines to minimize the risk of toxicity [257]. Medications in the thiopurine class (i.e., azathioprine, mercaptopurine, and thioguanine) are often monitored by drug levels in clinical practice as they are one of the oldest examples of drugs where metabolic differences lead to a variety of responses in

patients [45]. Thiopurines are antineoplastic agents commonly used for a variety of conditions, including acute lymphoblastic leukemia (ALL) and inflammatory bowel disease [259]. These agents are considered pro-drugs with three main metabolic pathways that include phosphorylation, methylation, and catabolism [41]. The metabolism is illustrated in Fig. 13.3.

It is thought that the phosphorylation pathway wherein inosine monophosphate dehydrogenase creates 6-thioguanine nucleotides (TGNs) is key for resulting in a therapeutic effect because the triphosphate can be incorporated into DNA and RNA as a false purine analog causing cell death [41]. These pro-drugs are also converted into inactive metabolites by xanthine oxidase (XO), TPMPT, and NUDT15 enzymes, leading to 6-thiouric acid (6-TUA), 6-methylmercaptopurine (6- MMP), 6-thioguanosine triphosphate (6-TGMP), and 6-thioguanine diphosphate (6-TGDP) [45]. The metabolite 6-MMP has been linked to thiopurine-induced liver toxicity [260]. In non-malignant conditions, thiopurine use is associated with potential toxicities such as gastrointestinal effects and myelosuppression, and over 20% of patients discontinue their thiopurine medication based on drug-related toxicities [259]. TPMT and NUDT15 deficiency is relatively rare but affects all thiopurine drugs; it is responsible for approximately 30% of all thiopurine-associated neutropenia cases [259]. NUDT15 deficiency is considered another risk factor for thiopurine-induced neutropenia. Patients with two normal function variants, considered TPMT or NUDT15 normal metabolizers, are expected to tolerate mercaptopurine at standard doses, while patients with a low or deficient activity variant and one normal function variant are classified as intermediate metabolizers [259, 261]. Finally, patients with two nonfunctional variants are considered poor metabolizers [262]. TPMT deficiency is observed more in Caucasian and African populations, whereas NUDT15 deficiency is relatively less frequent [263]. In contrast, NUDT15 deficiency is seen more in Asian and Hispanic populations, whereas TPMT deficiency is relatively less frequent [264]. Before starting this medication, it is recommended to have TPMT and NUDT15 genetic testing performed [41, 45]. If a patient has one nonfunctional TPMT or NUDT15 allele, it is recommended to start with reduced doses (by 30–70% with mercaptopurine and azathioprine). If the patient has two nonfunctional alleles, it is recommended to reduce the thrice-weekly dose by 90% or to consider an alternative agent [259]. Once a patient has started a thiopurine, ongoing dosing should be adjusted based on the degree of myelosuppression and advised through TDM [45].

TDM of thiopurines is an excellent example of the measuring of metabolites versus the usual drug level monitoring of the parent compound [41, 265]. Laboratories measure metabolite concentrations in red blood cells, by a quantification of the hydrolysis products from several metabolites [41]. With the use of high-performance liquid chromatography (HPLC), levels of TGN levels and methylated thioinosine derivatives are determined [266]. While higher 6-TGN levels are associated with improved clinical response and remission in inflammatory bowel disease patients, levels of 6-MMP are monitored to avoid toxicity [45, 267]. When applying TDM to patients who continue to have disease progression, patients with normal 6-TGN levels are unlikely to respond to treatment with thiopurines [268]. Patients with low concentrations of both 6-TGN and 6-MMP could be showing noncompliance or a suboptimal dosing strategy, and those with low 6-TGN levels and high 6-MMP levels are suggested to be metabolically shunting away from 6-TGN production and creating more of the toxic 6-MMP [269]. Thus, the metabolism of thiopurines and the polymorphism in their metabolizing enzymes illustrate the importance of measurement of metabolites as this could make a significant difference in achieving optimal therapeutic efficacy while avoiding toxicities.

13.3.3.2 Vitamin K Epoxide Reductase Complex Subunit 1 (VKORC1)

VKORC1 encodes the target enzyme of warfarin, vitamin K epoxide reductase [270]. It catalyzes vitamin K formation from vitamin K epoxide,

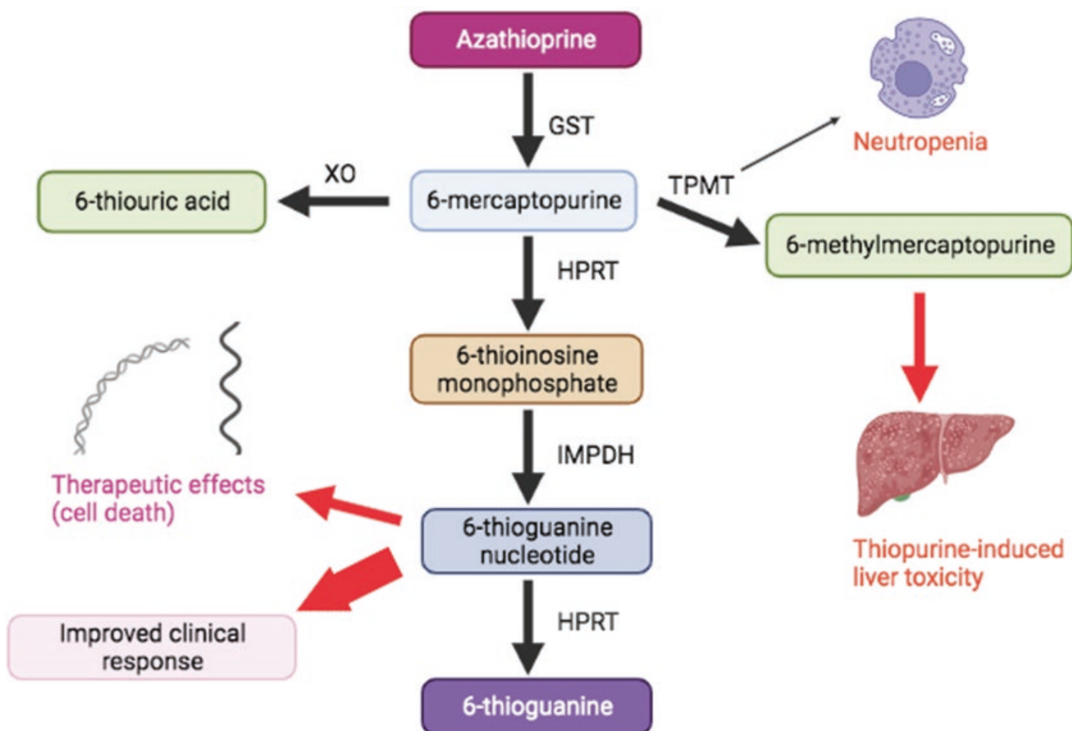

Fig. 13.3 Metabolism of thiopurine. Azathioprine is converted to 6-mercaptopurine by GST. Subsequently, 6-mercaptopurine can be converted to either 6-thiouric acid by XO or 6-methylmercaptopurine by TPMPT. The toxic metabolite 6-methylmercaptopurine has been linked to liver toxicity, and neutropenia can occur in cases of TPMT deficiency. Following the conversion of 6-mercaptopurine to 6-thioinosine monophosphate by HPRT, the metabolite is converted to 6-thioguanine nucleotide (6-TGN). 6-TGN is responsible for the therapeutic effects of thiopurine drugs, since the triphosphate can be incorporated into DNA and RNA and cause apoptosis through the mismatch repair system. As such, higher 6-TGN levels have been associated with improved clinical response. The thiopurine drug, azathioprine, is an immunosuppressive drug used in individuals with diseases such as rheumatoid arthritis, Crohn's disease, and systemic lupus, among others. Abbreviations: GST glutathione S-transferase, HPRT hypoxanthine-guanine phosphoribosyltransferase, IMPDH inosine-5′-monophosphate dehydrogenase, TPMT thiopurine S-methyltransferase, XO xanthine oxidase. (Figure created with BioRender.com)

which is the rate-limiting step in vitamin K recycling [271]. Upstream of VKORC1, one variant (c.-1639G>A, rs9923231) is associated with increased warfarin sensitivity. Individuals with one or two -1639A require lower warfarin doses than patients homozygous for -1639G, and a CPIC guideline exists to guide dosing when combined with other patient factors including CYP2C9 status [272]. There is a race-dependent variation in the expression of VKORC1 with Asian-Americans having a higher proportion of group A haplotypes and African-Americans displaying a higher proportion of group B haplotypes [273]. These and other types of polymorphism in VKORC1 expression play a key role in dose selection for warfarin [274].

In summary, there are many known variants of drug-metabolizing enzymes found for medications with narrow therapeutic index. Several guidelines exist for starting doses of certain medications based on a patient's pharmacogenomic profile; however, there are limited recommendations which guide therapeutic drug monitoring of metabolites. TDM of parent compounds remains the standard for monitoring efficacy and toxicity of drugs with a narrow

Table 13.2 Effect of polymorphism in drug-metabolizing enzymes on efficacy and safety of drugs with narrow therapeutic window

Drug	Enzyme class involved and polymorphic variants	Effect in poor metabolizers	Effects in extensive metabolizers	References
Tacrolimus	CYP3A4 and CYP3A5	May have higher troughs but no dosing changes recommended	Subtherapeutic troughs so initial dose is recommended to start higher	[6]
Thiopurines (i.e., azathioprine, mercaptopurine, and thioguanine	Thiopurine S-methyltransferase, glutathione S-transferase, hypoxanthine-guanine phosphoribosyltransferase, inosine-5′-monophosphate dehydrogenase, thiopurine S-methyltransferase, xanthine oxidase, nudix hydrolase 15	Dose reduction would be warranted based on level of enzyme deficiency	Myelotoxicity due to elevated 6-thioguanine nucleotide	[1]
Clozapine	CYP1A2	Increased serum clozapine level	Decreased plasma level and therapeutic resistance	[23, 24]
Warfarin	CYP2C9, VKORC1	Increased half-life, longer time to achieve goal INR, increased bleeding risk (2C9)		[25]
Phenytoin	CYP2C9	Increased risk for toxicity, require 30–50% dose reduction		[26]
Clopidogrel	CYP2C19	Decreased antiplatelet activity, alternative therapy recommended	Increased risk of bleeding	[27, 28]
Codeine	CYP2D6	Decreased conversion to morphine and decreased analgesic effect	May result in life-threatening adverse effects	[29, 30]
Carbamazepine	CYP3A5, UGT2B7	Higher carbamazepine serum concentration, patients may require lower doses, though this is disputed (CYP3A5)	Lower carbamazepine concentration and escalating dose requirements (UGT2B7)	[31]

therapeutic index in a clinical setting. The contribution of pharmacogenomics to drug metabolism and inherently the safety of these drugs suggest the need to monitor metabolite in TDM of drugs with narrow therapeutic window (Table 13.2).

13.4 Pro-drugs, TDM, and Metabolite Measurement

Pro-drugs are inactive substances that are metabolized in the body to their active metabolites [275]. Pro-drugs often contain functional groups

of esters, amides, phosphates, carbonates, or carbamates that are cleaved either enzymatically or chemically once in the body [276]. Upon biotransformation within the body, the active constituent is released, and the active metabolite can subsequently confer the intended pharmacological benefit of the pro-drug substance. While some examples of pro-drugs in clinical use are not associated with any clinical benefits and were only found to be pro-drugs retrospectively, other pro-drugs confer biological benefits [277]. For example, the cytotoxic drug cyclophosphamide is a pro-drug that only becomes active after undergoing hepatic metabolism [277]. Another clinical benefit of pro-drugs is to mask the polar or ionizable functional groups of the active molecules, ultimately improving oral bioavailability of the active substance [276]. For instance, tenofovir is a nucleotide inhibitor of reverse transcriptase involved in human immunodeficiency virus 1 (HIV-1) infections. Tenofovir was once a drug with limited clinical utility due to the high hydrophilicity of the phosphonic acid group, resulting in very low bioavailability in humans (<5%) [276]. However, the pro-drug "tenofovir disoproxil" had better oral bioavailability in humans (39%). The conversion of this pro-drug was demonstrated to occur by the chemical or enzymatic hydrolysis of tenofovir disoproxil to an intermediate substance, followed by spontaneous loss of CO_2 and formaldehyde, producing the monoester intermediate [276]. Phosphodiesterases were also suggested to play a key role in the final hydrolysis step of the monoester intermediate into the active drug "tenofovir" [276].

As was described in the previous sections of this book chapter, TDM of metabolites is important for many drugs in order to ensure that the drug remains within a safe therapeutic range. Similarly, TDM of the metabolites of pro-drugs is also performed as the outcomes are associated with comparable clinical utility. For example, therapeutic drug monitoring of the active component of the pro-drug fosphenytoin (phenytoin) is performed to guide anticonvulsant therapy [278]. Fosphenytoin is a phosphate ester pro-drug of phenytoin that is rapidly hydrolyzed (half-life of 5–15 min) and is associated with superior solubility and tolerance upon administration [278]. Due to its narrow therapeutic index, saturable elimination kinetics, and its concentration-dependent side effects, it is recommended that drug plasma concentrations be monitored in order to guide dose therapy individualization [278]. The first step in the enzymatic hydrolysis of the fosphenytoin sodium salt substance by phosphatase results in the formation of 3-hydroxymethylphenytoin (Fig. 13.4).

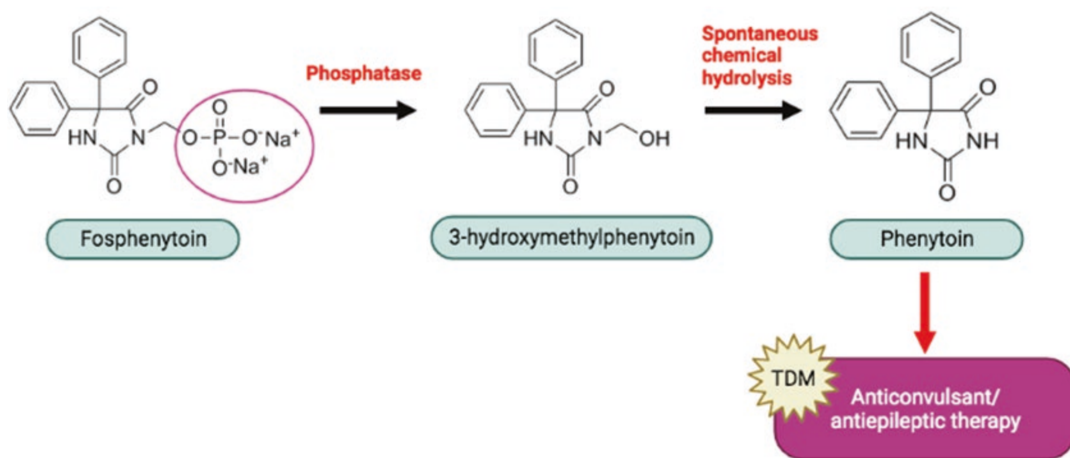

Fig. 13.4 Metabolic bioactivation of the pro-drug fosphenytoin. Fosphenytoin sodium salt contains a phosphate ester group (red circle) linked to an acidic amine of the antiepileptic agent, phenytoin, through an oxymethylene spacer. The first step in the enzymatic hydrolysis of fosphenytoin by phosphatase results in the formation of an unstable intermediate, 3-hydroxymethylphenytoin, which spontaneously converts to phenytoin. This is a good example of the need to monitor the blood levels of a compound which doubles as the active metabolite and one which merits TDM due to its narrow therapeutic window. (Figure created with BioRender.com)

This unstable intermediate subsequently spontaneously converts to phenytoin [279]. The conversion of the fosphenytoin to the active drug metabolite, phenytoin, occurs rapidly in the blood. Another example of the clinical utility of TDM of pro-drug metabolites can be demonstrated with the use of mycophenolate mofetil (MMF). MMF is a pro-drug that is converted to the active metabolite, mycophenolic acid (MPA), by esterase enzymes in the intestine, liver, and plasma [280]. MPA is associated with immunosuppressant activity. As such, MMF is often used after solid organ transplantation, including lung, heart, and renal transplantation [281]. Many studies have suggested that TDM of MPA in patients receiving these organ transplants may improve clinical outcomes and allow for dose individualization of MMF [282–286]. Effective dose individualization by using TDM of MPA ultimately increases the safety of the drug by potentially minimizing toxicity but also results in a minimal risk of organ transplant rejection, thus improving efficacy [282]. These findings also extend to pediatric transplant patients and children with pediatric lupus nephritis, wherein TDM of MPA was suggested to improve the efficacy of MMF [287, 288]. Altogether, these examples demonstrate the clinical utility of TDM of the active drug MPA as it can be used to guide clinical management and MMF dosing [282].

13.4.1 Toxicity of Metabolites of Pro-drugs

As shown above, the wide variation in the expression of drug-metabolizing enzymes among patient populations can impact the efficacy and/or toxicity of drugs used in clinical practice. Hence, in the case of pro-drugs which require prior metabolism before conversion to a therapeutically active molecule, individuals who do not express the relevant enzymes (slow or non-metabolizers) would not benefit from the use of such medications [44, 45]. On the other hand, patients who highly express these enzymes (rapid or ultrarapid metabolizers) produce a very high level of the active metabolite such that there may be safety concerns [289]. A clinical example of a direct relationship between metabolism and toxicity is in codeine, a pro-drug narcotic analgesic [44]. As a parent drug, codeine has no therapeutic effect [290]. However, metabolism mediated by hepatic CYP2D6 forms morphine, its active metabolite. Codeine may also undergo glucuronidation to produce codeine-6-glucuronide [291]. Morphine and codeine-6-glucuronide are two active metabolites with well-pronounced therapeutic effects for pain relief (Fig. 13.5).

In patients who undergo ultrarapid metabolism by CYP2D6, there is a dramatic shift of metabolism of codeine toward morphine. An increase in the levels of morphine leads to an increased risk of oversedation, respiratory depression, and death, even with a low dose of codeine. Alternatively, for patients who are poor CYP2D6 metabolizers, they often experience poor pain control from codeine therapy [292]. In 2013, the US FDA issued a warning to restrict the use of codeine by adding a contraindication to the drug label of codeine alerting that codeine should not be used to treat pain or cough in children younger than 12 years [293]. A similar example is tramadol, an opioid analgesic pro-drug which is transformed into O-desmethyltramadol, the active metabolite, via metabolic pathways that include CYP2D6 [44, 294]. Excessive levels of this metabolite lead to the same adverse effects as a morphine overdose which includes oversedation, respiratory depression, and death [44]. The FDA has also issued a similar warning to restrict tramadol use in children older than 12 years of age for pain management after adenotonsillectomy and in children younger than 18 years of age [293]. The examples above illustrate the importance of measuring the active metabolite which would be of greater clinical value to determine drug toxicity in comparison with measuring the parent compound.

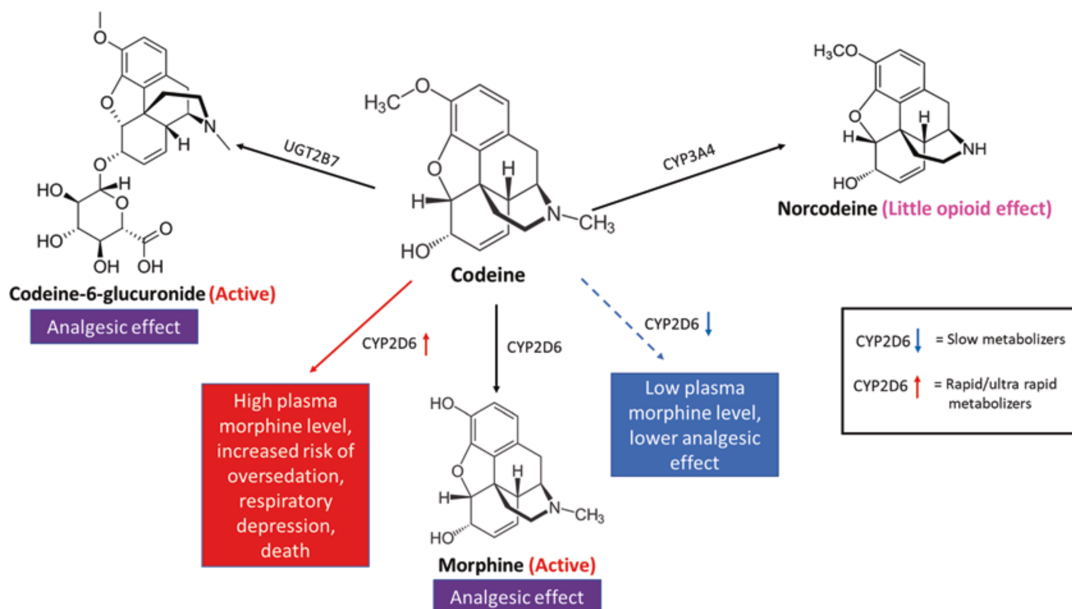

Fig. 13.5 Metabolism of codeine. Codeine undergoes hepatic metabolism into morphine (10%), norcodeine (5–10%), and codeine-6-glucuronide (70–80%). While codeine has no direct therapeutic effect, the active metabolites (morphine, codeine-6-glucoronide) have analgesic effects, exploited in pain management. Patients who exhibit rapid metabolism of codeine by CYP2D6 display a shift of metabolism toward morphine, resulting in adverse effects such as oversedation, respiratory depression, and possibly death. On the other hand, poor CYP2D6 metabolizers experience a lack of efficacy from codeine therapy. (Figure created with BioRender.com)

13.5 Conclusions and Future Directions

While the main focus of TDM over the years has been the determination of the level of the parent compound in order to achieve an optimal therapeutic benefit while also avoiding toxicity, this chapter has highlighted the importance of measuring metabolites of drugs subject to TDM. Through the various examples that have been provided in this chapter, where clinical decision-making based on the blood level of the administered medication leads to an incomplete picture of efficacy or toxicity, we hope to provide further justification for the value and importance of taking into consideration the impact of metabolic pathways. If this approach is adopted, the ability to provide a higher level of precision medicine for our patients will be optimized.

One major setback in the measurement of metabolites is the practicality and cost of running these assays on a routine basis. The first step is the screening of the entity involved in the toxicity of the drug of interest, to see if the toxicity of concern is a result of the parent compound or the metabolites. This will require establishing an association between the dose ingested, blood level of the parent compounds and/or metabolites, and emergence of toxicity. Since detection of metabolites at levels up to 25% of circulating drug levels can be a safety concern [74–76], such metabolites should be further investigated as to their possible role in the toxicity of the drug, particularly in cases of the adverse reactions. These results can then be followed with further investigations in a larger patient population to determine the association between the emergence of the adverse effect and metabolite detection. In developing an assay for the detection of the metabolites, the same standard processes and considerations can be implemented as those used for measuring parent compounds. Important considerations include the timing of sample collec-

tion, sample processing (including choice of anticoagulant to use for blood collection), and sample storage conditions [3]. These factors depend on the compound of interest and the metabolite of interest, and method development and validation are key in this endeavor. Key factors affecting the robustness of the chosen method include its accuracy, precision, limit of quantification/detection/identification, linear dynamic range, reproducibility, and repeatability [295]. To a large extent, techniques including radioimmunoassay, high-performance liquid chromatography (HPLC), immunobinding assays, fluorescence polarization immunoassay (FPIA), enzyme-multiplied immunoassay technique (EMIT), and enzyme-linked immunosorbent assay (ELISA) have been used in the determination of the blood level of parent compounds [296–298] and can be used in metabolite measurement. Another concern in metabolite measurement is in the fact that cross-reactivity has been reported between the metabolites and cellular macromolecules such as proteins and even antibodies, thereby resulting in an alteration in the measurable concentration of the metabolite in question. Therefore, cross-reactivity may ultimately interfere with the result of the assay [299, 300]. For this reason, other specific methods would need to be developed to accurately measure the concentration of metabolites. Furthermore, since reactive metabolites are mostly unstable, a delay between sample collection and analysis can result in an undetectable amount of the metabolite. This characteristic highlights the importance of developing a robust method for quantification of reactive metabolites.

Finally, another key consideration in the implementation of metabolite measurement in TDM is the cost of running such analyses vis-à-vis the benefit to the patients. The pharmacoeconomic impact of TDM has been studied to compare the advantage that accrues to the patients in comparison with the cost involved in running such analyses. While it is expected that adding metabolite measurement to standard TDM may be associated with considerable cost, the overall advantage to the patients in terms of quality of life, lower incidence of adverse reactions, and increased longevity may outweigh these costs [301]. As reviewed by Kang and Lee [3], the example of antiepileptic therapy was used to exemplify the benefit of monitoring metabolites in TDM. This review reported improved therapeutic outcomes including effective seizure control, a reduced incidence of adverse reactions, cost savings from reduced hospitalization per seizure, and greater chances of remission in patients who underwent TDM. Further, these factors enhanced the quality of life of the patients, resulting in better earning capacity, with reduced economic losses due to hospital stay [301]. A similar outcome has been reported for other therapeutic interventions in which TDM has been applied, including aminoglycoside drugs [302, 303], immunosuppressants [304], and other indications [305]. Of particular interest in these cases was the fact that dose optimization and individualization, especially upon consideration of pharmacogenetics, resulted in cost-effectiveness. A review by McNeill and Barclay [306] showed that TDM has proven to be cost-effective overall in the management of inflammatory bowel disease when drugs such as thiopurine were considered. Additional modeling data to predict the cost-effectiveness and beneficial outcomes of metabolite measurement in the TDM of thiopurine was reported in another study [307]. In addition to enhanced outcomes, it was predicted that metabolite measurement would result in approximately 18% reduction in the cost of treatment. Since the studies described above were based on a modeling data, further studies may be required to further demonstrate the cost-effectiveness of metabolite measurement in TDM with a particular focus on metabolites.

Although TDM focuses primarily on the measurement of parent compounds administered to patients in clinical practice, this chapter has highlighted the importance of including metabolite measurement in TDM as these measurements further enhance the quality of healthcare, assist to monitor and predict the emergence of drug-induced organ-system toxicity, and help optimize therapy. Not all drugs requiring TDM would benefit from metabolite measurement. However,

those which are known to be metabolized to bioactive or toxic metabolites need to be monitored, as they can have significant adverse impacts on patient outcomes. In order for an all-encompassing adoption of metabolite determination in TDM to be implemented, there is a need for a well-defined strategy for sample collection, analytical method development, and cost-benefit analysis. This would ultimately facilitate the robust implementation of TDM, resulting in a reduced incidence of drug toxicity and optimal utilization of therapeutics.

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14 Recent Advances in Nanosensors for Therapeutic Drug Monitoring (TDM)

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Abstract

Nanomaterials have at least one dimension that is less than 100 nm in size. This chapter focuses on the recent development seen in the use of nanomaterials and nanotechnology for therapeutic drug monitoring (TDM) in order to meet the urgent need of measuring drug concentrations in biological matrices like plasma, serum, saliva, or urine, at a lower cost and within a shorter time period than traditional immunoassays or chromatographic methods. Developments in the detection of different types of medications (e.g., antibiotics, anticonvulsants, anticoagulants) are described, using both optical and electrochemical approaches and including competing nanosensor technologies for the same drug analyte.

Keywords

Carbon nanotubes · Electrochemical determination · Fluorescence · Nanoparticles · Quantum dots

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14.1 Traditional TDM and the Need for Nanosensors

One factor that significantly impacts the rational prescribing process is therapeutic drug monitoring. Despite the fact that, in general, one can easily observe and measure the effects of many drugs that are administered for treatment purposes, the situation is different (and potentially life-threatening) for other specific drugs. In the case of the former group of drugs, their doses can be easily modified, or treatment can be halted in case of observed (and reversible) adverse events. However, the latter group of drugs requires therapeutic drug monitoring to attain the desired benefits of the drugs with reduced risk. Some of these medicines exhibit characteristics such as large inter-individual variation between the drug dosage and its benefits, which makes therapeutic drug monitoring useful in patients taking such medications. It is generally not advisable nor possible to take routine measurements of drug concentration at the site of action; however, considering the fact that plasma concentration of a drug can better predict the therapeutic outcome or adverse effects of the drug than the dosage, it has become necessary to monitor such drugs to improve outcomes [1]. The World Health Organization (WHO) has therefore proposed a

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list of medications that warrant therapeutic drug monitoring, as follows: phenytoin, amikacin, cyclosporine, lithium, valproic acid, methotrexate, digoxin, phenobarbital, gentamicin, carbamazepine, and vancomycin [2]. Therapeutic drug monitoring involves multiple sequential stages such as accurate determination of plasma concentration, interpretation of the obtained results, and, finally, analysis of how much dose is required for the patient to obtain benefits from the medication without any adverse effects. This chapter focuses mainly on the stage of precisely measuring the plasma concentration of the drugs proposed by WHO, or measuring their concentration in other biological matrices like saliva and urine, with the use of nanosensors (Table 14.1).

14.2 Traditional TDM Practice *Versus* Recent Advances

Therapeutic drug monitoring has evolved over the years, beginning with the use of chromatographic methods. Gas chromatography and high-performance liquid chromatography (HPLC) have been widely applied to determine the concentration of drugs in body fluids, especially plasma. Liquid chromatography with tandem mass spectrometry (LC-MS/MS) methods were later developed that exhibited high specificity in detecting the analyte. However, challenges remained, such as lower throughput and inability to get accurate results due to the interference of the matrix and co-eluting compounds during mass spectrometry, among others [3]. HPLC techniques were then combined with simple detectors (diode-array detection, flame ionization detection, or ultraviolet detection) that helped relieve some burden off equipment cost, operation time, and the need for expertise. The difficulty in resolving different analytes with similar retention times was further worsened by unknown constituents present in the biological matrices under study [4]. Immunoassays were later developed and have been widely employed in clinical laboratories for therapeutic drug monitoring. This technique offers many advantages such as affordability, high-throughput adaptability, high affinity, requirement of low sample volume, and ease to conduct. The demerit in using immunoassays lies in the contribution of cross-reactants or other nonspecific constituents in the sample to the observed analyte concentration that makes this technique less specific [4]. A more recent development in TDM is the introduction of nanotechnology for drug monitoring, at a lower cost and within a shorter time period than immunoassays or chromatographic methods. In addition, the use of nanosensors does not require highly trained personnel.

14.3 Definition of Nanomaterials

Nanomaterials (e.g., carbon nanotubes; Fig. 14.1) refer to materials that have at least one dimension of single or multiple phase that is less than 100 nm in size [5].

14.4 Definition of Nanosensors

Nanosensors are sensors that are made up of nanomaterials or nanoparticles and engineered to possess nanoscale dimensions in order to increase their surface area for detection of a specific molecule, an environmental circumstance, or a biological component [6]. These sensors thus exhibit improved selectivity and sensitivity compared to their macroscale analogs. Nanosensors are made up of (1) a recognition element that is specific for a particular analyte and helps to quantify them and (2) a signal transducer that produces a quantifiable signal based on the interaction between the analyte and the recognition element [6]. These signals are further amplified and processed to generate data that corresponds to the analyte concentration.

14.5 Current Nanosensor Techniques for TDM

Nanosensors are categorized into different types based on their signal transduction methods or the biological recognition element used (Table 14.1).

Table 14.1 TDM nanosensor design details discussed in this chapter

Drug	Nanosensor design	Linear range reported	Detection limit reported	Biological matrix investigated	Reference
Gemcitabine	*Optical*: silver-associated carbon quantum dots (Ag-CQD)	0.003 μM–0.1 μM	0.002 μM	Plasma and urine	[20]
Flucytosine	*Raman spectroscopy*: nitrocellulose/inkjet-fabricated paper sensor	10 μg/mL–150 μg/mL	12.1 μg/mL	Undiluted serum and whole blood	[21]
Linezolid	*Electrochemical*: cobalt nanoparticle-incorporated 2-hydroxypropyl-β-cyclodextrin (HP-βCD)	1.0×10^{-10} M–1.0×10^{-4} M	1.7×10^{-11} M	Plasma	[22]
Amikacin	*Optical*: surface plasmon resonance (SPR) immunoassay	0.33 ng/mL–5.77 ng/mL	0.13 ng/mL	Serum	[23]
Gentamicin	*Optical*: epicatechin coated silver nanoparticles (Ag-NP)	0 μM–100 μM	1.28 μM	Serum and plasma	[24]
Digoxin	*Electrochemical*: silver nanoparticle-decorated graphene oxide composite (AgNP-GO)	1 pM–0.1 μM	0.3 pM	Plasma	[27]
Digoxin	*Optical*: aqueous gold nanoparticles (AuNP)	N/A	Colorimetric? 571 pM Fluorescence? 392 pM	Serum	[28]
Warfarin	*Electrochemical*: magnetic iron oxide nanoparticle-modified carbon paste	0.5–1000 μM	0.21 μM	Urine and serum	[31]
Warfarin	*Electrochemical*: cadmium sulfide (CdS) quantum dots on carboxylated multiwalled carbon nanotubes (MWCNT)	0.05–80 μM	8.5 nM	Serum, breast milk, and urine	[32]
Warfarin	*Electrochemical*: manganese ferrite nanoparticles and a MWCNT paste matrix	0.10–447 μM	0.08 μM	Serum and urine	[35]
Warfarin	*Electrochemical*: molecularly imprinted polymer (MIP) nanosensor with MWCNT and gold nanoparticles	0.031–0.616 ng/mL	0.024 ng/mL	Serum	[37]
Warfarin	*Electrochemical*: MWCNT with cobalt oxide nanoparticles	0.008–800 μM	0.0033 μM	Serum and urine	[39]
Heparin	*Optical*: rhodamine B-labeled peptides	0.01– 0.1 nM and 1.0–70.0 nM	7.5 pM	Serum	[45]
Aspirin	*Electrochemical*: carbon electrode with chitosan capped with gold nanoparticles	1 pg/mL–1 μg/mL	0.03 pg/mL	Urine and saliva	[51]

(continued)

Table 14.1 (continued)

Drug	Nanosensor design	Linear range reported	Detection limit reported	Biological matrix investigated	Reference
Tramadol	*Electrochemical*: MIPs on decorated graphene nanosheets with Ag-NPs	3.5×10^{-9}–1.0×10^{-2} M	2.04×10^{-9} M	Urine	[61]
Sulfasalazine	*Electrochemical*: nickel oxide + carbon nanotube (NiO/CNT) composite	5.0×10^{-7}–8.0×10^{-4} M	9.0×10^{-8} M	Urine	[64]
Theophylline	*Electrochemical*: screen-printed carbon electrodes + graphene quantum dots	1.0–700.0 μM	0.2 μM	Urine	[69]
Theophylline	*Optical*: RNA aptamer + graphene oxide/cryonase-mediated fluorescence amplification	50 nM–5 μM	47 nM	Serum	[72]
Levothyroxine	*Electrochemical*: melamine Au-NPs + mercaptoethanol-modified MWCNT	10–120 nM	2.84 nM	Serum	[74]
Lithium	*Optical*: ruthenium-based metallacrown complexes	N/A	N/A	Serum	[77]
Carbamazepine	*Electrochemical*: cerium-doped zinc oxide (ZnO) and reduced graphene oxide	0.05–100 μM	1.2 nM	Urine	[80]
Phenobarbital	*Optical*: MIPs on green source carbon dots (GSCDs)	0.4–34.5 mM	0.1 mM	Plasma	[88]
Phenobarbital	*Electrochemical*: platinum NPs + MWCNT	0.4–60 μM	0.1 μM	Urine	[93]
Valproic acid	*Optical*: thioglycolic acid (TGA)-capped CdTe quantum dots	0.3–7.5 mg/L	0.24 μg/mL	Serum and urine	[95]
Phenytoin	*Optical*: branched gold nanoparticles (B-AuNPs)	67–670 ng/mL	21 ng/mL	Plasma	[96]

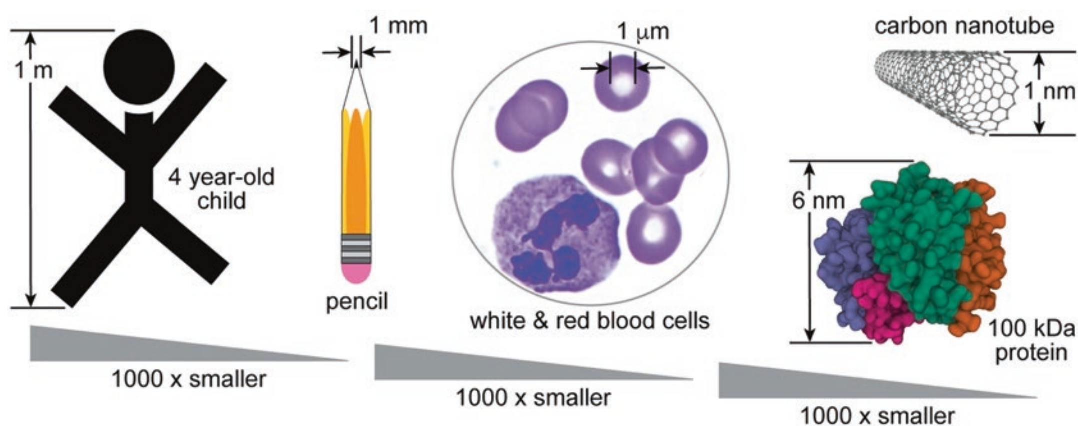

Fig. 14.1 Relative sizes of various objects to highlight the extreme scale of nanomaterials. "Nano-" is defined as one billionth (10^{-9}) part of an arbitrary unit of measurement, hence the use of the term "nanomaterials" to describe materials like carbon nanotubes. The diameter of a typical carbon nanotube is 10^9 shorter than a typical 4 year-old child

According to their signal transduction methods, nanosensors are classified into optical, electrochemical, nanomechanical, or piezoelectric sensors [7]. Affinity and catalytic biosensors are differentiated based on the kind of biological recognition element they possess: whereas catalytic biosensors depend on chemical reactions catalyzed by enzymes, affinity biosensors depend on complex formation between the biorecognition element and the analyte [8]. Detection of the analyte-receptor complex can be achieved via labeling (e.g., fluorescent or enzymatic), or a change in the physical properties of the signal transducer [9].

Electrochemical sensors are nanosensors that determine the concentration of an analyte in a medium or matrix based on the degree of change in current or voltage after an interaction between a bioreceptor and the analyte [10]. Therefore, nanosensors which produce signals that are potentiometric, amperometric, or impedimetric (which includes conductimetric) are electrochemical in nature [11]. This kind of sensor has been widely used in monitoring several drugs in which some examples are listed below. For a successful electrochemical measurement, the setup requires electrodes, a potentiostat/galvanostat, and a solution [12]. The three kinds of electrodes used in the setup are pretreated and polished to enhance reproducibility and eliminate contaminants. They include the reference electrode (which creates an avenue for measuring the potential of other electrodes based on the stable potential it maintains), working electrode (where the reaction with analyte and electrochemical measurement occurs), and counter/auxiliary electrode (which completes the electrical circuit) [12]. The analyte is present in the solution together with some ions that facilitate the flow of charges between the counter electrode and the working electrode. The potentiostat connects to all the electrodes and measures the given response [12]. Modification of the electrodes with nanostructures as well as biorecognition elements improves the overall performance of the sensor. The surface of the electrode can be modified with nanostructures in many ways such as addition of graphene/graphene oxide carbon nanotubes, conducting or metallic or carbonic polymer nanomaterials, and/or quantum dots [13]. Various classes of biorecognition elements that can be incorporated onto an electrode (depending on the type of matrix and analyte) include redox reaction molecules, binding-only molecules, and conformation changing molecules [12]. Examples of redox reaction molecules are enzymes and redox-reactive species. Some binding-only molecules that are used include molecularly imprinted polymers (MIP) and antibodies. Aptamers are conformation changing molecules, often based on nucleic acids, that can also be used to modify an electrode to enhance its performance [12].

Optical sensors are sensor devices that determine the concentration of an analyte based on the degree of change in properties of light such as fluorescence, absorption, light scattering, or refractive index after a molecular recognition type reaction [14]. Two categories of optical sensors have been described: (1) evanescent field-based sensors and (2) bio-optrodes. Evanescent field-based sensors are characterized by production of an evanescent wave due to transmission of light by total internal reflection in an exposed fiber core. This generated evanescent wave can be absorbed in the presence of an absorbing molecule in the field, resulting in diminished amplitude of the wave being propagated in the fiber core [15]. Examples of such sensors include total internal reflection fluorescence (TIRF) sensors, reflectometric interference spectroscopy (RIfS) sensors, surface-enhanced Raman scattering (SERS) sensors, and surface plasmon resonance (SPR)-based sensors [8]. Bio-optrodes are built from an optical fiber with a sensing layer at the end point of the fiber containing biorecognition molecules and dyes. A reaction between the analyte and the constituents of the sensing layer causes a measurable change in the optical properties of the transducer. A fiber optic device is an example of a bio-optrode [16].

Piezoelectric sensors are sensors that possess an oscillating piezoelectric crystal capable of vibrating at its natural frequency to produce resonance. A signal produced due to an interaction between an analyte and a recognition element causes a change in frequency which in turn

results in a change in electric current being measured. This change in electric current determines the concentration of analyte detected [17].

Nanomechanical sensors measure the concentration of the target analyte, via either an optical or piezoelectric system, when their detection through molecular interaction on the surface of the sensor causes a cantilever flection [18]. Both the piezoelectric and nanomechanical types of sensors are seldom used in therapeutic drug monitoring, but they are popular in performing quality control procedures in industry and protein evaluations, respectively.

14.6 Nanosensors for Clinical Use

14.6.1 Nanosensor for Gemcitabine

The anticancer chemotherapeutic agent gemcitabine (an analog of cytarabine) is primarily used to manage solid tumors occurring in the lung, bladder, pancreas, breast, and ovary [19]. Gemcitabine is given by intravenous administration in the hydrochloride form, infused over 30–60 min, and subsequent doses are adjusted depending on both the therapeutic and toxicologic responses observed. Rapid clearance of gemcitabine occurs after absorption in the blood stream. Metabolism of this drug occurs in the blood, kidney, liver, and other tissues by cytidine deaminase [19]. Almost the entire dose of gemcitabine is eliminated in urine with 1% excreted in feces. Its half-life varies between 42 and 94 min based on age and sex; clearance in men is 25% higher than in women [19]. Bone marrow suppression has been identified as the main adverse effect that limits the dose of gemcitabine; however, this effect does not generally require therapy to be stopped. Interstitial pneumonitis, acute respiratory distress syndrome, as well as pulmonary fibrosis can occur with therapy. Such pulmonary toxicity generally requires therapy to be stopped [19]. Caution must also be taken when using gemcitabine in patients with impaired renal or hepatic function due to reports of transient liver enzyme elevations, proteinuria, hematuria, severe hepatotoxicity like liver failure, and death. Initial signs of microangiopathic hemolytic anemia warrant the stoppage of gemcitabine treatment [19].

For the reasons outlined above, there is the need to develop a suitable monitoring tool for detection of gemcitabine in body fluids for optimum therapeutic outcome. Methods of detection that have previously been identified have posed several challenges due to how expensive, less sensitive, and time-consuming they are. Therapeutic monitoring of gemcitabine using nanosensing technology has of late shown a promising solution to these aforementioned problems. Carbon quantum dots (CQDs) are fluorescent nanomaterials that have favorable optical and electrical properties for the detection of gemcitabine [20]. The ease of modification of this nanomaterial through simple deposition or coating with metal particles or organic molecules, as well as their biosafety and simple preparation procedure, offers an advantage for extensive research and development into TDM nanosensors. Studies have investigated the potential of CQDs associated to metal nanoparticles (M-CQDs) to detect gemcitabine in the biological matrices of human plasma and urine. Silver-associated carbon quantum dots (Ag-CQD) were seen to exhibit higher photostability and detectable responses to gemcitabine compared to other metals tested (e.g., Cu, Mn, Ni) [20]. Optimal detection conditions were identified as a pH of 6 and 7 min of incubation time, with a linear range of drug detection from 0.003 μM to 0.1 μM of gemcitabine [20]. Hence, Ag-CQD can be used to measure gemcitabine in nanomolar concentrations. Subsequent tests performed to validate the application of Ag-CQD gemcitabine sensor in human plasma and urine revealed no interference between the matrix and the different concentrations of gemcitabine [20]. Therefore, this Ag-CQD nanosensor design has the capability to meet the need to measure gemcitabine in biological specimens during cancer chemotherapy treatment.

14.6.2 Nanosensors for Specific Antibiotics

14.6.2.1 Nanosensor for the Antifungal Flucytosine

Flucytosine (5-fluorocytosine; 5FC) is an antifungal drug indicated for the treatment of systemic fungal infections. Cryptococcal meningitis and severe systemic candidiasis are commonly treated with a combination therapy of flucytosine and fluconazole or amphotericin B. The therapeutic range for flucytosine is narrow: spanning from 20 to 80 μg/mL [19]. Rapid and almost complete absorption of flucytosine occurs in the gastrointestinal tract, reaching a bioavailability ranging from 78% to 89%. A trough plasma concentration of 25–50 μg/mL is targeted when adjusting the dose in patients, especially patients living with AIDS who are highly susceptible to bone marrow toxicity [19]. Flucytosine can be given by oral, intravenous, or topical administration. Fungi such as *Cryptococcus neoformans* and *Candida* spp. are known to develop resistance against flucytosine. However, less resistance issues have been associated with application of the combination of flucytosine and amphotericin B [19]. The distribution of flucytosine is extensive in the body tissues, with 65–90% of concentration in the serum present in the cerebrospinal fluid (CSF) [19]. The drug is filtered in the glomerulus, and about 90% is excreted in the unchanged form; fluorouracil is a product of metabolism of a minor amount of flucytosine. Orally administered flucytosine that does not get absorbed in the gastrointestinal tract is eliminated unchanged in the feces. This drug has an elimination half-life of 2.5–6 h, a time span that rises with a decline in renal function. Combination therapy with amphotericin B, poor renal function, as well as plasma concentrations exceeding 100 μg/mL has been associated with bone marrow suppression, notably thrombocytopenia and leucopenia [19]. Plasma concentrations greater than 100 μg/mL also pose a higher risk of hepatotoxicity and gastrointestinal toxicity [19]. Significant variations in renal clearance have been observed among patients, especially when combined with amphotericin B, posing a challenge to determine the physiological load in real time.

This justification above explains the need for real-time therapeutic drug monitoring of flucytosine. Complicated microbiological assays, as well as methods like HPLC or LC/MS, have posed challenging in measuring flucytosine levels due to their time-consuming and expensive natures and their advanced training requirements. A dual substrate paper/membrane system utilizing inkjet-fabricated, surface-enhanced Raman spectroscopy (SERS) nanosensors has been used for rapid detection of flucytosine in undiluted serum and whole blood [21]. Rapid monitoring of drugs from such biological matrices as serum and whole blood requires physical separation of the analyte from the blood components since these components can obscure signal detection by non-discriminately coating the surface of the paper-based SERS sensors. Nitrocellulose membranes combined with paper SERS sensors in a passive vertical flow scheme help to achieve this physical separation goal [21].

14.6.2.2 Nanosensor for Linezolid

Linezolid is used for the treatment of infections caused by gram-positive bacteria occurring in the respiratory tract and the skin. It is active against nosocomial infections caused by multidrug-resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci [19]. It can be administered via the oral or intravenous route. It is rapidly absorbed into the blood stream after 1–2 h of oral administration. There is extensive distribution of linezolid into the muscle, fat, cerebrospinal fluid, lungs, bone, and skin blister fluids. It undergoes extensive metabolism, with only 30% being excreted in urine in the unchanged form. Linezolid has an elimination half-life of 5–7 h [19]. Clearance of the drug is more rapid in children compared to adults. Resistance in staphylococci (including methicillin-resistant *Staphylococcus aureus*, *S. auricularis*, and *S. epidermidis*) and enterococci (*Enterococcus faecium* and *Enterococcus faecalis*) has been reported in its use [19].

The variable pharmacokinetics of linezolid and the emergence of resistance warrant the need for therapeutic monitoring of linezolid. One study reported utilizing nanoparticle-enhanced potentiometric ion-selective electrodes (ISE) for the detection of linezolid in human plasma. In addition to its reported advantages of being sensitive, affordable, portable, and selective, little or no sample pretreatment was found to be needed. According to the study, four fabricated sensors were used, namely, a cationic exchanger phosphotungstate sensor, a 2-hydroxypropyl-β-cyclodextrin (HP-βCD) sensor, a copper nanoparticle (NP)-incorporated HP-βCD sensor, and a cobalt NP-incorporated HP-βCD sensor [22]. The cobalt NP-incorporated HP-βCD sensor was the most sensitive among the four sensors tested. HP-βCD promotes recognition of the molecular structure of linezolid as well as formation of inclusion complexes. Moreover, incorporation of the metal oxide NP (cobalt) improved the sensor's selectivity and sensitivity, given the NP's characteristics of having rich electronic and chemical properties, high surface area, thermal stability, and high mechanical strength even though ultra-lightweight. Its selectivity was maintained when used for monitoring linezolid in plasma in the presence of co-administered drugs (meropenem and theophylline) [22].

14.6.2.3 Nanosensor for Amikacin

Amikacin is a member of the group of antibacterial agents classified as aminoglycosides. It is used in the treatment of severe gram-negative infections and is active against gentamicin- and tobramycin-resistant bacteria. Amikacin is commonly seen in neonatal therapy since newborns are highly prone to infections [19]. Enzymes that are known to cause acquired aminoglycoside resistance by degrading other aminoglycosides are unable to break down amikacin. Hence, cross-resistance with other aminoglycosides does not often occur, highlighting amikacin's efficacy against resistant strains. During treatment with amikacin, care must be taken not to drop below trough plasma concentrations of 5–10 μg/mL nor exceed peak plasma concentrations of 30–35 μg/mL [19]. Amikacin has the tendency to cross the placenta but does not readily enter the cerebrospinal fluid. Its half-life is 2–3 h. Almost the entire drug dose is filtered into the urine within 24 h [19]. The drug has a narrow therapeutic range (1–30 μg/mL) and is associated with adverse effects such as irreversible, cumulative ototoxicity and reversible nephrotoxicity [19].

Due to toxicities associated with amikacin use, there is the need for therapeutic drug monitoring in order to optimize therapy and improve patient outcomes. An SPR-based competitive immunoassay has been developed for therapeutic drug monitoring of amikacin in serum [23]. This sensing technique employs an indirect competitive inhibition assay due to the low molecular weight of amikacin (585.6 Da), which can lead to low detectability in the case of a direct assay given that SPR signal is proportional to the molecular weight of the analyte. This technique enables the use of a small volume of the patient sample, which is subsequently diluted for analysis [23]. Moreover, the indirect assay offers more advantage for the therapeutic monitoring of amikacin because the immobilization of antibodies to a solid surface in the direct assay poses many challenges to accurate measurement of the analyte. Indiscriminate (random contact) attachment of the antibodies to a solid surface could tamper with the native structure of the antibodies resulting in loss of affinity [23].

14.6.2.4 Nanosensor for Gentamicin

Gentamicin is an antibiotic that is used in the treatment of systemic infections caused mainly by gram-negative organisms [19]. These diseases include brucellosis, cystic fibrosis, pneumonia, otitis media, meningitis, endocarditis, and septicemia. Therapeutic drug monitoring of gentamicin is paramount to prevent the incidence of antibiotic resistance as well as prevent adverse effects that arise from overdosage such as irreversible ototoxicity and reversible nephrotoxicity. Factors such as high doses over prolonged courses, renal impairment, and age, each of which may predispose an individual to toxicity, must be considered in therapeutic drug monitoring of gentamicin.

Since there are no fluorophores or chromophores in the molecular structure of gentamicin, a nanosensor was developed using epicatechin coated silver nanoparticles to determine the concentration of gentamicin in samples of human serum and plasma [24]. The principle of measuring gentamicin using this design is based on the fact that gentamicin can quench the absorbance intensity of silver nanoparticles at a pH ranging between 1 and 12. The detection limit for this nanosensor was 1.28 μM, and the linear range was observed to be from 0 μM to 100 μM [24].

14.6.3 Nanosensors for Digoxin

Digoxin is a drug used for the management of heart failure and cardiac arrhythmias. It is classified as a cardiac glycoside sourced from the plant *Digitalis lanata*, and it exerts its effects on the vascular smooth muscle and the heart [25, 26]. It increases the myocardial force of contraction as well as reduces conduction of impulse to the heart through the atrioventricular node [19]. Moreover, digoxin increases vagal activity in vascular smooth muscles. In a nutshell, digoxin produces effects such as negative chronotropy, positive inotropy, and reduced atrioventricular nodal activity. The therapeutic effect of digoxin begins 2 h after oral administration and peaks around the sixth hour. Steady-state plasma concentrations are reached between 1 and 3 weeks based on the renal status [19]. Patient therapy begins with a loading dose of digoxin if "digitalization" of the patient (initial dose establishment by fractionation) is needed, followed by a maintenance dose which is adjusted to individual patients based on age, renal function, electrolyte balance, lean body mass, disease nature, thyroid status, and degree of tissue oxygenation to achieve therapeutic efficacy. This drug can be administered through the oral route, intravenous route, or sometimes the intramuscular route. Principally, it has a narrow therapeutic window between 0.5 and 2.0 ng/mL [26]. Confounding this narrow therapeutic window, there is the existence of inter-individual variation. Therapeutic drug monitoring may not be needed in patients who respond well to treatment without any adverse effects depicting toxicity. However, factors such as poor compliance, inadequate response, fluctuating renal function, lack of history whether patient has previously taken a cardiac glycoside, confirmation of clinical toxicity, and drug interactions all warrant the need for therapeutic monitoring of digoxin. Digoxin toxicity can result in cardiotoxicity, neurological symptoms like mental confusion and visual disturbances, as well as hypersensitivity reactions. It has a large volume of distribution with higher concentrations in myocardium compared to the plasma. Digoxin has an elimination half-life ranging from 1.5 to 2 days. It is excreted in urine in the unchanged form via tubular secretion and glomerular filtration [19].

1. An electrochemical aptasensor using silver nanoparticle-decorated graphene oxide (AgNP-GO) has been investigated for use in analyzing digoxin concentration in biological samples [27]. A previous method of employing an immunoassay for digoxin detection in the clinic posed challenges that included the interaction with digoxin-like molecules (e.g., ouabain). Aptamers are single-stranded oligonucleotides that have shown great specificity and affinity for various targets including drugs. They provide benefits such as reproducibility for various targets, great stability, and easily modified with electroactive enzymes and other suitable substances. Electrochemical aptasensors provide great benefit because of their simple operation, high sensitivity, low cost, and great stability. An AgNP-GO nanocomposite was used as a redox tag for this digoxin sensing technique [27]. The surface area of the biosensing platform was also increased with electrodeposited gold nanoparticles (GNP) [27]. These strategies provided a dual signal amplification, better detection limit, and greater dynamic range to the aptasensor. It has been successfully used in detection of digoxin concentrations in human plasma samples without the need for pretreatment. Its linear dynamic range is reported as 1 pM to 0.1 μM, with a detection limit of 0.3 pM

[27]. There is also no need for continuous calibration considering its high degree of reproducibility. However, this biosensor cannot be used multiple times and must be disposed after use.

2. An optical aptasensor based on aqueous gold nanoparticles (AuNP) has independently been developed for the determination of digoxin [28]. Due to the ability of aptamers to bind to a wide range of targets as well as exhibit high affinity and sensitivity to these targets, they were employed in this digoxin sensor. The fluorescence produced by aptamers can be quenched through fluorescence resonance energy transfer (FRET) when these aptamers are adsorbed onto the AuNP surface [28]. AuNPs were selected in fabricating this sensor because they are recognized as strong quenchers. Digoxin binding to the aptamers leads to AuNP aggregation (resulting in a colorimetric change from red to blue) and recovery of the fluorescence produced by the aptamers. The detection limit for the colorimetric aptasensor was 571 pM while that of the fluorescence quenching aptasensor was 392 pM [28]. Based on the outcome, the fluorescence quenching aptasensor is said to be more sensitive than the colorimetric aptasensor. This nanosensor design has subsequently been applied to the detection of digoxin in serum samples [28].

14.6.4 Nanosensors for Blood-Thinning Agents

14.6.4.1 Nanosensors for Warfarin

Warfarin is an oral anticoagulant used to treat and prevent venous thromboembolism [29]. Warfarin therapy is initiated shortly after heparin administration, and this combination is given up to at least 5 days before stopping heparin. It is also beneficial in stroke management and the prevention of myocardial infarction. Warfarin-based anticoagulant therapy must be monitored, and doses individualized, due to increased risk of bleeding in patients, which could cause complications like anemia or hematomas. Warfarin is commonly monitored by checking the clotting status of the patient's plasma, and it is defined by an international normalized ratio (INR). Warfarin has a narrow therapeutic concentration ranging from 2.0 to 5.0 µg/ml and a long half-life between 20 and 60 h [30]. Moreover, warfarin acts indirectly to depress synthesis of vitamin K-dependent clotting factors II, VII, IX, and X. Therefore, there is the need to directly monitor its concentration in plasma during treatment [19].

1. A magnetic iron oxide (Fe_3O_4) nanoparticle-modified carbon paste electrode (CPE) was used to build an electrochemical sensor for warfarin determination [31]. The electrochemical method is claimed to provide a cheap, sensitive, and simple sensor for drug detection. Fe_3O_4 nanoparticles were employed because they offer a large ratio of surface area to volume as seen with other nanomaterials as well. Determination of warfarin was successfully conducted in urine and serum with little interference. The linear range was 0.5–1000 µM, and the limit of detection was 0.21 µM [31].
2. A warfarin nanosensor has been developed in an independent effort based on covalent immobilization of cadmium sulfide (CdS) quantum dots onto carboxylated multiwalled carbon nanotubes and a chitosan composite film modified electrode [32]. Quantum dots (QDs) are semiconductor nanocrystals that possess electronic as well as optical characteristics capable of being modified on their surface using various functional groups [33]. QDs serve as a platform for the attachment of multiwall carbon nanotubes (MWCNTs) to improve electronic transmission as well as electrode sensitivity. The presence of carbon nanotubes conferred good stability, a wider available surface area, and high conductivity [34]. In addition to the low-cost advantage of chitosan, this polycationic polymer substance exhibits high permeability, good adhesive properties, and acceptable film-forming capacity. Warfarin determination was conducted in three potentially interfering biological matrices (viz., serum, breast milk, and urine), where this method still exhibited

acceptable selectivity [32]. The sensor that was developed exhibited a wider linear range compared to previously reported sensors and good sensitivity as well as a low detection limit: The linear range for the detection of warfarin with this method was from 0.05 to 80 μM, and the detection limit was reported to be 8.5 nM [32].

3. A nanosensor made up of $MnFe_2O_4$ and a multiwalled carbon nanotube (MWCNT) paste matrix was built for the determination of warfarin [35]. $MnFe_2O_4$ is a magnetic nanoparticle that possesses favorable properties such as excellent thermal stability, good magnetic property, good catalytic activity, and excellent electronic conductivity [36]. These nanoparticles are combined with MWCNTs to promote high sensitivity, resistance to surface fouling, low limits of detection, and reduction of overpotentials. Previous warfarin electrochemical nanosensor design using Fe_3O_4 magnetic particles [31] was considered by these designers [35] as limited by instability in the external environment [35]. Therefore, the choice of manganese ferrite ($MnFe_2O_4$) nanoparticles in their newly developed electrode was to help resolve stability issues experienced in previous nanosensors [35]. The detection limit for the MWCNT/$MnFe_2O_4$/CPE electrode was found to be 0.08 μM, and the linear dynamic range was 0.10–447 μM [35]. The only substance reported to interfere with this method was free cysteine, which is not abundant in biological matrices under examination [35]. This procedure could therefore be considered for determination of warfarin in serum and urine samples.
4. A molecularly imprinted polymer (MIP) nanosensor has also been described for warfarin determination [37]. The sensitivity and electronic transmission of the nanosensor were improved by incorporating multiwall carbon nanotubes (MWCNTs) containing carboxylic functional groups onto a glassy carbon electrode [37]. The limit of detection for this method was 0.024 ng/mL, and the linear range was 0.031–0.616 ng/mL [37]. Molecularly imprinted polymers possess specific binding sites that recognize the warfarin target. In addition, MIPs are cheap, highly stable, and robust and possess a strong affinity to their template molecule, warfarin. MIPs had previously been used as a thin layer on the modified electrode [38]; the sensitivity of the nanosensor was further enhanced by the addition of MWCNT and Au nanoparticles (NP) on both sides of the MIP layer [37]. This form of nanosensor exhibited good selectivity to warfarin when probing human serum samples.
5. A newer warfarin nanosensor was made by incorporating cobalt oxide nanoparticles onto a multiwalled carbon nanotube-modified glassy carbon electrode [39]. The choice of cobalt oxide nanoparticles enhanced the electrochemical response of warfarin [39]. Carbon nanotubes are commonly employed as a modifier in such setups given their characteristics such as large surface areas, good adsorptive ability, catalysis, improved mass transport, and high porosity [40]. Incorporating cobalt oxide nanoparticles increased warfarin adsorption that occurs as a result of cobalt ions and warfarin forming a complex [41]. The electrochemical signal of warfarin was amplified, and this nanosensor eventually widened the range of measurement as well: the limit of detection for this method was 0.0033 μM, and the linear range was 0.008–800 μM [39]. Good selectivity as well as long-term stability was established in this nanosensor when used in the determination of warfarin without any form of interference [39], facilitating the measurement of warfarin in both human serum and urine samples [39].

14.6.4.2 Nanosensor for Heparin

Heparin functions as an anticoagulant by promoting antithrombin III activity in plasma. Hence, the rate of inhibition by antithrombin III of blood clotting factors such as thrombin and factor Xa is potentiated by heparin in a dose-dependent manner [19]. Heparin is used to treat and prevent thromboembolic disorders such as pulmonary embolism and deep vein thrombosis. It is highly bound to plasma proteins and excreted mainly in

a metabolized form. Heparin therapy serves as a precursor to oral anticoagulation until the full effect of the oral anticoagulant is exerted. At this point, heparin can then be withdrawn [19]. Heparin is administered intravenously, and its effect is monitored daily by measuring activated partial thromboplastin time (APTT) [42, 43]. However, this APPT method of monitoring is indirect and may not provide accurate measurements due to interference from other substances. Moreover, the risks of hemorrhage and heparin-induced thrombocytopenia warrant the need for heparin detection and quantification during treatment [44].

An optical nanosensor based on fluorescence has been developed for the detection of heparin. This nanosensor is made up of rhodamine B-labeled peptides (RBP) that serve as sensitive bioreceptors for heparin given their provision of numerous heparin-binding sites [45]. The binding to RBP of heparin quenches the fluorescence of RBP due to an induced conformational change of RBP from the unfolded form to the folded form [45]. The RBP-based nanosensor was reported to have two linear ranges of operation: 0.01–0.1 nM and 1.0–70.0 nM; the limit of detection turned out to be 7.5 pM [45]. This method was used for the determination of heparin in human serum samples, exhibiting high selectivity and good sensitivity. This sensor performance was speculated to arise from the spatial conformation assumed by the heparin and the electrostatic nature of the heparin/RBP binding interaction [45]. An arginine-rich peptide sequence was specifically designed to serve as the basis for the RBP heparin receptor because previous studies had shown that arginine was critical to nearly all heparin-binding peptides from the G domain of the laminin α1 chain [46–50]. This arginine-rich peptide was then conjugated with the fluorophore rhodamine B, affording a measurable change in fluorescence when the peptide changed conformation upon binding heparin.

14.6.4.3 Nanosensor for Aspirin

Aspirin (also known as acetylsalicylic acid) has historically been used in low doses as an anti-thrombotic measure in the initial management of cardiovascular diseases and prophylaxis of cardiovascular diseases in high-risk patients, as well as for the treatment of mild to moderate pain. After its ingestion, acetylsalicylic acid is hydrolyzed into acetic acid and salicylic acid, and the latter moiety is responsible for aspirin's pharmacological activity. It exhibits its action via inhibition of cyclooxygenase enzyme leading to a decreased production of prostaglandins and thromboxanes [19]. Aspirin causes adverse effects such as NSAID-induced gastric ulceration, increase in bleeding time, and hypersensitivity reactions. Salicylism, metabolic acidosis, and hyperventilation may also occur with high intoxication of aspirin, which can be controlled if dosage is reduced. Salicylate intoxication involves the ingestion of more than 125 mg/kg of aspirin, which warrants monitoring of plasma salicylate concentration to predict the severity of the poisoning.

An electrochemical nanosensor was built on a screen-printed carbon electrode with chitosan capped with gold nanoparticles (Cs + AuNPs) [51]. Its linear range in detection of aspirin was from 1 pg/mL to 1 μg/mL. The limit of the detection was 0.03 pg/mL [51]. An electrochemical method of determination was chosen as it affords various advantages for this kind of drug nanosensor design including cheaper costs, high sensitivity, simple operation, and its capacity to analyze real samples in complex biological matrices [52–60]. Chitosan, a polysaccharide, is a poor electrical conductor and therefore needed to be attached with conductive metallic loads to enhance its conductivity. Gold nanoparticles were therefore selected to augment this function, providing a reduction in response times and improvement in signal-to-noise ratio. These nanoparticles also promoted signal amplification by increasing the adhesion that exists between the analyte and the substrate. Screen-printed electrodes were considered to provide several convenience factors sup-

porting real-time monitoring that included low cost, small volume of sample requirements, as well as simplicity. Quantitative determination of aspirin was carried out in both human urine and saliva [51].

14.6.5 Nanosensor for Tramadol

Tramadol is an opioid drug that produces analgesia mainly via opioid receptors. However, it also exerts its actions via noradrenergic and serotonergic means and therefore also considered a member of the serotonin-norepinephrine reuptake inhibitor (SNRI) class of antidepressant drugs [19]. There is the need for this drug to be monitored in patients who have mild renal impairment, consume large amounts of alcohol, or have hepatic impairment. Tramadol also lowers seizure threshold in patients with epilepsy; hence, there is the need for dosage adjustment for this potential adverse effect as well. Metabolism of tramadol occurs in the liver by O-demethylation and N-demethylation via CYP2D6 and CYP3A4 metabolizing enzymes. Sulfation or glucuronidation of tramadol can also occur. Its metabolism produces O-desmethyltramadol, which is itself pharmacologically active. Tramadol has an elimination half-life of about 6 h, and its metabolites are mainly excreted from the body in urine.

An electrochemical nanosensor has been described which has a carbon paste matrix composed of ionic liquid, decorated graphene nanosheets with silver nanoparticles, and graphite powder [61]. Tramadol-imprinted polymer nanoparticles were combined with the carbon paste matrix to form a "nanosensing layer" of the resultant potentiometric nanosensor [61]. Quantitative determination of tramadol using this method was conducted successfully in human urine samples. The linear range for this electrochemical determination was found to be from 3.5×10^{-9} to 1.00×10^{-2} M with a Nernstian slope of 59.85 ± 0.13 mV per decade [61]. The limit of detection was 2.04×10^{-9} M [61]. Tramadol-imprinted polymer nanoparticles function in this design as the sensing agent, while the ionic liquid is used in this method as a conductive paste binder. The specificity property conferred by carbon paste electrodes, even in complex samples, served as a central justification for its use in this method of determination [62, 63]; selection of the type of modifier used for ultimate fabrication of the carbon paste electrode was considered a central mechanism by which the selectivity and sensitivity of the nanosensor were improved.

14.6.6 Nanosensor for Sulfasalazine

Sulfasalazine is a drug used in the treatment of inflammatory bowel disease. It can be metabolized into mesalazine (5-aminosalicylic acid), which is mainly responsible for its activity, and into sulfapyridine, which has a significant impact on the adverse reactions of sulfasalazine. Sulfasalazine is also classified as a disease-modifying antirheumatic drug (DMARD), which helps to alleviate the joint pain, swelling, and stiffness experienced in rheumatoid arthritis [19]. The small intestine absorbs about 15% of intact sulfasalazine, with some of it later undergoing enterohepatic cycling. Most of the intact drug is cleaved in the colon into mesalazine and sulfapyridine prior to absorption. Both mesalazine and sulfapyridine are extensively metabolized by acetylation. In addition, sulfapyridine is also metabolized by glucuronidation and hydroxylation. Trace amounts of intact sulfasalazine is excreted in urine in an unchanged form with significant amounts excreted as metabolites. Therapeutic drug monitoring is therefore needed here since higher dosage of sulfasalazine is associated with toxicity, especially in slow acetylators with whom it is two to three times more probable to develop overt toxicity symptoms.

An electrochemical nanosensor made up of ionic liquid and nickel oxide (NiO)/CNTs nanocomposite was used to fabricate a modified carbon paste electrode [64]. The linear range for this nanosensor design was from 5.0×107 to 8.0×10^{-4} M, and the limit of detection was 9.0×10^{-8} M [64]. The ionic liquid that was used in this design as a conductive paste binder was reported to have high thermal stability, extensive

electrochemical window, fast electron transfer, and high conductivity [64]. Nickel oxide nanoparticles were chosen as they were considered to possess satisfactory biological compatibility, electron transfer capacity, and high chemical stability [65–67]. This nanosensor was used to detect sulfasalazine in urine samples.

14.6.7 Nanosensors for Theophylline

Theophylline is a xanthine used in the management of respiratory disorders like asthma and chronic obstructive pulmonary disease. It is said to exert its bronchodilatory effect via inhibition of phosphodiesterase, leading to increased levels of cyclic adenosine monophosphate (cAMP) [19]. Other schools of thought believe that the action of theophylline arises from its antagonistic role on adenosine receptors as well as its anti-inflammatory effect [68]. Theophylline is rapidly absorbed and reaches peak serum concentrations 1–2 h after oral administration. The therapeutic window for theophylline is narrow, ranging from 10 to 20 μg/ml [19]. Factors such as smoking, medications, age, and diet may modify the pharmacokinetics of theophylline. Therefore, there is the need for the dose of theophylline to be individualized during treatment based on plasma levels, adverse effects, and therapeutic response. 40–60% of the drug is bound to plasma proteins; moreover, variations in theophylline metabolism in different individuals affect the ultimate plasma concentration of theophylline. Products from theophylline metabolism are mainly excreted in urine, with only 10% excreted in the unchanged form in adults [19]. Monitoring theophylline levels would help prevent toxicity and, eventually, serious adverse effects.

1. An electrochemical nanosensor design for theophylline has been described, using screen-printed electrodes (SPEs) modified with graphene quantum dots (GQDs) [69]. This electrochemical method was tested on urine samples; the linear dynamic range of this electrochemical nanosensor for theophylline was from 1.0 to 700.0 μM, with a limit of detection of 0.2 μM [69]. Merits of using screen-printed carbon electrodes (SPCE) in these electrochemical measurements have been described as including affordability, quick response, small size, and simple fabrication [70, 71]. Graphene quantum dots increase the surface area that has contact with the analyte. Therefore, the electroactive analyte interacts with an expanded electrochemical active surface area [69].
2. A biosensor for detection of theophylline, based on an RNA aptamer for theophylline coupled with a graphene oxide (GO) and cryonase-mediated amplified fluorescence system, has been independently developed [72]. Rather than establishing theophylline detection on the basis of an RNA aptamer-attuned nuclease, a GO/cryonase-mediated amplified fluorescence system was employed, to achieve signal amplification regardless of which nucleic acid molecular probe (NAMP) was ultimately used in the design. GO has affinity for both single-stranded and double-stranded NAMPs. Moreover, the ability of cryonase to digest both DNA and RNA probes enzymatically facilitates its universal application for all forms of NAMP sequences. This method can be used to design new aptasensors for various analytes. The fluorescence produced by dye-labeled aptamers is quenched through fluorescence resonance energy transfer (FRET) when these aptamers are adsorbed onto the GO surface. GO was selected in fabricating this nanosensor because it is recognized as a strong fluorescence quencher. However, this quenching is disrupted by the binding of theophylline to the aptamer, resulting in the reoccurring of fluorescence produced by the aptamers. Release of the aptamer/theophylline complex from the GO surface becomes available for cryonase digestion. Afterwards, the free theophylline binds to another aptamer to facilitate another cycle of cleavage. This cycle continues repeatedly until all aptamer probes are consumed. This cycling enables the activation of all fluorophores and consequently amplifies the fluorescent signals. The limit of detection of

theophylline was 47 nM [72]. This biosensor was used for determination of theophylline in serum samples.

14.6.8 Nanosensor for Levothyroxine

Levothyroxine (T4) is a drug used to treat diffuse goiter and Hashimoto's thyroiditis due to its ability to suppress the production of thyroid-stimulating hormone (TSH) [19]. Given its ability to suppress TSH secretion, T4 is also used in differential diagnosis of hyperthyroidism and beneficial in thyroid carcinoma treatment. This drug is essentially a thyroid hormone that is commonly given orally, intravenously, or intramuscularly and does not immediately change therapeutic response when its dose is adjusted. It is often used as a replacement therapy in hypothyroidism [73]. Moreover, its absorption is greatly impaired with food. During treatment, TSH levels are monitored, and the dosage of levothyroxine is adjusted accordingly to attain normal TSH levels following total thyroidectomy. The physiological significance of T4 speaks to the necessity in monitoring its levels in serum to bring insight to the diagnosis of hypothyroidism and hyperthyroidism. Adverse effects are commonly experienced in cases of T4 overdosage, which are similar to symptoms of hyperthyroidism like insomnia, cardiac arrhythmias, muscle weakness, and tachycardia. Chronic intoxication results in events such as thyroid storm, cardiac arrhythmias, heart failure, and death. Due to its narrow therapeutic index, extra precautions should be taken when administering T4 to patients with preexisting cardiovascular disorders, long-standing hypothyroidism, adrenal insufficiency, diabetes, and epilepsy to prevent worsening their prognosis.

A nanocomposite made up of melamine attached to gold nanoparticles by means of mercaptoethanol-modified multiwalled carbon nanotubes (MWCNTs/CC-SH/AuNPs) was developed as an electrochemical nanosensor for the detection of levothyroxine [74]. The linear dynamic range for the proposed nanosensor was from 10 to 120 nM, and the nanosensor's limit of detection was 2.84 nM [74]. Carbon nanotubes were chosen as modifiers due to the fact that they are relatively chemically unreactive and possess good chemical stability, ordered structure, and electrical conductivity [75, 76]. The incorporation of gold nanoparticles helped to enhance the sensitivity as well as the selectivity of the electrochemical nanosensor. The nanocomposite was immobilized onto the surface of a glassy carbon electrode (GCE), and this electrochemical method enabled by this nanosensor design was subsequently applied in human blood serum.

14.6.9 Nanosensor for Lithium

Lithium is a drug used to manage conditions such as bipolar disorder, recurrent unipolar depression, and mania. It has a narrow therapeutic index that warrants the need for this drug to be monitored in patients taking it. While its mechanism is unknown, it is said to be involved in a competition with sodium ions in various locations of the body [19]. Lithium is entirely absorbed in the gastrointestinal tract and excreted mainly by the kidneys into urine.

An optical nanosensor based on fluorescence spectroscopy was developed for the detection of lithium ions. A 12-metallacrown-3 complex in the lithium nanosensor played the role of recognition component due to its high selectivity and affinity for lithium ions [77]. This nanosensor exhibited very good selectivity for lithium over Na^{+} and Mg^{2+} even while these latter ions are more concentrated in biological matrices compared to lithium [77]. Prior to determination of lithium in human serum using this optical nanosensor, a simple precipitation procedure was employed to remove the large proteins which could have caused interference via autofluorescence and competitive coordination to (arene) Ru complexes that constituted the 12-metallacrown-3-based recognition component.

14.6.10 Nanosensors for Anticonvulsants

14.6.10.1 Nanosensor for Carbamazepine

Carbamazepine is an antiepileptic and psychotropic drug that is commonly used to manage generalized tonic-clonic seizures as well as partial seizures [19, 78]. Carbamazepine requires dose adjustment from one patient to the other to achieve plasma concentrations usually between 4 and 12μg/mL [79]. The dose must be adequate to control the seizure and yet minimize adverse effects like neurotoxicity. Carbamazepine undergoes hepatic metabolism, with its metabolites almost entirely excreted in urine with trace amounts excreted in feces. Reports show that 70%–80% of the drug is bound to plasma proteins and elimination of the drug is slower in adults, resulting in longer retention of more active metabolites; the half-life of carbamazepine also shortens with repeated drug administration because it can induce its own metabolism [19]. Based on the characteristics of this drug including complex pharmacokinetics and its serious adverse effects, therapeutic drug monitoring would help to optimize therapy in patients who depend on it to live seizure-free.

A modified glassy carbon electrode which comprises of a nanocomposite of cerium-doped zinc oxide (ZnO) and reduced graphene oxide was developed for the electrochemical sensing of carbamazepine [80]. The linear range for carbamazepine determination was from 0.05 to 100 μM, and the limit of detection was 1.2 nM [80]. This nanosensor was reported to exhibit high selectivity, long-term stability, high sensitivity, and good reproducibility. This method of detection was successfully executed in urine samples. ZnO has become an important metal oxide that is used in electrochemical nanosensors as a semiconductor for the determination of drugs [81, 82]. To increase the selectivity and sensitivity of the nanosensor, ZnO was doped with a different rare earth metal (Cerium) [80]. The sensing performance of the electrode was further improved by forming a nanocomposite of cerium-doped zinc oxide (ZnO) with reduced graphene oxide (rGO) [83]. rGO was chosen to reduce the aggregation of active materials and improve the electrocatalytic properties of the nanosensor design [80].

14.6.10.2 Nanosensors for Phenobarbital

Phenobarbital is an antiepileptic medication that is prescribed to control seizures such as status epilepticus and partial seizures, as well as generalized tonic-clonic seizures [19, 84, 85]. It has a half-life of about 75–120 h in adults with 45%–60% bound to plasma proteins. The dosage required to bring the seizure under control varies from individual to individual with its usual therapeutic plasma concentration ranging from 15 to 40 μg/mL [19]. This therapeutic window may need to be increased with time due to the development of tolerance with this drug [86]. Hence, there is the need for dose adjustment to be made based on the patient. Therapeutic drug monitoring of phenobarbital helps to avoid adverse effects like cardiovascular and respiratory depression, ataxia, and coma that result from overdosage [87].

1. A fluorescence nanosensor based on green source carbon dots (GSCDs) coated with molecularly imprinted polymers (MIPs) was developed for the determination of phenobarbital [88]. The limit of detection for this nanosensor was 0.1 mM, and the linear range was between 0.4 and 34.5 mM [88]. This optical method of determination was conducted in human blood plasma samples. The molecular imprinting technique was used in building this nanosensor, creating a recognition site with polymeric materials that is selective to the target, phenobarbital [89]. Moreover, as previously reported, MIPs are cheap, are easy to prepare, are thermally stable in basic and acidic medium, and confer a high degree of selectivity to the proposed nanosensor [90]. The fluorescent characteristic of carbon dots, together with their simplicity in terms of synthesis, surface functionalization, and good stability, made these nanoparticles stand out as suitable for the development of this nanosensor [91, 92].

2. Independent of the optical design mentioned above, a modified multiwalled carbon nanotube (MWCNT) paste electrode was set up with the incorporation of platinum (Pt) nanoparticles for the detection of phenobarbital [93]. The linear range for phenobarbital for this electrochemical nanosensor was from 0.4 to 60 μM, and its limit of detection was 0.1 μM [93]. This electrochemical method was investigated in human urine samples. Carbon nanotubes are commonly employed as a modifier in such setups due to their favorable characteristics such as large surface areas, good adsorptive ability, catalysis, improved mass transport, and high porosity [40]. Electrocatalytic processes and surface areas of the composite matrix were reported to improve significantly when Pt nanoparticles are combined with the carbon nanotubes, as compared to the individual component properties [94].

14.6.10.3 Nanosensor for Valproic Acid

Valproic acid is a drug used to treat primary generalized seizures and partial seizures. It has been shown to be beneficial in the treatment of absence and myoclonic seizures. Despite the fact that its mechanism is not fully understood, valproic acid is said to facilitate GABAergic transmission in the central nervous system as well as inhibit sodium channels [19]. There is the need for dose adjustment in patients taking valproic acid to gain adequate seizure control. The use of valproic acid can result in adverse effects like liver dysfunction which requires the discontinuation of treatment if left unchecked. The dose needs to be adjusted in patients that experience elevated liver enzymes within the first few months of treatment because this adverse effect is dose related. The metabolites of valproic acid are excreted entirely in urine.

An optical method was developed for the determination of valproic acid. The nanosensor was developed with thioglycolic acid (TGA)-capped CdTe quantum dots (QDs) [95]. These quantum dots are said to possess properties such as high photoluminescence quantum efficiency, photostability, and tunable emission spectrum. The extent of fluorescence of QDs is dependent on the change in pH of the environment [95]. Hence, the weakly acidic nature of valproic acid causes a pH change that results in fluorescence quenching of CdTe QDs. The linear range of the engineered nanosensor was from 0.3 to 7.5 mg/L, and the detection limit was 0.24 μg/mL [95]. This nanosensor was used for the detection of valproic acid in urine samples and human serum.

14.6.10.4 Nanosensor for Phenytoin

Phenytoin is an antiepileptic drug that is used to manage generalized tonic-clonic and partial seizures [19]. Treatment of patients with phenytoin requires the dose to be individualized to control seizures due to the narrow therapeutic index of this drug. The concentration range within which therapeutic outcomes can be effectively achieved is between 10 μg/mL and 20 μg/mL. The half-life of phenytoin varies based on the dose administered but averages about 22 h. Moreover, the potential for phenytoin to inhibit its own metabolism prolongs the time it takes to achieve steady-state concentration.

Monitoring of phenytoin was reported to be possible in plasma samples with a nanosensor developed using branched gold nanoparticles (B-AuNPs). The B-AuNPs possess a localized surface plasmon resonance (SPR) property which helps in spectrophotometric determination of phenytoin in situ. Phenytoin forms a complex with Au (III) leading to the aggregation of B-AuNP. Therefore, at 540 nm, the intensity of the characteristic SPR band of B-AuNP is reduced, followed by a change in color of the solution from red to blue [96]. The method is sensitive, rapid, and simple based on the detection limit of 21 ng/mL, the capacity to obtain results in less than 15 min, and being able to visually observe the color changes [96]. The low detection limit allows the plasma samples to be well diluted prior to determination to prevent interactions from species present in the sample. However, interference studies revealed that drugs like lamotrigine and paracetamol can interfere with the detection of the analyte. The linear range was determined to be from 67 to 670 ng/mL [96].

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15 Organ Toxicity by Immunosuppressive Drugs in Solid Organ Transplantation

George J. Dugbartey and Alp Sener

Abstract

Solid organ transplantation is the preferred therapeutic option that confers significant survival advantage on patients suffering from end-organ dysfunction and provides improved quality of life together with its cost-effectiveness. During perioperative and post-transplant periods, organ transplant recipients receive immunosuppressive therapy, which reduces the high risk of allograft rejection, minimizes infections, improves allograft quality, and prolongs recipient survival. While there is no optimal immunosuppressive protocol in solid organ transplantation across transplant centers, some centers have adopted a triple combination therapy consisting of a calcineurin inhibitor, a purine synthesis inhibitor, and glucocorticoid. Other centers have also used dual therapy comprising inhibitors of mammalian target of rapamycin and belatacept to achieve the goal of immunosuppression. However, chronic immunosuppression is associated with post-transplant complications including organ toxicity, resulting in significant mortality of transplant recipients and hindering long-term success of organ transplantation. This chapter discusses organ toxicity induced by commonly used immunosuppressive drugs in solid organ transplantation and strategies to reduce the burden of immunosuppression after solid organ transplantation.

Keywords

Allograft rejection · Immunosuppression · Immunosuppressive drugs · Organ toxicity · Solid organ transplantation

15.1 Introduction

15.1.1 Historical Account of Immunosuppressive Drugs in Clinical Organ Transplantation

Solid organ transplantation is a revolutionary field in modern medicine that has become the treatment of choice for patients with end-stage

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organ failure. It improves quality of life of transplant recipients and confers significant survival advantage together with its cost-effectiveness and can rightly be considered as one of the medical miracles of the twentieth century. This challenging life-saving intervention has shown remarkable growth and has also made great strides over the past few decades to the present era, illustrating the evolution of a complex and technical procedure from its preclinical origin in the mid-twentieth century to becoming a routine clinical practice today, with continuous refinement including the introduction of immunosuppressive drugs to prevent allograft rejection. Against the background of failure, the first successful kidney transplantation was performed in 1954 in the United States, in which the donor and recipient were identical twins [1]; hence, the transplant recipient did not require immunosuppression therapy. This outstanding clinical outcome paved way for research into immunosuppression, which suggests that organ transplantation could be extended beyond the boundaries of genetic pairs. Unlike isotransplantation in which the organ donor and recipient are genetically identical, thus requiring no immunosuppression as seen in identical twins, allotransplantation (the most common form of transplantation) requires immunosuppression since the donor and recipient are genetically unidentical. It is important to note that immunosuppression is greatest during peri-operative and early post-transplant periods to reduce the high risk of allograft rejection, minimize infections, improve graft quality, and prolong recipient survival due to cold preservation injury of the allograft and the sudden exposure of recipient's immune system to abundance of foreign antigens. Later in the post-transplant period, immunosuppression is then reduced (maintenance immunosuppression) to an optimal level depending on allograft function and tolerability. Thus, while maintenance immunosuppression prevents allograft rejection, it is also a pharmacological strategy to avoid organ toxicity. Total body irradiation (TBI), a radiotherapy to suppress the patient's immune system and prevent allograft rejection, was identified (after the first successful renal isotransplantation) for allotransplantation [2]. Although TBI achieved immunosuppression, it also resulted in high patient mortality due to overwhelming infections arising from profound bone marrow aplasia [2]. By the end of the mid-twentieth century, it became obvious that TBI could not be used as an immunosuppressive therapy in organ transplant recipients.

Following the withdrawal of TBI, 6-mecaptopurine (6-MP), an anticancer drug which successfully suppressed the immune system and prolonged the survival of organ grafts in previous experimental transplantation in rabbits and dogs [3, 4], entered into human clinical trials with a 40–50% 1-year success rate [5]. Later, 6-MP was replaced by its less toxic derivative azathioprine, which is equally as effective as 6-MP. Corticosteroids were also introduced to treat the unavoidable allograft rejection and also served as an adjunct to azathioprine therapy for maintenance immunosuppression. Development of corticosteroid-resistant allograft rejection soon after transplantation led to the introduction of anti-thymocyte globulin, the first immunosuppressive agent to treat corticosteroid-resistant acute allograft rejection, with about 70% 1-year allograft survival rate in several clinical trials [6–8]. The "azathioprine era" ended in the early 1980s due to high mortality of transplant recipients (as high as 40% at 1-year post-transplant). This led to the arrival of a new era in clinical transplantation, with the introduction of another immunosuppressive drug, cyclosporine – a calcineurin inhibitor, producing over 80% 1-year allograft survival rate characterized by significantly reduced renal graft loss from irreversible rejection and markedly improved quality of other highly immunogenic solid organ allografts such as heart, lungs, liver, and intestine [2]. Subsequent decades of the twentieth century after the "cyclosporine era" produced a significant armamentarium of immunosuppressive agents with different efficacy and safety. These new drugs include tacrolimus, everolimus, sirolimus, and mycophenolate mofetil. In addition, monoclonal antibodies such as basiliximab (anti-CD25/IL-2R), rituximab (anti-CD20), alemtuzumab (anti-CD52), and belatacept

(anti-CD80/86) directed against T and B lymphocytes – the primary mediators of alloimmune response and effectors of the rejection process – were also developed and introduced into clinical practice to induce immunosuppression or treat allograft rejection episodes. All these new immunosuppressive drugs that followed the "cyclosporine era" produced over 90% 1-year allograft survival rate, thus facilitating major improvement compared to the early era of clinical organ transplantation.

In the face of these great strides in clinical organ transplantation is the growing concern of long-term post-transplant complications, which hinders the long-term success of organ transplantation. Post-transplant complications including organ toxicity due to prolonged exposure and chronic immunosuppression are associated with significant mortality of transplant recipients even though the combination of immunosuppressive drugs increases the efficacy of immunosuppression and allows maintenance immunosuppression at reduced doses [9–12]. This suggests the need for further studies to expand our understanding of the human immune system to develop newer and more efficient drugs that target newly discovered mechanisms and pathways of immune response to provide immunotolerance without the burden of organ toxicity and other side effects associated with current regimens. This chapter discusses organ toxicity induced by commonly used immunosuppressive drugs in solid organ transplantation and strategies to reduce the burden of immunosuppression after solid organ transplantation.

15.2 Immunosuppressant-Induced Organ Toxicity After Solid Organ Transplantation

Optimal immunosuppressive protocol in solid organ transplantation varies widely across transplant centers. As summarized in Table 15.1, the most frequently adopted regimen is a triple combination therapy consisting of a calcineurin inhibitor such as cyclosporine or tacrolimus, a purine synthesis inhibitor (antimetabolite) such as azathioprine or mycophenolate mofetil, and glucocorticoid such as prednisone, prednisolone, or methylprednisolone, all of which interfere with different steps in the immune response to the allograft. Some transplant centers have also adopted a double combination therapy comprising a calcineurin inhibitor and other immunosuppressive agents, while other centers have also used inhibitors of mammalian target of rapamycin (mTOR; proliferation inhibitors) such as sirolimus and everolimus, with belatacept. The classification of these drugs is based on their mechanisms of action. Although induction and maintenance immunosuppression therapy reduces the rate of acute allograft rejection and improves allograft half-life and patient survival, it also has long-term adverse effect on virtually all organ systems. As illustrated in Fig. 15.1, chronic immunosuppression following organ transplantation has resulted in increasing evidence of a number of post-transplant long-term complications such as toxicity of organ systems, hypertension, dyslipidemia, opportunistic infections, malignancies, and diabetes, all of which contribute to high post-transplant mortality [9–15].

15.2.1 Immunosuppressant-Induced Nephrotoxicity

Among the effects of immunosuppressive drugs on organ systems after transplantation is nephrotoxicity, one of the most common and significant complications which eventually leads to renal graft loss in transplant recipients [16]. While calcineurin inhibitors such as cyclosporine and tacrolimus are the main class of immunosuppressive agents used across the globe as part of immunosuppressive regimens during peri- and post-transplant periods, they are major contributors to the development of chronic kidney disease and renal failure after transplantation, with an increased risk of mortality of transplant recipients [17]. Long-term use of calcineurin inhibitors, for example, has been reported to cause

Table 15.1 Commonly used immunosuppressive agents in solid organ transplantation

Classification of immunosuppressant	Example	Indication
Calcineurin inhibitors	Cyclosporine, Voclosporine, Tacrolimus	Maintenance of immunosuppression
Purine synthesis inhibitors (antimetabolite)	Azathioprine, Mycophenolate mofetil Mycophenolate sodium	Maintenance of immunosuppression
mTOR inhibitors (proliferation inhibitors)	Sirolimus, Everolimus	Maintenance of immunosuppression
Corticosteroids	Prednisone, Prednisolone, Methylprednisolone	Induction of immunosuppression
Antibodies	Basiliximab, Daclizumab, Muromonab, Alemtuzumab, Thymoglobulin	Induction of immunosuppression

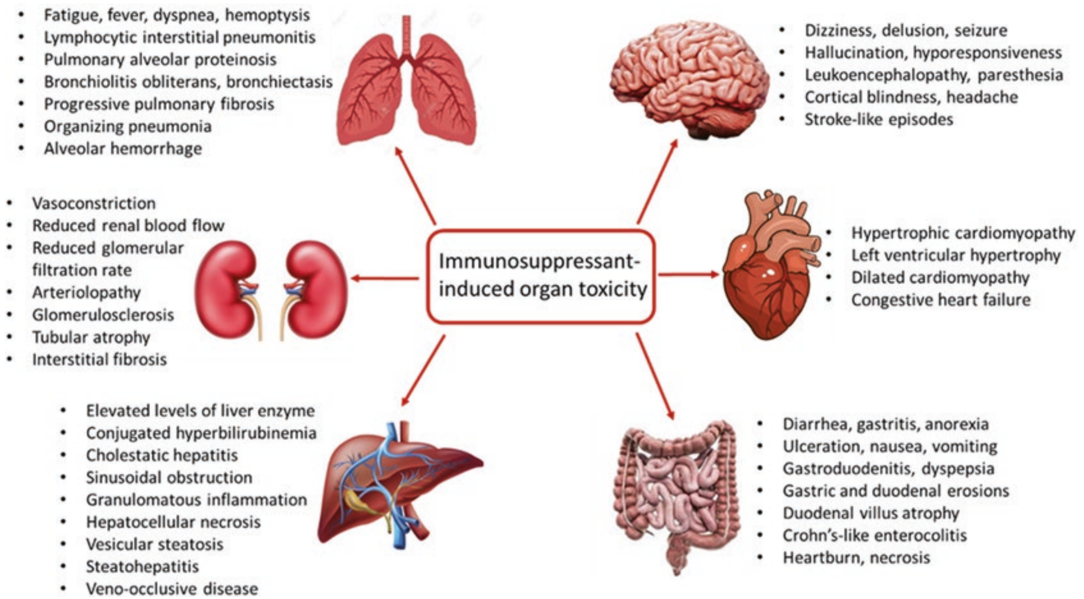

Fig. 15.1 Summary of clinical presentation of organ toxicity induced by immunosuppression therapy after solid organ transplantation

acute allograft nephropathy. This includes impaired production of vasodilating mediators such as nitric oxide and prostanoid, increased sympathetic activation, and enhanced production of vasoconstrictive mediators such as endothelin, thromboxane, angiotensin II, and platelet-activating factor [9, 18–25]. Such hemodynamic changes ultimately increase vascular resistance at the level of afferent arterioles to a greater extent than efferent arterioles and altogether negatively impact renal hemodynamics, resulting in vasoconstriction and consequently reduced renal blood flow and glomerular filtration rate in preclinical and clinical studies [9, 18–25] (Fig. 15.1).

There is also evidence of chronic allograft nephropathy induced by calcineurin inhibitors, which include irreversible renal pathological changes such as arteriolopathy, glomerulosclerosis, damaged endothelium and smooth muscle of afferent arterioles, tubular atrophy, and tubulointerstitial fibrosis characterized by increased expression of transforming growth factor-beta (TGF-β; a well-defined central mediator of renal fibrosis), which appear to be dependent on angiotensin II but independent of the acute renal hemodynamic changes [26, 27] (Fig. 15.1). At the molecular level, treatment with calcineurin inhibitors has been reported to

increase renal production of reactive oxygen species (ROS; natural by-product of cellular oxidation metabolism and destructive tissue mediator) with reduced levels of antioxidants (e.g., glutathione). This observation correlated with increased expression of pro-apoptotic genes (e.g., p53, Bax and Fas-L) and decreased expression of anti-apoptotic genes (e.g., Bcl-2), resulting in apoptosis of renal tubular epithelial cells [28, 29]. Hauser et al. [30] also provided additional evidence of the nephrotoxic effect of calcineurin inhibitors in which they showed in a clinical study that renal allografts with increased expression of P-glycoprotein (a transmembrane drug transporter whose function determines drug concentration in plasma and tissues) are less vulnerable to immunosuppressant-induced nephrotoxicity, suggesting that P-glycoprotein is a major determinant of calcineurin inhibitor-induced nephrotoxicity.

Considering the nephrotoxic effect of calcineurin inhibitors discussed above, there are a number of suggestions including a dose reduction or withdrawal of calcineurin inhibitors or their combination with significantly less toxic immunosuppressive agents such as sirolimus and everolimus or with non-nephrotoxic immunosuppressants such as mycophenolate mofetil as part of the immunosuppression regimen to reduce the risk of post-transplant nephrotoxicity. While there is currently no general consensus on the choice of combined immunosuppression therapy to avoid allograft rejection and minimize nephrotoxicity, such immunosuppressant combinations resulted in enhanced calcineurin inhibitor-induced nephrotoxicity in phase III clinical studies [31, 32]. This observation was attributed to changes in renal tissue distribution of calcineurin inhibitors and inhibition of glycolytic pathway by the anti-proliferation immunosuppressive drugs [33]. Also, withdrawal of calcineurin inhibitors during early post-transplant period in an attempt to start de novo transplant recipients on so-called “calcineurin inhibitor-free” immunosuppressive protocol resulted in significant increase in acute and chronic allograft rejection [34–38]. In fact, a 9-year clinical study from 1996 to 2005 involving 25,045 kidney transplant recipients with well-functioning renal allograft showed that withdrawal of calcineurin inhibitors or mycophenolate mofetil from the immunosuppression regimens or reduction of their doses below certain thresholds during early post-transplant period was associated with significant risk of allograft loss with a hazard ratio of 1.52–1.73 relative to continuing treatment [39]. These clinical observations suggest that administration of calcineurin inhibitors is crucial especially during early post-transplant period and that their withdrawal may be considered a double-edge sword. Thus, acute allograft rejection and allograft loss will remain a major concern when considering withdrawal or discontinuation of calcineurin inhibitors. It further suggests the need to develop pharmacodynamic monitoring tools and pharmacogenetic screening strategies to optimize individualization of immunosuppression therapy preferably during early post-transplant period based on organ-specific and patient-specific factors such as age, race, comorbidities, genetic polymorphism, immunologic risk, concomitant and previous pharmacotherapy, and overall immunosuppression burden. In summary, immunosuppressant-induced nephrotoxicity is a major post-transplant complication, which places a considerable metabolic burden on the renal allograft and contributes in part to post-transplant renal dysfunction. Therefore, new agents will be needed as an adjuvant to or replacement of current calcineurin inhibitor to provide alternative mechanisms of action and reduce nephrotoxicity.

15.2.2 Immunosuppressant-Induced Neurotoxicity

Immunosuppressant-induced neurotoxicity is one of the most significant post-transplant complications with over 59% prevalence in organ transplant recipients. As shown in Fig. 15.1, chronic immunosuppression with calcineurin inhibitors, for example, is associated with hallucinations, seizures, paresthesias, delusions, dizziness, hypore-

sponsiveness, headaches, depression, stroke-like episodes, progressive multifocal leukoencephalopathy, and cortical blindness in organ transplant recipients [40, 41]. Several mechanisms have been implicated in calcineurin inhibitor-induced neurotoxicity. While calcineurin inhibitors do not readily cross the blood-brain barrier (BBB), they alter its permeability by inducing apoptosis of brain capillary endothelial cells and inhibiting the function of P-glycoprotein and thereby enhancing their diffusion across the BBB into the central nervous system (CNS) [42, 43]. In the CNS, calcineurin inhibitors selectively alter mitochondrial function in glial cells and oligodendrocytes, resulting in increased mitochondrial ROS production and thereby inducing oxidative stress and ultimately damaging these cells that provide structural, trophic, and metabolic support to neurons in the CNS [44–46]. Indeed, mitochondrial dysfunction was reported in the brains of rats following administration of calcineurin inhibitors such as cyclosporine, resulting in reduced ATP production and increased ROS formation [41, 47]. Arnold et al. [48] also reported additional mechanism of calcineurin inhibitor-induced neurotoxicity by altering excitability properties, causing neuronal membrane depolarization via modulation of receptors of excitatory and inhibitory neurotransmitters [49, 50]. As with nephrotoxicity, calcineurin inhibitors also activate vasoconstriction system and increase sympathetic activity, leading to inhibition of nitric oxide-mediated and other vasodilation pathways. The consequent disruption of the endothelial function causes systemic hypertension, local ischemia, and cerebral edema [51].

Besides calcineurin inhibitors, antimetabolites (purine synthesis inhibitors) such as azathioprine and mycophenolate mofetil also deplete purine stores in neuronal cells and inhibit DNA and RNA synthesis and lymphocyte proliferation. In addition, administration of high doses of glucocorticoids or prolonged administration at low doses has been reported to negatively affect the hypothalamic-pituitary-adrenal axis (the main physiological system that mediates the body's stress response) and induced neuronal structural remodeling with synaptic loss, glial malfunction and increased the risk of developing metabolic, neurodegenerative, and neuropsychiatric disorders [52, 53]. Furthermore, anti-proliferation agents (mTOR inhibitors) such as sirolimus have been reported to increase ROS production via interaction with calcineurin inhibitors, leading to oxidative stress and its negative metabolic effects in the cells of the CNS [54] as is also observed in nephrotoxicity. For this reason, further clinical and preclinical studies are needed to better understand the pathogenesis and mechanisms as well as relationship between immunosuppressant-induced neurotoxicity and nephrotoxicity to allow proper clinical decisions to be taken on the choice of immunosuppression therapy during peri- and post-transplant periods to manage the delicate balance between immunosuppression, neurotoxicity, and nephrotoxicity. This will require a close collaboration between neurologists and nephrologists.

15.2.3 Immunosuppressant-Induced Cardiotoxicity

Cardiotoxic effects of immunosuppressive drugs are less common, but serious adverse effects have been observed in both adult and pediatric transplant recipients with no previous risk factors for cardiac disease prior to organ transplantation. These cardiotoxic effects have manifested as hypertrophic obstructive cardiomyopathy, dilated cardiomyopathy, and severe concentric left ventricular hypertrophy with congestive heart failure [55–59] (Fig. 15.1). Interestingly, most of the known cases of immunosuppressant-induced cardiotoxicity are associated with tacrolimus therapy, while others are isolated with other immunosuppressants. In five pediatric patients without any history of cardiac diseases prior to bowel and liver transplantation, Atkinson et al. [55] observed various degrees of left ventricular hypertrophic obstructive cardiomyopathy and heart failure within 2 months of starting tacrolimus therapy after transplantation. Conversion to cyclosporine resulted in improvement in their heart conditions. Several subsequent studies also reported tacrolimus-related ventricular hypertrophy

characterized by thickening of left ventricular wall and interventricular septum in adult and pediatric transplant recipients, which resolved after tacrolimus was discontinued [56–62]. However, a unique case of tacrolimus-associated hypertrophy was reported by McLeod et al. [63] in which they observed rapid progression from cardiac hypertrophy to dilated cardiomyopathy with severely reduced systolic function in adult liver transplant recipients shortly after post-transplant initiation of tacrolimus therapy. Interestingly, the change in cardiac function remained the same even after tacrolimus withdrawal. The authors surmised that the progression to dilated cardiomyopathy could be due to tacrolimus, considering the short time period between initiation of tacrolimus-based immunosuppression and the sudden changes in cardiac function. This conclusion, however, warrants investigation. The mechanism underlying tacrolimus-induced hypertrophic cardiomyopathy is unclear. Proposed mechanisms may be related to inappropriate intracellular calcium handling. There are studies showing that FK506 binding protein (FKBP) in cardiac (and skeletal) muscle closes cardiac ryanodine receptor, blocking calcium release from its storage in the sarcoplasmic reticulum [64, 65]. Tacrolimus inhibits the effect of FKBP by enhancing the opening of the ryanodine channels and subsequent calcium release into the sarcoplasm of cardiomyocyte, which induces cardiac hypertrophy [66]. Another study also proposed coronary artery arteritis and tacrolimus-induced hypertension from sympathetic activation [67].

Apart from tacrolimus, sirolimus and MMF were recently reported to cause recurrent pericardial effusion in a cohort of heart transplant patients and required their discontinuation [68]. However, another study reported that conversion from calcineurin inhibitors to sirolimus improved diastolic function and filling pressure, attenuated myocardial fibrosis progression, and reduced left ventricular mass of cardiac allografts [69–71]. Considering these contradictory outcomes involving sirolimus, further studies are indicated to clarify its mechanism of interaction with MMF. The attenuation of myocardial fibrosis progression by sirolimus was shown in animal models to be due to inhibition of transforming growth factor-β pathway and fibrogenic activation of myofibroblasts [72]. Roberts et al. [73] also observed cardiomegaly with preferential septal hypertrophy at autopsy in pediatric and adult patients who received tacrolimus and cyclosporine after liver transplantation and had no risk factors to cardiac diseases prior to transplantation. In addition, chronic immunosuppression with prednisone and cyclosporine caused mid-apical hypertrophy, a phenotype of hypertrophic cardiomyopathy in an adult heart transplant recipient [74]. Overall, the incidence of immunosuppressant-induced cardiotoxicity, though less common, draws attention to the need for careful evaluation of transplant patients on these drugs, particularly tacrolimus. As only a few cases of cardiotoxicity by other immunosuppressive agents have been reported at single centers, a larger dataset based on consistent multicenter investigations is needed.

15.2.4 Immunosuppressant-Induced Pulmonary Toxicity

Immunosuppressant-induced pulmonary toxicity is one of the major causes of post-transplant complications, accounting for 20% incidence of allograft failure and mortality of transplant recipients [75–77]. It usually presents in the form of fatigue, fever, lymphocytic interstitial pneumonitis, bronchiolitis obliterans, pulmonary alveolar proteinosis, organizing pneumonia, bronchiectasis, dyspnea, nonproductive cough, hemoptysis, alveolar hemorrhage, and progressive pulmonary fibrosis with extensive bilateral patchy or diffuse alveolo-interstitial infiltrates as revealed by imaging tests such as chest radiograph and high-resolution computerized tomography [78–82] (Fig. 15.1). There are published reports of isolated cases showing mTOR inhibitors such as sirolimus and everolimus inducing pulmonary toxicity in the absence of infectious causes (after bronchoscopy with bronchoalveolar lavage) or other pulmonary diseases in recipients of deceased donor kidneys after conversion from

calcineurin inhibitors, whereby dose reduction or drug withdrawal and replacement with corticosteroid therapy resulted in clinical and radiologic improvement in both adult and pediatric transplant recipients [83–88]. While the exact mechanism of pulmonary toxicity by mTOR pathway inhibition is unknown, some studies suggest a possible pathogenic mechanism including an idiosyncratic cell-mediated autoimmune response after exposure of cryptic antigens or a T cell-mediated delayed type of hypersensitivity reaction [83, 89].

Besides mTOR inhibitors, tacrolimus and MMF have also been reported to cause dyspnea, hemoptysis, and extensive pulmonary fibrosis at 1-month post-deceased donor kidney transplantation, which was temporarily improved after discontinuation of the drugs. However, resumption of MMF resulted in deterioration of respiratory function and subsequent death from hypoxic respiratory failure [90], suggesting that MMF, if necessary, must be used with utmost care in transplant recipients with underlying pulmonary conditions with poor baseline pulmonary function. Other studies in adult and pediatric transplant recipients also showed improvement in pulmonary conditions when MMF therapy was stopped and replaced with azathioprine and corticosteroid therapy [91–98]. In summary, dose reduction and conversion from one immunosuppressive agent to another should be considered when unexplained pulmonary complaints develop in patients post-transplant. Given that the aforementioned examples represent single-center experiences, a larger dataset based on consistent multicenter trials would provide a more comprehensive understanding and useful information regarding the incidence of immunosuppressant-induced pulmonary toxicity and its management.

15.2.5 Immunosuppressant-Induced Gastrointestinal Toxicity

Gastrointestinal (GI) toxicity commonly occurs after solid organ transplantation and is one of the major causes of allograft loss, morbidity, and mortality of immunosuppressed organ transplant recipients, often presenting as diarrhea, gastritis, anorexia, ulcerations, gastric and duodenal erosions, nausea, vomiting, gastroduodenitis, dyspepsia, heartburn, necrosis, duodenal villus atrophy, and Crohn's-like enterocolitis [99, 100] (Fig. 15.1). Non-immunosuppressant co-medications (e.g., antibiotics, diuretics) and concurrent diseases (e.g., diabetes) also play a contributing role in the pathogenesis and progression of GI toxicity [101, 102]. The GI tract is involved in the metabolism of almost all immunosuppressive agents; hence, it is exposed to immunosuppressants and their metabolites. This makes the GI tract highly vulnerable to all forms of GI disorders and complications, thereby affecting the quality of life of organ transplant recipients, which could lead to low patient compliance and in turn increase the risk of allograft loss. In a meta-analysis of randomized controlled trials and a cross-sectional study with 543 kidney transplant recipients who underwent upper endoscopy due to GI complications, the incidence of erosive lesions in the upper GI tract increased when tacrolimus was added to the immunosuppressive regimens [103, 104]. Tacrolimus and cyclosporine were also associated with acute pancreatitis and fatality of kidney and heart transplant recipients 2 years after transplantation [105–107]. In addition, administration of these calcineurin inhibitors and sirolimus (an mTOR inhibitor) resulted in chronic severe diarrhea and constipation in kidney transplant recipients [103, 108, 109], which is attributed to altered expression of Na^+/H^+ exchanger 3 in apical membranes of the intestines and lipid malabsorption [110, 111] with gastroduodenal ulcer bleeding in kidney and liver transplant recipients who were on sirolimus therapy [112, 113]. Interestingly, these symptoms were reversed following sirolimus withdrawal [113].

Antimetabolites (purine synthesis inhibitors) such as mycophenolate and azathioprine have also been implicated in GI toxicity following their administration in organ transplant recipients. Mycophenolate comes as mycophenolate mofetil (MMF) and enteric-coated mycophenolate sodium (EC-MPS; to reduce incidence of GI adverse effect), which are hydrolyzed to the

active drug mycophenolic acid (MFA) by esterases in the stomach, small intestine, liver, blood, and other tissues after oral administration. In three clinical trials involving kidney transplant recipients on either MMF-cyclosporine or MMF-tacrolimus therapy, the incidence of severe diarrhea was reported during the first post-transplant year [114–117], with the occurrence of duodenal villus atrophy and erosive inflammation in the small intestine, in which the patients responded following MMF dose reduction or withdrawal [118, 119]. There are also reports of colonic and oral ulcerations and enterocolitis similar to Crohn's disease in kidney and liver transplant recipients, which were linked to direct GI mucosal injury by the active drug, MPA, and were resolved following discontinuation of MMF [120–122]. The mechanism of action of MPA is linked to its anti-proliferative properties, by selectively inhibiting inosine monophosphate dehydrogenase, the main enzyme in de novo pathway of synthesis purine in GI epithelial cells for growth and replication [123]. This suggests that MPA could inhibit GI epithelial cell growth and replication and consequently disrupt fluid absorption in the GI tract, resulting in diarrhea. The enteric-coated form of mycophenolate, EC-MPS, which was developed as an alternative to MMF with the goal of reducing GI adverse events, indeed resulted in improved GI tolerability after conversion from MMF to EC-MPS in kidney transplant recipients [124, 125]. This observation suggests that indeed EC-MPS could serve as a useful alternative in transplant recipients who are intolerant to MMF. However, comparative studies of EC-MPS with equipotent dose of MMF showed no difference in GI adverse event profile in other kidney transplant recipients [126–128]. This contradictory outcome warrants further investigations. As with MMF, azathioprine administration also led to severe persistent diarrhea with severe small-bowel villus atrophy, gastroenteritis, and malabsorption in kidney transplant recipients, which were reversed upon discontinuation of the drug [129–131]. Azathioprine administration also resulted in acute pancreatitis as observed in tacrolimus and cyclosporine administration [132]. Besides MMF and azathioprine, a meta-analysis also revealed a significant increase in the risk of GI hemorrhage or perforation in corticosteroid-treated patients [133]. Corticosteroid therapy also caused acute pancreatitis and colonic malakoplakia, a chronic granulomatous disease in a kidney transplant recipient [134, 135]. In two phase III clinical trials, diarrhea, constipation, nausea, and ulcerative colitis were observed in kidney transplant recipients who were on belatacept-based immunosuppression, which was comparable to those placed on cyclosporine-based immunosuppression therapy [136–139].

As GI toxicity is commonly associated with chronic immunosuppression following organ transplantation, it is important to develop an effective approach to reduce these adverse events to prevent allograft rejection and subsequent loss. Also, a systemic and individualized approach should be developed to optimize the management of transplant recipients' GI toxicity, which overlaps with non-immunosuppressant co-medications and comorbidities. In the absence of critical illness, a watchful waiting approach should be considered to determine whether the GI disorder will spontaneously resolve without pharmacological intervention, as was observed in 65% of 130 kidney transplant recipients with diarrhea [128]. In cases where a transplant recipient develops severe or prolonged GI complications requiring pharmacological treatment, the underlying cause should be determined. Moreover, once a GI complication occurs at a sufficient post-transplant period such that any possible transplantation-induced adverse effects can be eliminated, other possible etiologies such as non-iatrogenic causes should be considered before altering the patient's immunosuppressive therapy. In the absence of non-iatrogenic etiologies, the patients' immunosuppressive regimen should be modified in a controlled manner with careful evaluation of each component of the regimen.

15.2.6 Immunosuppressant-Induced Hepatotoxicity

Following organ transplantation, transplant recipients often have more aggressive liver diseases compared to their non-transplant counterparts. For example, 20% of transplant recipients with hepatitis C (HCV) disease recurrence progress to cirrhosis within 5 years of liver transplantation [140]. Immunosuppressants and other concomitant drugs used by transplant recipients often cause hepatotoxicity characterized by increased levels of liver enzymes and conjugated hyperbilirubinemia, with histological patterns such as cholestatic hepatitis, sinusoidal obstruction, granulomatous inflammation, macro- and/or micro-vesicular steatosis with or without steatohepatitis, hepatocellular necrosis ranging from single-cell dropout to broad necrosis, veno-occlusive disease, or a combination of any of these injury patterns [141–151] (Fig. 15.1). Dual therapy with cyclosporine and MMF or tacrolimus with MMF significantly increased hepatic apoptotic markers and contributed to inflammation and the development of liver graft injury after liver transplantation [149]. The authors supported their observation with results from their in vitro study in which they treated primary mouse hepatocytes with various combinations of cyclosporine, tacrolimus, and MMF and observed significant reduction in hepatocyte viability and increased expression of hepatic apoptotic markers such as cleaved caspases 3 and PARP. However, sirolimus/MMF combination prevented apoptosis and enhanced hepatocyte viability [149], which also confirms a previous study in which sirolimus/MMF combination reversed hepatotoxicity caused by tacrolimus and cyclosporine at 17 weeks after bilateral lung transplant [152]. This suggests that withdrawal of calcineurin inhibitors from the immunosuppression regimens could protect against hepatotoxicity in transplant recipients.

It is worth noting that calcineurin inhibitors have been previously implicated in the development of hepatotoxicity after organ transplantation. Cyclosporine, for example, was reported to have caused development of cholestatic hepatitis, bile duct stones, and sledge formation resulting from reduced bile acid secretion in a subset of kidney transplant recipients [153–155]. Animal studies also revealed sinusoidal dilatation and congestion, infiltration, hydropic degeneration, and loss of glycogen storage in the liver after cyclosporine treatment [148]. Mechanistically, cyclosporine decreased the levels and activities of hepatic antioxidants such as glutathione, catalase, and superoxide dismutase while increasing hepatic ROS production with increased liver enzymes and total bilirubin levels [148]. Also, tacrolimus-induced hepatotoxicity characterized by hyperbilirubinemia, increased levels of liver enzymes, centrilobular cholestasis, hepatocellular necrosis, and cholestatic jaundice was reported in previous studies in kidney transplant recipients after all other possible etiologies of hepatic dysfunction including viral hepatitis were ruled out. However, dose reduction or conversion to other immunosuppressive drugs such as prednisolone resulted in complete resolution of the liver condition [147, 152, 156–158]. Although the exact molecular mechanism underlying tacrolimus-induced hepatotoxicity is unknown, a preclinical study in rats showed inhibition of biliary excretion of glutathione and bicarbonate following tacrolimus administration [159].

Contrary to the hepatoprotection by sirolimus and MMF in transplant recipients [149, 152], MMF administration in a subset of liver transplant recipients induced hepatotoxicity as evidenced by hepatic mitochondrial stress and abnormalities similar to those observed in primary and secondary mitochondrial abnormalities [151]. This observation was supported by an in vitro study by the same authors in which MMF induced various stress changes in hepatic mitochondria of mice and markedly increased mitochondrial number, size, and number of lipid droplets per cell compared to untreated mice [151]. However, reduction or withdrawal of MMF resulted in improvement of liver function. Mycophenolate sodium, another form of MMF that has been proven to be as safe and effective as MMF [126, 127], also induced centrilobular cholestatic hepatitis in a kidney transplant recipient, which was completely reversed after withdrawal

of the drug [145]. Sirolimus administration also produced sinusoidal congestion with elevated levels of liver enzymes in liver and kidney transplant recipients after exclusion of possible etiologies. A quick normalization of liver function was observed after sirolimus was discontinued [143, 144]. Everolimus, another immunosuppressant in the class of mTOR inhibitors with sirolimus, also increased the levels of liver enzymes within a week of therapy in a liver transplant recipient, with corresponding histopathological changes, notably the presence of eosinophils, rare acidophil bodies, and focal mild portal inflammation after other possible etiologies of liver dysfunction including concomitant medications have been ruled out [150]. Finally, azathioprine use post-transplant has also been linked to elevated levels of liver enzymes and induced hepatotoxicity characterized by jaundice, chronic hepatitis, chronic active hepatitis, chronic persistent hepatitis, and cirrhosis in kidney transplant recipients, which were enhanced by hepatitis B virus or hepatitis C virus. However, azathioprine withdrawal normalized the liver condition and levels of liver enzymes [141, 142, 146]. Taken together, immunosuppressant-induced hepatotoxicity is a common complication in organ transplant recipients. Therefore, dose reduction or early identification and withdrawal of a suspected immunosuppressive agent from the treatment regimen are important to avoid progression or deterioration of the liver condition. Thus, close monitoring of liver tests in organ transplant recipients and early evaluation of unexplained persistent liver function test abnormalities using biopsy and possibly electron microscopic examination should be requested.

15.3 Reducing the Burden of Immunosuppression After Solid Organ Transplantation

As discussed in the above section, minimization of immunosuppressive drugs in the treatment algorithm following organ transplantation with the goal of individualizing organ-specific immunosuppression and limiting the impact of chronic immunosuppression should be considered at all transplant centers. This suggests development of tools such as peripheral and intra-graft expression markers of immune activation as tools to carefully guide patient selection and closely monitor drug minimization trials including allograft function. Also, there is the need to develop newer and more efficient drugs that target novel mechanisms and pathways of both cellular and humoral mechanisms of adaptive immune response including mechanisms related to ischemia-reperfusion injury in order to provide immunotolerance without the burden of organ toxicity and other complications induced by the currently used immunosuppressive regimens. At the same time, these proposed drugs should achieve acceptable graft rejection rate and prolong survival of organ transplant recipients. In addition, while there are reports of rare cases of immunotolerance using existing drugs (with inexplicable underlying mechanisms), reliable methods and assays should be developed to identify organ transplant recipients who can be weaned of immunosuppression since the current clinical practice requires that organ transplant recipients are bound to lifelong immunosuppression. Furthermore, high-throughput "omic" techniques such as genomics, proteomics, and metabolomics should be considered in identifying allograft injury-specific mechanisms in order to develop biomarkers for acute and chronic rejection as well as tolerance. Finally, future investigations should consider polymorphism studies involving gene interaction with immunosuppressive agents as an additional tool towards the management of organ transplant recipients. In conclusion, individualization of immunosuppression, development of novel drugs to provide immunotolerance without organ toxicity, identification of biomarkers of allograft rejection, and close monitoring of drug minimization trials may prevent immunosuppressant-induced organ toxicity and facilitate lasting tolerance of the human immune system in the future.

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16 Artificial Intelligence-Based Techniques to Assess Drug Toxicity in Drug-Induced Liver Injury (DILI) Disease

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Abstract

Drug-induced liver injury (DILI) is difficult to detect, and a rare condition which is responsible for drug removal from the market. DILI poses a great drug safety concern for the Foods and Drug Authority (FDA) and pharma industry. It is challenging for pathologists to differentially diagnose DILI injury patterns and to assess hepatotoxicity and other closely related liver disease phenotypes such as non-alcoholic steatohepatitis (NASH). Early detection of DILI can save a person from morbidity associated with this condition and also cost of hospitalization. It is generally difficult to detect complex and overlapping DILI injury patterns by human eyes under microscope. Computational pathology and artificial intelligence can be helpful for diagnosing DILI injury patterns, liver toxicity, and disease management. Deep learning models in artificial intelligence can be tested on the whole slide digital images (WSI) of liver biopsy to classify the DILI injury patterns such as fibrosis and steatosis.

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Keywords

Automated machine learning · Computational biomarker · Diagnostic support system · Drug-induced liver injury · Feature-based detection

16.1 Introduction

Drug-induced liver injury (DILI) or hepatotoxicity is a common cause of acute liver failure. DILI is a rare disease, responsible for the withdrawal of drugs from the market due to its late detection. High cost of drug development process and a long time to reach the market create disappointment and waste of scientific efforts. The cause of DILI-related injury is poorly understood and still unknown today. It's very hard to detect an agent responsible for DILI and challenging to assess the injury levels induced on the liver, because of multiple drugs taken by patients. DILI is a growing concern in the drug development research community because of the increasing number of drugs used in medical care and the increasing number of individuals who takes them routinely. Hepatotoxicity is the highest concern of adverse drug reactions in the pharmaceutical industry [1]. DILI injury is induced by prescription drugs taken together with over-the-counter drugs and alternative medicines, such as herbal products and supplements.

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There is no treatment available to cure injured liver other than discontinuation of drug and removal of offending agents which can be helpful to avoid risk involved in pre-existing liver disease patients. In clinical practice, pathologists study liver biopsy under microscope to assess DILI injury patterns and score affected liver. There is a great inter- and intra-variability of observations among the pathologists to read and diagnose DILI injury and to assess the state of disease severity. Other major challenges are overlapping DILI injury patterns with other common liver diseases.

Digital pathology is a potential area of interest for pathologists to understand DILI injury patterns using machine learning imaging algorithms. Artificial intelligence and machine learning technologies are rapidly gathering attention in the digital imaging space due to its pattern recognition features. Deep learning, a sub-type of artificial intelligence algorithm, successfully tested on millions of images (with great accuracy and efficiency) for pattern recognition [2]. Artificial intelligence can be used to assess DILI-induced hepatotoxicity, disease severity, and DILI pattern recognition on liver digital biopsy images as an AI-based diagnostic tool.

16.2 Etiology

DILI is classified as intrinsic and idiosyncratic. Intrinsic DILI is predictable and dose-dependent, whereas idiosyncratic DILI is unpredictable and dose-independent and has variable latency period. The etiology of DILI remains complex and unclear and makes it harder for a pathologist to perform differential diagnosis to classify if these are drug-induced patterns [3]. In a liver biopsy, DILI injury patterns are widely resembled with commonly expressed histologic patterns of chronic hepatitis and fatty liver disease ranging from inflammation, necrosis, and fibrosis. Pathogenesis of DILI is complex and unpredictable and could be related to the activation of the immune system. Liver biopsy is not recommended for DILI diagnosis but can be useful in excluding other etiologies to assess the closely associated phenotypes. Clinical features associated with DILI etiologies described in patients are closely related to herbal medications and dietary supplements [4]. DILI risk factors related to patients include age, gender, alcohol, genetics, drug-dose, and metabolism.

16.3 LiverTox

National Institute of Health's (NIH) LiverTox website is the important resource to study liver toxicity. LiverTox is a freely available resource which provides clinical and research information on DILI drugs, herbals, and dietary supplements responsible for causing DILI liver injury. LiverTox platform is designed by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and widely used by the medical community, mostly physicians, clinicians, patients, researchers, and academicians for the updated DILI-related hepatotoxicity information [5]. The LiverTox can be accessed at https://www.ncbi.nlm.nih.gov/books/NBK547852/ maintained by NCBI National Library of medicine website. The information documented in LiverTox is taken from scientific literature, public databases, various drug development studies, and clinical trials.

DILI liver injury relies on diagnosis of exclusion and is very closely associated with other liver diseases. There is no diagnosis available for DILI-related injury, other than causality assessment and disease management which is again challenging and relies on the timing of onset of allergic reactions and starting and stopping of medication. There is no treatment available to reverse DILI injury but discontinuation of the drug and toxins.

16.3.1 Phenotype of DILI

DILI is categorized by phenotypes, clinical findings, and symptoms, which are closely associated with other liver disease and hepatotoxicity patterns. LiverTox broadly categorizes DILI expression into 12 phenotypes, namely, acute

hepatic necrosis, acute hepatitis, non-alcoholic fatty liver (NAFL), and others which could be helpful in diagnosis, disease management, causality assessment, and understanding the disease pathogenesis. These phenotypes have distinct immunologic features and adverse outcomes which may lead to liver failure and cirrhosis. DILI diagnosis is hard due to its resembling and overlapping phenotypes with other liver diseases and fatty liver, especially in patients with pre-existing disease conditions and who take multiple drugs of different doses.

16.3.2 Classification of DILI Drugs

There is no clear-cut categorization recommended for classification of direct DILI or idiosyncratic DILI. Based on the level of injury induced by DILI drugs, frequency of drug taken, commonly or uncommonly, reported injury levels, etc., NIDDK created a "Likelihood Score" on five-point categorization of the likelihood that a medication is associated to DILI liver injury. The network can be accessed at https://dilin.org/ and is known as Drug-Induced Liver Injury Network (DILIN) [6].

DILIN collects and analyzes cases of severe injury and documents toxin agents that can cause DILI injury in normal or overdose conditions.

Based on the five-point system adopted by DILIN, tabulated in Table 16.1, the severity of DILI is assessed as mild, transient, liver and other organ failure, jaundice, and any hospitalization conditions.

Table 16.1 DILI grading system

Score	Condition	Assessment
1+	Mild	Raised serum aminotransferase or alkaline phosphatase levels or both, but total serum bilirubin <2.5 mg/dL and no coagulopathy (INR <1.5)
2+	Moderate	Raised serum aminotransferase or alkaline phosphatase levels or both and total serum bilirubin level ≥2.5 mg/dL or coagulopathy (INR ≥1.5) without hyperbilirubinemia
3+	Moderate to severe	Raised serum aminotransferase or alkaline phosphatase levels and total serum bilirubin level ≥2.5 mg/dL and hospitalization (or preexisting hospitalization is prolonged) because of the drug-induced liver injury
4+	Severe	Raised serum aminotransferase or alkaline phosphatase levels and serum bilirubin ≥2.5 mg/dL and at least one of the following: Prolonged jaundice and symptoms beyond 3 months, or signs of hepatic decompensation (INR ≥1.5, ascites, encephalopathy), or other organ failure believed to be related to drug-induced liver injury
5+	Fatal	Death or liver transplantation for drug-induced liver injury

Adopted from LiverTox
[a]The international correction ratio (INR) is a calculation used to monitor individuals who are being treated with blood-thinning medication

16.4 Liver Causality Assessment Tools

DILI casualty is assessed and weighted on other important factors which include hypersensitivity, recurrence, and drug allergies. Commonly used scale for capturing adverse drug reactions known as Naranjo Probability Scale [7] is also used for causality assessment. Broadly categorized, there are three liver-specific causality assessment tools currently available in clinical practice for the diagnosis of drug-related DILI:

1. Roussel Uclaf Causality Assessment Method (RUCAM): the Roussel Uclaf Causality Assessment Method (RUCAM) was developed in 1989 by the Council for International Organizations of Medical Scientists (CIOMS) for the diagnostic criteria or DILI injury assessment [8].
2. Digestive Disease Week Japan 2004 scale (DDW-J): DDW-J is superior to CIMOS and M & V scales and includes in vitro Drug Lymphocyte Stimulation Test (DLST) [9]
3. Clinical Diagnostic Scale (CDS). CDS is more stringent tool in assessing DILI in comparison to RUCAM and DDW-J [10].

Mostly all three assessment tools behave similarly in selection of variables and exhibitions of casualty assessment with minor variations which

may likely benefit for improvement by using data-driven and computer-based techniques.

16.5 Computational Pathology for DILI Injury Assessment

Deep learning algorithms are tested to understand chemical or molecular behavior of DILI drugs [11]. Very few studies are available on computational pathology and DILI detection on histology images [12]. Biopsy tissue glass slides scanned digitally at higher magnification provide a rich source of disease phenotypic information which can be helpful to assess the DILI injury patterns. Computational pathology and artificial intelligence [13] used with feature engineering, a technique in computer vision to extract morphometric disease information from digital images, can be helpful for a computer algorithm to extract enriched disease information which is challenging for a human pathologist to interpret. Image magnification and staining variability add another level of complexity. Artificial intelligence and deep learning techniques could be incorporated in this space of imaging-based diagnostic tools to analyze DILI injury patterns.

16.6 Liver Toxicity and Fatty Liver

Liver is the largest organ in the body performing more than 500 tasks; major functions include detoxification, protein synthesis, and bile production. Liver cells are known as hepatocytes. Toxicity caused to hepatocytes is called hepatotoxicity which is induced by the intake of toxins or overdose of prescribed drugs. One of the phenotypes of liver toxicity is fatty liver. In some patients, autoimmune hepatitis and DILI injury patterns present and manifest very closely associated overlapping pathological features [14]. DILI present with autoimmune features is known as AI-DILI. Patients with DILI features show positive markers for autoimmunity with IgG immunoglobulin. Few drugs are listed here that carry similar features: nitrofurantoin, minocycline, methyldopa, or hydralazine [15]. The presence of autoimmune features such as antinuclear antibodies (ANAs), smooth muscle antibodies (SMAs), and elevated immunoglobulin G (IgG) levels is diagnostically challenging for DILI disease management.

DILI drugs in patients with non-alcoholic fatty liver disease (NAFLD) share common pathophysiological features and induce macrovesicular steatosis which can trigger the inflammations and hepatotoxicity. Steatosis manifests as the accumulation of droplets of triglycerides inside the cytoplasm of hepatocytes. Hepatotoxicity in NAFLD exhibits necroinflammation and fibrosis which belongs to pharmacological class of few of the DILI drugs such as acetaminophen, halothane, methotrexate, rosiglitazone, and tamoxifen [16]. Obesity is also closely associated with NAFL characterized by accumulation of hepatic lipids which may progress towards NAFLD and later to non-alcoholic steatohepatitis (NASH), an advanced form of NAFLD. NAFLD tends to develop in patients who are overweight or obese or have diabetes, high cholesterol, or high triglycerides. In a pathological setting, the NASH scoring system commonly considers main histopathological features such as ballooning degeneration of hepatocytes (liver cells), inflammation, steatosis, and fibrosis. Liver fibrosis developed by deposition of an extracellular matrix is a major parameter guiding the prognosis of liver diseases.

Necrosis is another phenotype induced by the DILI drug. Acetaminophen (paracetamol, N-acetyl-p-aminophenol; APAP) is a widely used over-the-counter analgesic drug and considered safe at therapeutic doses; acetaminophen produces a hepatic necrosis that can be fatal if taken in higher doses [17]. Some other drugs such as bromobenzene, beryllium, quinidine, Iodoform, ferrous sulphate also cause zonal hepatotoxic injury that spreads from central vein to portal vein [18].

16.7 Artificial Intelligence in DILI Pattern Detection

Artificial intelligence is a computer-based technique used for process automation and pattern recognition which is gathering attention of the

medical community and widely used in medicine for various applications [19]. Artificial neural network (ANN) is the basic machine learning architecture in AI which mimics the human brain to learn, process, and perform a computational task with minimal human interference. ANN is used as a computational tool for process automation and high throughput in the drug development pipeline, extensively used and adopted in the pharma industry [20, 21]. ANN can be used on histology digital images which are scanned at high magnification. Digitally scanned biopsy images provide rich disease information in varying morphology in the shape of imaging features to assess disease diagnosis, severity, and progression [22].

Some important areas of bioinformatics such as computational pathology, machine learning (ML), morphology feature engineering, and data integration [23] have been widely accepted in the digital pathology community. Computational analysis is helpful to assess phenotype disease progression, in finding hotspots of disease activity [24], in reducing inter-/intra-observer variability [25], and for accurate disease diagnosis [26]. In this range of computational tools used in biomedicine, automated machine learning (AutoML) is another fully automated computational model (with no human interference) which works on the principle of learning from experience and mistakes and in which ANN is designed by another neural network. The AutoML model of ML is highly successful in industry and in accurately predicting outcomes and pattern recognition. AuML is a Google's machine learning (ML) model supported with the latest graphical processing units (GPUs). AuML is available in open-source public domain for high-performance artificial intelligence model processing and computing designed for non-ML experts on the concept where an artificial neural network is designed by another neural network with minimal human interference. In a DILI hepatotoxicity study, an AutoML model is tested to classify DILI injury patterns on whole slide pathology images on commonly used DILI drugs [12].

In this study, a deep learning model was designed and tested on the publicly available open-source toxicogenomics database imaging drug dataset popularly known as the Toxicogenomics Project—Genomics Assisted Toxicity Evaluation Systems (TG-GATEs) [27]. Machine learning algorithm was able to detect necrotic DILI injury levels induced by the use of commonly overdosed DILI drugs on biopsy images using mathematical geometry of fractals and lacunarity. Fractal-based texture analysis is used in detecting tumor geometry and growth in digital mammogram images [28].

16.7.1 AuML for DILI Pattern Detection

A study is designed as a proof of concept (PoCP) to test the AutoML AI algorithm for DILI pattern detection. The histology image dataset used in this study is taken from a public open source which is available at Gtex biobank maintained by the National Institutes of Health's Genotype-Tissue Expression (GTEx) project [29].

Liver toxicity image dataset is divided into four classes as fibrosis, steatosis, necrosis, and normal liver (2000 images in each class) as shown in Fig. 16.1.

AuML model is set to divide the dataset into training the model as 80% training data, 10% validation, and 10% out-of-box testing. AuML model performance is tested by the confusion matrix which is created between true and predicted labels, as shown in Fig. 16.2; the matrix shows how often the model classifies each label correctly (in blue) and which labels are most often confused for that label (in orange). The ML model is able to classify true and predicted values with 98.5% accuracy in case of normal, 95.6% in case of steatosis, 94.9% in necrosis, and 92.7% in fibrosis.

AuML model is validated on blinded test images (which the model has never seen during training). Model can perform 98% accurately in detecting test images of necrosis, fibrosis, steatosis, and normal. Few prediction examples are shown in Fig. 16.3 where randomly picked test images are tested in all four classes to validate the model. Figure 16.4 shows the model evaluation

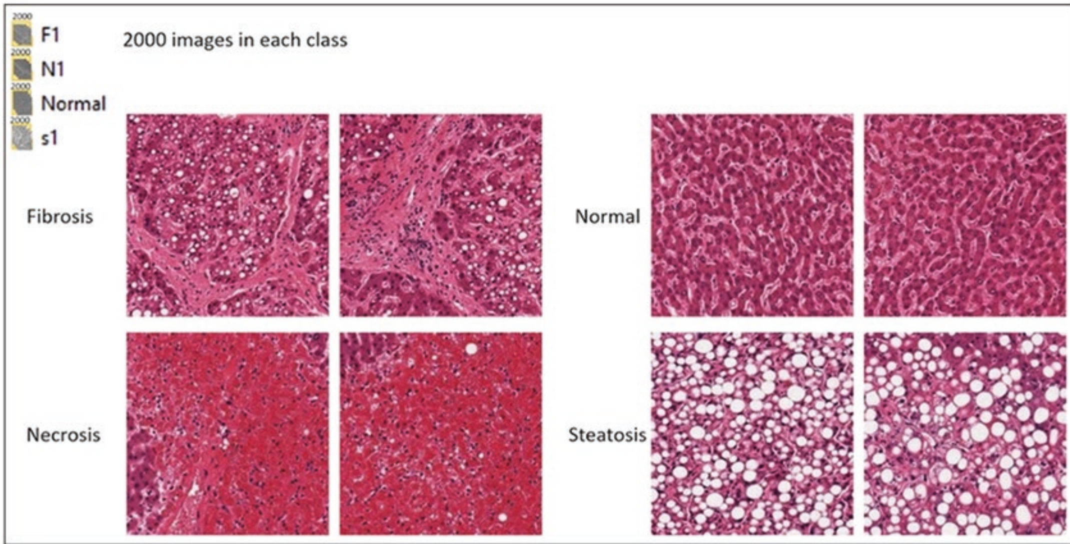

Fig. 16.1 Image dataset: Four classes of images (2000 images in each class are set for training ML model); F1 (Fibrosis), N1 (Necrosis), Normal (Normal liver images), & s1 (Steatosis). Two images of each class are shown here in the bottom rows

matrix and the test images which have mixed classes, i.e., necrosis with fibrosis and necrosis with steatosis. In most of the cases, the model can predict accurately. Figure 16.5 shows the accuracy curve of ML model during training and validation.

16.7.2 Deep Learning Model for DILI

Deep learning model in AI is made up of three major layers as input layer, hidden layer, and output layer. Data handling architecture layers in between input and output layers are referred to as "hidden layers," of which deep neural networks have many hidden layers; originally, "deep" means having more than one hidden layer. The DILI image data processing happens in these three layers. The original input data is first fed to the "input layer," and the "output layer" pushes out data that represents the model's prediction. Learning by ML model during the training process can be seen through internal hidden deep layer architecture. For example, here layer 5 learning is picked in the case of fibrosis and steatosis that illustrates how a deep learning model is learning the features of input image and pushing data towards prediction, shown in Figs. 16.6 and 16.7.

In fatty liver, steatosis develops in stages which can provide information in diagnosis severity for the liver disease. In pathology lab, progression of steatosis is assessed on H & E images under microscope. This process is highly error-prone and subject to inter-/intra-observer variability. High-throughput computer-aided image analysis tools and machine learning algorithms are widely accepted in pathology labs for scoring stages of steatosis.

In deep learning, there are two ways to automate the process, by classification or by segmentation technique. In steatosis segmentation, a deep learning model is proposed as DeEp LearnINg stEATosis sEgmentation (DELINEATE) both at patch-wise and whole slide for steatosis prediction analysis [30]. Proposed steatosis scoring method presents a strong correlation with pathologist annotations. In case of DILI, it's hard to implement preclinical models into clinical practice; drugs that cause DILI in humans typically do not show clear hepatotoxicity in animals. Molecular structure-based

True label \ Predicted label	fibrosis	steatosis	necrosis	normal
fibrosis	92.7%	2.9%	2.0%	2.4%
steatosis	3.3%	95.6%	-	1.1%
necrosis	2.6%	-	94.9%	2.6%
normal	0.5%	-	1.0%	98.5%

Fig. 16.2 Confusion matrix illustrates how ML model predicts the true class correctly (blue) and incorrectly (orange) as other class

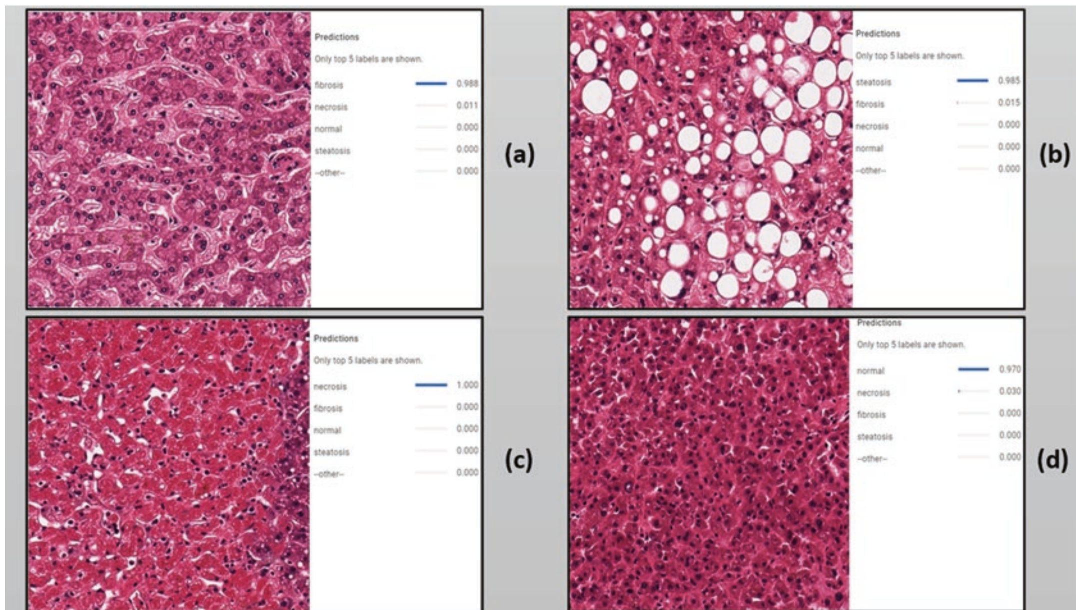

Fig. 16.3 Prediction on test images (**a**–**d**); prediction for fibrosis, steatosis, necrosis, and normal liver on test images by ML model

deep learning model was proposed and tested as UGRNN neural network architecture [31]. It's always a good practice to utilize the transfer learning technique in deep learning, instead of developing a new deep learning model from scratch. For scoring developing stages of fibrosis, different machine learning-based models using pre-trained AlexNet-CNN were tested which automatically score liver fibrosis stages with a level of accuracy similar to pathologists [32].

16.8 Conclusion

Studies that assess DILI provide considerable insights into the importance of hepatotoxicity in drug development. DILI is responsible for drug removal from the market, even in the final stages of drug approval, which poses a great concern for FDA and pharma industry. LiverTox and DILIN are the open-source resources available for the updated DILI-related information. Liver function

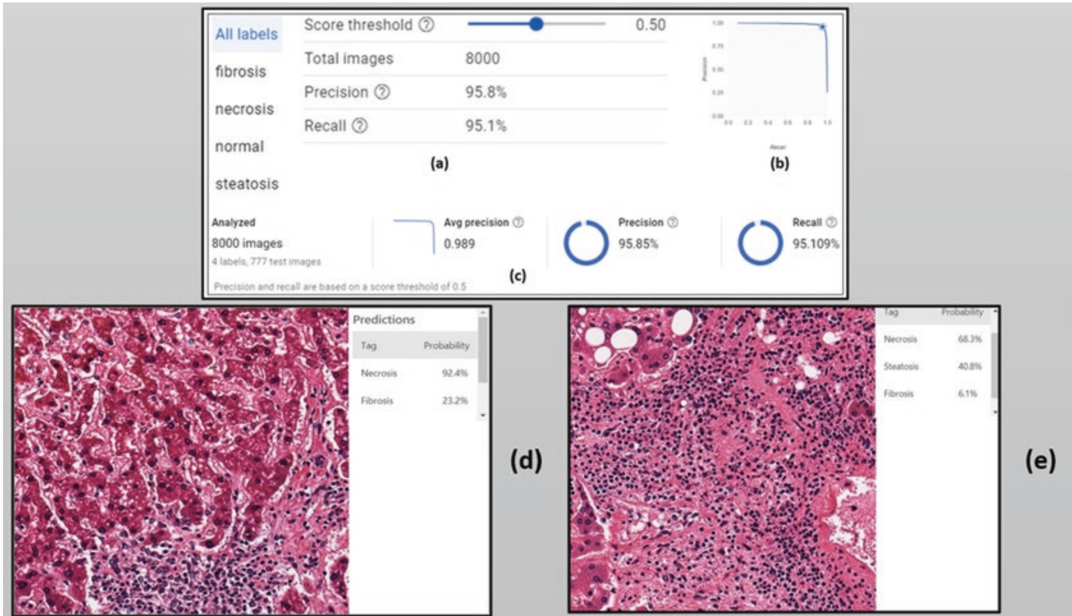

Fig. 16.4 Model evaluation matrix and prediction: (**a**) Threshold slider to adjust precision and recall. (**b**) ROC precision recall curve at 0.5 threshold. (**c**) Average precision, precision, and recall at 0.5 threshold. Precision and recall change by adjusting threshold. ROC receiver operating curve. (**d**) Prediction on necrosis, fibrosis. (**e**) Prediction on necrosis, steatosis, and fibrosis

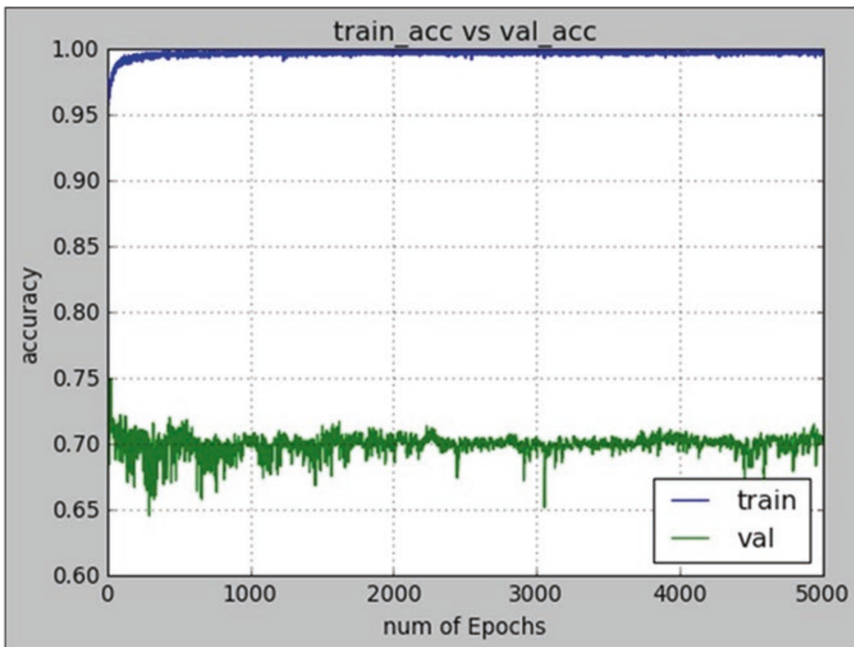

Fig. 16.5 Accuracy curve: ML model accuracy during training and validation

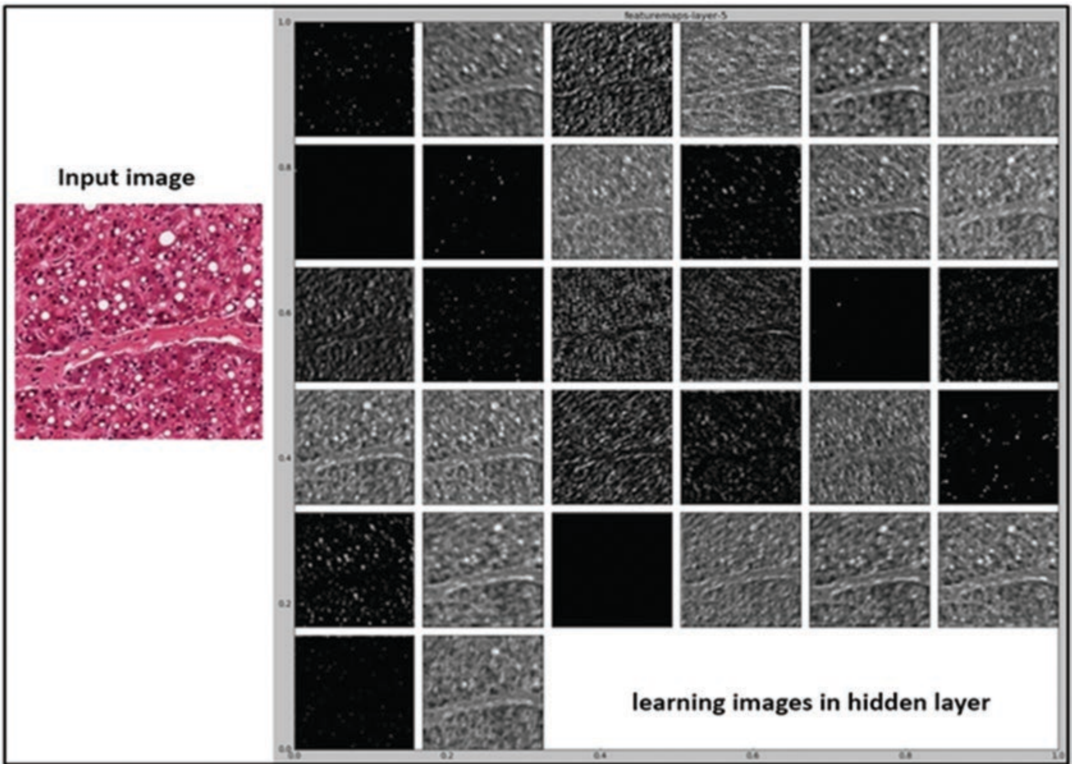

Fig. 16.6 Fibrosis learning representation: hidden layer 5 learning process in deep learning model, input image (left) and internal learning images (right)

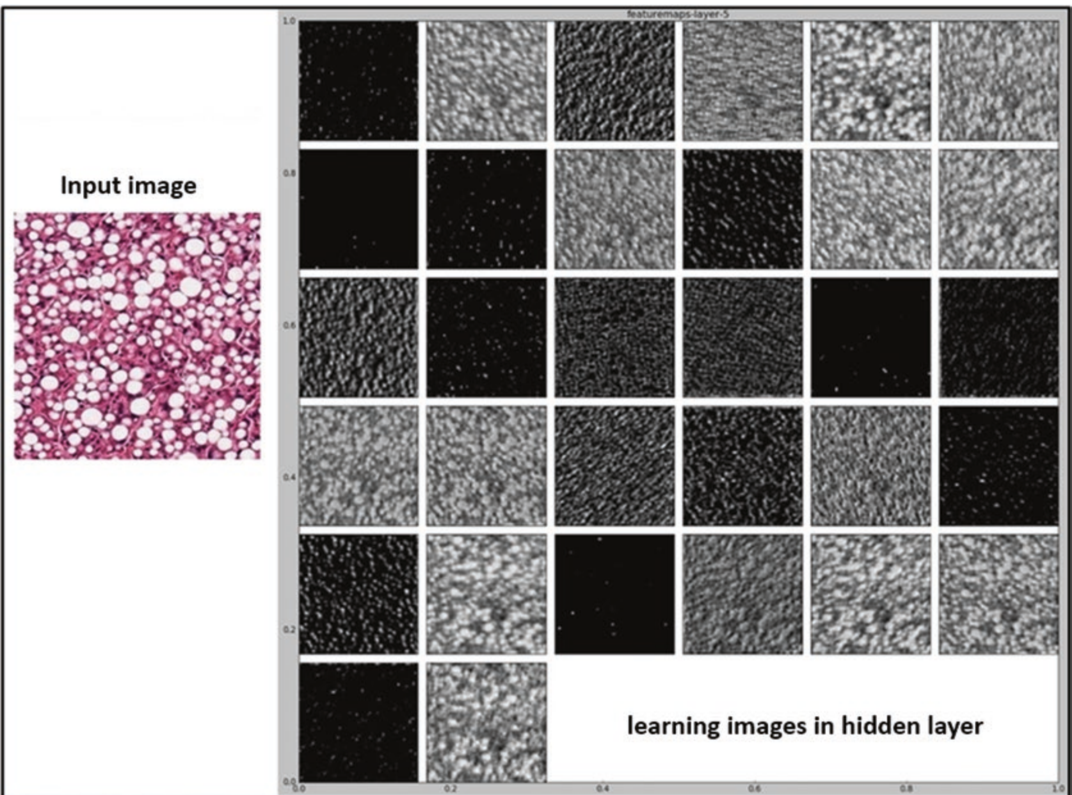

Fig. 16.7 Steatosis learning representation: hidden layer 5 learning process in deep learning model, input image (left) and internal learning images (right)

is highly complex, and it is hard to differentially diagnose hepatotoxicity injury patterns. Unknown pathogenesis and disease etiologies are poorly understood which makes the pathologists' task complex and challenging. An integrated approach will be helpful to adopt computational pathology in the clinical setting for DILI detection at early stages. Computational pathology with artificial intelligence can add valuable contributions in helping pathologists in diagnosing DILI injury patterns and for better prognosis and disease management.

Key Points

- DILI is a rare condition, a common cause of acute liver failure, and responsible for drug withdrawal from the market.
- No treatment is available, but discontinuation of the drug is often the best approach to reduce injury.
- NIH LiverTox website is a great resource for updated information on DILI drugs, herbals, dietary supplements, and toxins that cause DILI-associated hepatotoxicity.
- DILI diagnosis is challenging because of overlapping symptoms with other common liver disease phenotypes.
- DILI is a disease of exclusion, which makes it challenging for human pathologists to assess DILI related injury.
- Artificial intelligence has a great potential for detecting DILI injury patterns.
- AuML architecture of ML model is a resource for a non-ML expert to run a deep learning model on diagnosing DILI injury patterns.

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17 Drug Dose and Therapy Individualization

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Abstract

This chapter discusses how drug dosing depends on a variety of factors including those of the drug itself and those of the recipient. Principles of pharmacokinetics and pharmacodynamics can aid drug dosing, as well as prevent drug toxicity and overdose, which is especially important as the amount of opioid and over-the-counter medication overdoses is on the rise within the United States and worldwide. Utilizing drug dosing algorithms and understanding the varied dosing of the common medications warfarin, glipizide, codeine, and clozapine can help clinicians make informed decisions on patient care. This chapter explores the effects of patients' comorbidities and pharmacogenetics, specifically in regard to cytochrome P450 (CYP450) metabolism, infection, obesity, and renal insufficiency in order to highlight how important it is to understand a patient's individualism when prescribing based on treatment algorithms.

Keywords

Enzyme polymorphisms · Opioid overdose · Medication overdose · Therapy individualization · Pharmacodynamics · Drug dosing

17.1 Introduction to Pharmacodynamics and Pharmacokinetics

There are multiple subspecialties of medical science that focus on the importance of drug dosing. *Pharmacokinetics* is the study of how drug dosage is affected by its absorption, distribution, metabolism, and excretion. Alongside influencing drug delivery methodologies, pharmacokinetics determines drug dosing, as different drugs will have differing *bioavailability*. Bioavailability is a ratio of the amount of drug available after oral consumption versus IV administration; in drugs with lower bioavailability, physicians will have to prescribe more of the drug when given orally than when given intravenously [1]. Oral drugs can undergo first-pass metabolism, where

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they must pass through the liver before being absorbed into the body. IV drugs do not undergo this first-pass metabolism and thus do not require high dosage [2]. Pharmacokinetics also includes the study of *therapeutic indexes*, or the ranges within which a drug is clinically effective but nontoxic. Understanding therapeutic indexes is essential to preventing overdose as well as making sure sufficient dosage is given for an actual effect on the disease process to occur [3].

Other areas of medical science that home in on drug personalization are pharmacogenetics and pharmacogenomics. *Pharmacogenetics* explores how individual genes affect a person's response to drugs, while *pharmacogenomics* explores how a person's entire genome can provide a spectrum of quantifiable and qualitative responses to particular subcategories of drugs [4]. Specific genetic polymorphisms are found in specific ethnic groups or are associated with family histories, and understanding these differences can help with adjusting dosages or preventing adverse outcomes. Specific patients have different *isoenzymes*, or enzymes with the same action but slightly different structure or ability to catalyze a reaction. Most notable of these enzymes is cytochrome P450, found in the liver, kidneys, and GI tract and throughout the body. Different permutations of these cytochrome enzymes are associated with increased bleeding with warfarin administration, reduced metabolism of lornoxicam, and poor metabolism of proton pump inhibitors [4].

Another helpful tool in the clinician's arsenal to individualize prescribing practices is *targeted therapy*. Targeted therapy is the usage of drugs that will respond to a specific enzyme mutation, genetically polymorphic protein, or other unique aspects of the disease of interest. For instance, erlotinib is a tyrosine kinase inhibitor that can specifically be used for patients whose cancer cells express an overactive epidermal growth factor receptor (EGFR). By being "targeted" to a specific mutation or abnormality, these drugs are less likely to have dangerous off-target side effects and are better suited for that individual's disease and prognosis [5]. Other important tools include genetic profiling, PBPK (physiology-based pharmacokinetic) models, and propagation models of the diseases to see which drugs directly affect them [6].

Overall, drug individualization is rooted in an understanding of not only drug profiles, but the patient who will be receiving that drug as well as the environmental factors that might affect that drug's ultimate effectiveness for that particular patient. This chapter will explore concepts of drug dosing, adjusting prescription practices, and medication prescription individualization in order to reiterate the importance of drug therapy personalization.

17.2 Influence of Pharmacodynamics and Pharmacokinetics on Dosing

By definition, pharmacokinetics means looking at how a medication is processed throughout the body, while pharmacodynamics is looking at how the body is affected by the medication [7]. Both of these processes greatly impact how much of a certain drug should be given to a patient and whether or not it should be given at all.

There are four steps that contribute to pharmacokinetics – how the drug is absorbed into the body, how it gets to different parts of the body, how it is processed by the body itself, and how it leaves the body [7]. Multiple factors are involved in each of the steps to further contribute to dosing. For example, it is important to consider if a medication is long-acting or short-acting which is based off how it is metabolized. Giving too high of a dose of a long-acting drug can be toxic if it's in the body for longer than it is supposed to be. Furthermore, anything that changes the ability of a part of the body to absorb a medication can affect the pharmacokinetics, such as a surgery in the gastrointestinal tract which may impact absorption of oral drugs. A condition that is detrimental to blood flow can alter how the drug is distributed throughout the body if it is taken in an intravenous method. A disorder in the body's ability to break down the medication can have an effect on the metabolism, while some-

thing that influences the end of the gastrointestinal tract can affect excretion. In these ways and more, the differences in pharmacokinetics among people can be markedly significant, making it more difficult to generalize a treatment strategy. Thus, it is important to consider the specific patient as much as possible in making the decisions regarding dosing.

Therapeutic drug monitoring is a system greatly impacted by these processes. It requires knowing how much of the drug is in the plasma to decide what patients' accurate dosing will be, thus making it a more precise way of treating patients [8]. The Bayesian method of predicting dosing influences therapeutic drug monitoring. It works specifically with pharmacokinetics by looking at the differences between the concentration of the drug in the patient and how much it should be and the characteristics of the patients and the general population [8]. The comparison to average makes it easier to differentiate the patient's specific characteristics so that the therapy can be further tailored to them in the best way possible.

17.2.1 Drug Dosing and Weight

As mentioned previously, several factors must be considered when dosing drugs, and one of those considerations is the weight of the patient. Because weight can fluctuate throughout the years and it can be so varied among people, it is important to think about when trying to individualize the treatments of patients to what suits them best over the period of their care. A heavier or lighter weight makes enough of an important difference when trying to understand how much of a drug should be administered in a specific case.

When thinking of obese patients, the ratio of the weight of fat tissue to weight of lean tissue changes so that there are more of both types of tissues in the body [9]. How fast the drug leaves the body is proportional to how much lean tissue there is, so having more would mean that the drug would leave faster [9]. If a drug is taken out of the system at a more rapid rate, it may be important to recognize that and adjust accordingly so that the patient is still receiving an amount that is clinically effective as a treatment. On the other hand, if a drug is slower in leaving the system, it is necessary to recognize that having too high of a dose present in the body at once may lead to toxicity.

Another consideration is volume of distribution which can be related to weight if it is a drug that normally disburses in the fat tissue [9]. When put together, these are important factors that may change how much of the drug is given to the patient, although it does require a knowledge of properties of the drug itself as well. Not all drugs need to consider all of the same factors when being dosed, as exemplified with the volume of distribution. Some common calculations relating to weight when dosing are total body weight, ideal body weight, adjusted body weight, lean body weight, and body surface area [9].

17.2.2 Dosing Algorithm as Population Based

There are numerous factors that play a part in developing a dosing algorithm for each drug. For example, age, gender, weight, clinical conditions present in the patient, and characteristics of the drug itself are all considered when figuring out how much of a drug a patient should receive. As such, various methods can contribute to figuring out the ideal dosing for drugs in different populations.

One of the aforementioned methods is through modelling and simulation [10]. This allows for more information to be collected regarding how a drug works without doing an extensive clinical trial, thus making the process a lot faster [10]. The efficiency can greatly contribute to developing dosing algorithms for large populations, as it quickly generates the amount of data needed for patient dosing. Furthermore, it is less common to find information regarding doses that are in between the ones that are already suggested, which modelling and simulation can find [10]. This allows information to be gained in a new realm of doses for medications. Moreover, it can be used to look at how the safety and efficacy of

a drug are impacted by prognostic factors [10]. This is extremely important in coming up with guidelines for dosing, as it greatly changes which medications a patient can or cannot take and how much they should be using. These nuances in guidelines are essential and can have life-threatening effects if not considered.

17.3 Examples of Commonly Individualized Medications and Their Pharmacology

17.3.1 Warfarin

In the event of bleeding, a thrombus is formed via the following sequence of events: vasoconstriction, platelet plug formation, and activation of the coagulation cascade to ultimately form a cross-linked fibrin mesh which stops the bleeding. In the coagulation cascade, vitamin K is critical for the biosynthesis of clotting factors II, VII, IX, and X and antithrombotic proteins C and S [11, 12]. To demonstrate the role of vitamin K, the prothrombinase complex will be discussed. This complex requires factor II (prothrombin) as the substrate, factor Xa as the protease, factor Va as the cofactor, calcium, and anionic phospholipids such as phosphatidylserine found on the activated platelet plasma membrane [12]. Vitamin K is functionally necessary for the enzyme gamma-glutamyl carboxylase in the conversion of glutamate to gamma-carboxyglutamate. Gamma-carboxyglutamate residues in the 579-amino-acid-containing-prothrombin chelate calcium involved with the platelet plasma membrane. This allows for factor Xa to cleave prothrombin at codons Arg271 and Arg320 from the remainder of the protein, to form thrombin (IIa) [13] (Fig. 17.1).

Gamma-glutamyl carboxylase is a vitamin K hydroquinone-dependent hepatic enzyme residing in the endoplasmic reticulum that converts the prozymogen clotting factors into gamma-carboxylated prozymogens at the time of protein synthesis. Upon this conversion, vitamin K epoxide is released which is then reduced to vitamin K via the hepatic enzyme vitamin K epoxide reductase (VKOR encoded by vitamin K epoxide reductase complex 1 (*VKORC1*) gene). Vitamin K is then further reduced to vitamin K hydroquinone, now ready for use with gamma-glutamyl carboxylase [14] (Fig. 17.2).

It is now obvious that the role of vitamin K in the conversion of glutamate to gamma-carboxyglutamate is absolutely necessary in the clotting cascade, as demonstrated in the production of clotting factor IIa (thrombin), but recall that the vitamin K-dependent factors also include VII, IX, X, and proteins C and S. Without vitamin K, blood clotting is severely impaired, but this is taken advantage of in medications used to prevent inappropriate clotting in hypercoagulable patients. Warfarin is a clinically important vitamin K antagonist and, by way of its similar ring structure to vitamin K, binds to and inhibits VKOR [15].

Warfarin is commonly taken by patients to prevent venous thromboembolism and to reduce the risk of ischemic cerebrovascular accident in patients with atrial fibrillation [16]. Warfarin response is monitored clinically to maintain a patient's international normalized ratio (INR) between 2.0 and 3.0 for most medical conditions, but between 2.5 and 3.5 for patients with cardiogenic embolus, antiphospholipid syndrome, or mechanical heart valve. Healthy patients fall between 1 and 2. The INR measures the extrinsic pathway of coagulation and is necessary in place of the prothrombin time, because of differences in manufacturers of biological tissue factor [17, 18]. Recall that the extrinsic pathway involves tissue factor and factor VIIa converting X to Xa [18, 19].

It is especially critical to monitor a patient's initial response to warfarin as there is wide variability in each patient's pharmacokinetics and pharmacodynamics with this drug, and the INR can help direct the clinician to prescribe the appropriate dose. Of note, since gamma-carboxylation of glutamate residues occurs shortly after protein translation, warfarin therapy must be bridged with another anticoagulant to allow pre-existing clotting factors (especially factor X and prothrombin) to naturally deplete. Prothrombin has the longest half-life at 3 days

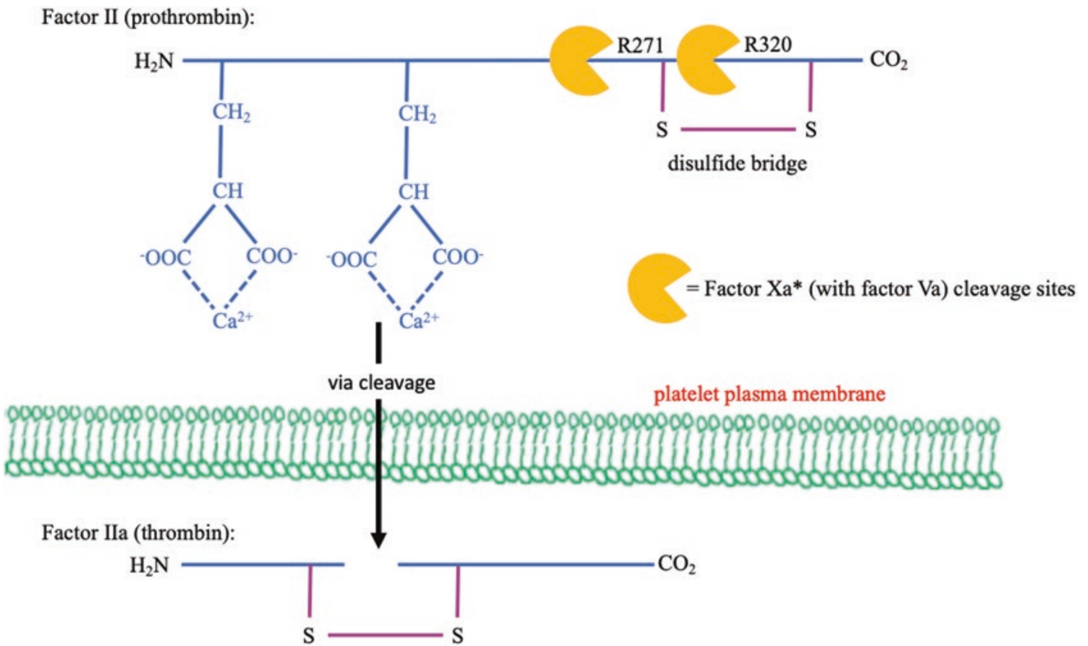

Fig. 17.1 This figure demonstrates the conversion of prothrombin to thrombin. Gamma-carboxyglutamate residues on prothrombin chelate calcium associated with the platelet plasma membrane, allowing factor Xa (with Va as a cofactor) to cleave prothrombin at sites Arg271 and Arg320, resulting in the thrombin product. Of note, thrombin is composed of two protein chains connected by a disulfide bridge. Note this figure is not to scale

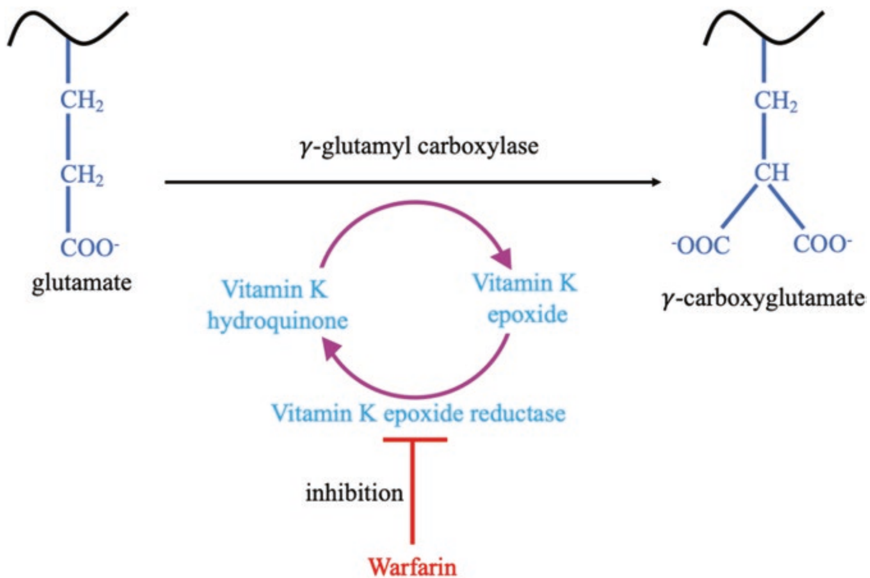

Fig. 17.2 This figure demonstrates gamma-glutamyl carboxylase's role in the conversion of glutamate to gamma-carboxyglutamate which is critical for proper clotting factor function. Gamma-glutamyl carboxylase is a vitamin K-dependent enzyme; this figure shows vitamin K's role and how warfarin inhibits it and thus the clotting factor

[20, 21]. Thus bridging must last for 3 days after warfarin initiation. Interestingly, proteins C and S, which naturally inhibit factors V and VIII, are also vitamin K-dependent; therefore, there is an initial paradoxical procoagulant effect of warfarin that is short-lived and rarely clinically significant [22].

VKORC1 and CYP2C9 are key in warfarin pharmacogenetics and individual patient response. Warfarin primarily inhibits vitamin K epoxide reductase. Single-nucleotide polymorphisms in *VKORC1* have been found to alter warfarin sensitivity [23]. Additionally of note, warfarin is a racemic drug. Cytochrome P450 2C9 (CYP2C9) primarily metabolizes S-warfarin, and studies demonstrate that *CYP2C9* polymorphisms affect patient response [24]. In terms of clinical significance, it is recommended that if testing of these genetics is not performed, then a clinician should begin a patient on a low starting dose and then increase this dose slowly while monitoring the INR carefully. If testing is completed and shows variations, a safer, lower dose can be initiated [24]. However, routine genetic testing is not recommended at this time [26].

17.3.2 Glipizide

Pancreatic beta cells are constantly measuring blood glucose concentrations so that they are able to release commensurate amounts of insulin. This is accomplished via the glucokinase receptor, present only in the liver and pancreas [27, 28]. First, glucose enters the pancreatic beta cell via the low affinity GLUT2 transporter, which will increase with chronic hyperglycemia [29, 30]. The rate-controlling step in glucose-stimulated insulin release involves glucokinase phosphorylating glucose to glucose-6-phosphate [28]. Glucokinase differs from hexokinase in terms of enzyme kinetics; glucokinase is low affinity and of high maximum velocity, and hexokinase is the opposite, though both catalyze glucose to glucose-6-phosphate. The low affinity of glucokinase is critical to allow the remainder of the body to obtain glucose via the high-affinity hexokinase, because glucokinase, with its high Vmax, will not saturate at physiological concentrations of glucose and would otherwise starve the remainder of the body.

Upon phosphorylation via glucokinase, the glycolysis, citric acid cycle, and oxidative phosphorylation pathways are completed resulting in an increased intracellular adenosine triphosphate (ATP)/adenosine diphosphate ratio. The increased ATP inhibits ATP-sensitive potassium channels located on the cell membrane, preventing hyperpolarization and subsequently resulting in depolarization of the cell. Voltage-gated calcium channels open, and increased intracellular calcium concentrations result in exocytosis of insulin-containing granules (Fig. 17.3).

Increased insulin release benefits patients with type II diabetes mellitus. Glipizide is a sulfonylurea medication that inhibits ATP-sensitive potassium channels in pancreatic beta cells, thus inducing insulin release [31]. Glipizide is metabolized by the cytochrome P450 2C9 (CYP2C9). Two variants, *CYP2C9*2* (R144C) and *CYP2C9*3* (I359L), have been shown to cause reduced enzymatic activity and thus decreased glipizide metabolism [32]. One study showed that patients with two variant alleles were 3.4× more likely to reach a target hemoglobin Alc of <7, compared to patients with two wildtype alleles, and that patients with variant alleles were less likely to fail sulfonylurea monotherapy[33]. In patients carrying the *CYP2C9*3* allele, oral clearance of glipizide is reduced, resulting in increased plasma drug concentrations. In one study, sulfonyl-treated patients with the *CYP2C9*3/*3* or **2/*3* genotypes had a 5.2× increased risk of a severe hypoglycemic incident [33]. Knowing a patient's pharmacogenomics can assist a physician in choosing a medication or a certain dosage to avoid adverse effects and achieve an ideal treatment goal.

17.3.3 Codeine

Codeine is a mild/moderate agonist of opioid receptors. Clinical indications may include nociceptive pain, psychogenic pain, and breakthrough pain. Opioid receptors are G_i-protein coupled

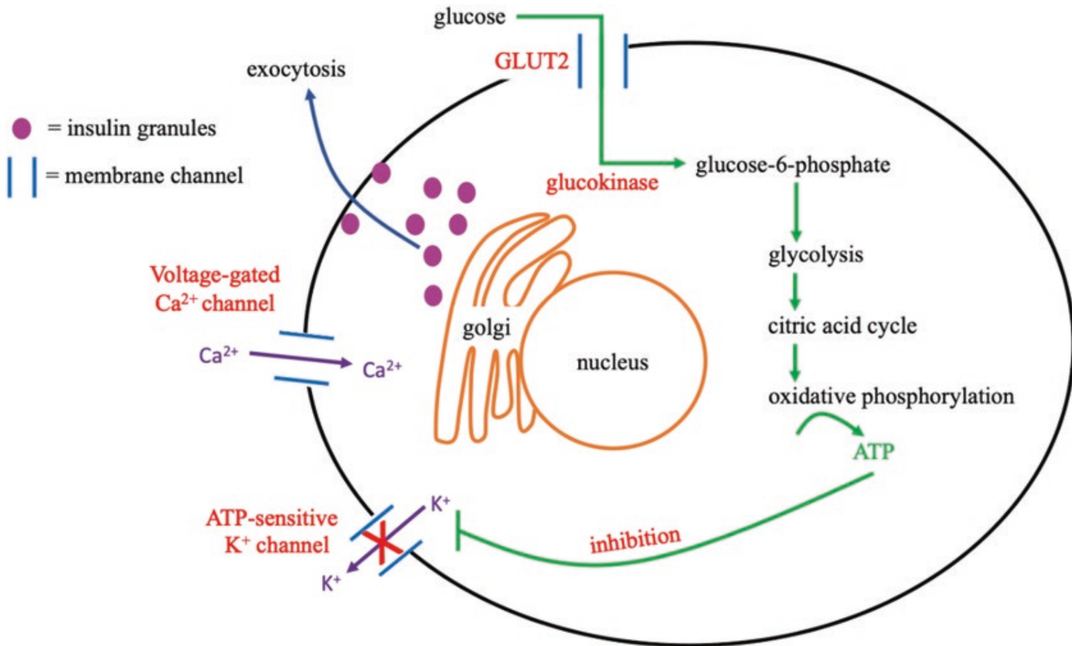

Fig. 17.3 This figure demonstrates the biochemistry of insulin secretion in a pancreatic beta cell. Glucose undergoes uptake via GLUT2 and is converted by glucokinase to glucose-6-phosphate, which then undergoes typical metabolism resulting in ATP production. The increased ATP: ADP ratio inhibits ATP-sensitive potassium channels, preventing hyperpolarization of the cell. Subsequently, the cell depolarizes, activating voltage-gated calcium channels and resulting in an influx of calcium. Increased intracellular calcium causes exocytosis of insulin granules

receptors (G_iPCR) located in areas including the brain, spinal cord, and gastrointestinal tract and are approximately found in the same areas that endorphins are synthesized. Endorphins are endogenous peptides that act on opioid receptors [33]. There are three clinically important categories of opioid receptors with similar structure and function though different distribution: mu, delta, and kappa [].

G_iPCRs inhibit adenylate cyclase which subsequently decreases concentrations of cyclic adenosine monophosphate (cAMP). This directly reduces neurotransmitter release. Opioid receptors also have two other mechanisms; they reduce the likelihood of action potentials by hyperpolarizing neurons by stimulating potassium efflux, and they reduce neurotransmitter release by inhibiting voltage-gated calcium channels [35]. Recall that calcium influx is critical to bind to SNARE proteins and thus fuse intracellular vesicles to the cell membrane for exocytosis of neurotransmitters.

Activation of opioid receptors located within the periaqueductal gray (PAG) reduces emotional response to pain by reducing signals from the PAG to the forebrain and amygdala and reduces perception of pain by inhibiting the inhibitory gamma-aminobutyric acid neurons within the PAG, which subsequently stimulates endorphin release onto spinal cord neurons [36].

Codeine is a prodrug and is clinically effective once converted into morphine via CYP2D6. Per the Clinical Pharmacogenetics Implementation Consortium (CPIC), in CYP2D6 ultra-rapid metabolizers, codeine should be avoided secondary to the risk of toxicity [37, 38]. In CYP2D6 poor metabolizers, codeine should be avoided secondary to lack of efficacy [25].

17.3.4 Clozapine

Antipsychotics are often used to treat severe psychotic disorders such as schizophrenia. Clinically effective concentrations of antipsychotics correlate with dopamine D2 receptor antagonism. Antipsychotics inhibit D2 receptors along the mesocortical (ventral tegmental area releases dopamine onto frontal cortex), mesolimbic (ventral tegmental area releases dopamine onto nucleus accumbens), nigrostriatal (substantia nigra releases dopamine onto striatum), and tuberoinfundibular (hypothalamus releases dopamine on pituitary gland) pathways, though therapeutic effects arise from antagonism of the mesolimbic and mesocortical pathways. D2 is a $G_{i/o}$-protein coupled receptor, which will inhibit adenylate cyclase and subsequently decrease concentrations of cAMP.

Clozapine was the first developed second-generation antipsychotic, so-called atypical because it is less likely to cause extrapyramidal symptoms like the typical antipsychotics, e.g., haloperidol. This is possibly due to its transient binding with D2 receptors. Clozapine also binds to D1; D3; D4; D5; histamine H1; acetylcholine muscarinic M1; serotonin 5-HT2A, 5-HT2C, 5-HT6, and 5-HT7 receptors; and alpha 1 adrenoreceptors [39].

Studies have shown clozapine to be more effective than other typical antipsychotics in treatment-resistant patients [40], and in treating schizophrenic patients with persistent suicidal ideation, clozapine reduces suicide attempts [41]. However, use is restricted to the aforementioned patients due to its potential severe side effect profile including agranulocytosis [42], seizures [43], and myocarditis [41], among others.

HLA-DQB1 is a genetic marker found to be disproportionately represented in patients who later resulted with a leukocyte count <500/μL while being treated with clozapine, compared to patients treated for at least 1 year with clozapine and with normal leukocyte and absolute neutrophil counts. PGxPredict:Clozapine (Clinical Data, Inc., New Haven, CT) is a tool that will utilize this marker to allow for the identification of patients at risk of agranulocytosis secondary to clozapine use [45]. If a clinician were to identify a patient at risk, they would likely avoid clozapine in the treatment of schizophrenia and select another antipsychotic for treatment.

17.4 Drug Dosing in Patients with Comorbidities

17.4.1 Involvement of CYP450 in Drug Metabolism

Cytochrome P450 is a collection of enzymes, mainly found in the liver but also in the small intestine, lungs, kidneys, and placenta, involved in the metabolism of drugs and toxins. There are six specific enzymes in this family, CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5, which are involved in the metabolism of 90% of drugs. The activity of these enzymes can be affected in comorbid conditions in the patient which may require adjusting the dosages of drugs that are metabolized by it. This section will look at drug dosing in patients with comorbidities, specifically infection, obesity, hepatic insufficiency, renal insufficiency, and pregnancy.

17.4.2 Infection

Infection and inflammatory states have been shown to decrease the activity of CYP450. The interferon (IFN) hypothesis states that depression of CYP450 enzymes is a common property of interferons and factors that induce interferons. Studies have shown that many inflammatory cytokines are also involved in the depression of CYP450 during inflammatory states. Due to the inhibition of the CYP450 system, infection and inflammatory states are thus implicated in impaired drug metabolism [46]. In an infection, drug concentrations may be increased due to not being able to be metabolized by CYP450.

Current viral infections that have been found to depress the CYP450 system in humans include hepatitis A, influenza A and B, adenovirus, HSV, and HIV. Specifically, influenza A has been

shown to decrease clearance of theophylline in the pediatric asthmatic population. This is likely due to the increase in endogenous IFN, which is released in response to influenza, which depresses CYP1A2, the primary enzyme responsible for the metabolism of theophylline [46].

17.4.3 Obesity

Drug dosing for patients based on weight is dependent on four main pharmacokinetic factors: absorption, distribution, metabolism, and elimination. There are many biometrics that a clinician can use to quantify the size of a patient: body mass index (BMI), total body weight, ideal body weight, body surface area, and lean body weight [48].

Oral drug absorption has not been shown to be significantly affected in obese patients. Animal and human studies have found that some parenteral drug absorption is negatively affected by an increase in BMI. Specifically, subcutaneous insulin and enoxaparin injections are associated with delayed systemic absorption, as weight increases. Obesity is also associated with gastric bypass surgery, which can lead to a decrease in absorption of certain nutrients and drugs, specifically those that require an acidic environment or require duodenal intestinal transporters. Specifically, cyclosporine, tacrolimus, thyroxine, phenytoin, and rifampin have been shown in case reports to have diminished absorption following gastric bypass surgery [48].

Volume of distribution (V_d) of a drug mainly appears to rely on the lipophilicity or hydrophilicity of the drug, as it relates to obesity. In general, charged hydrophilic drugs, such as lithium, are contained to the water compartments of the body, and their distributions are not significantly affected by changes in adipose tissue. Hydrophilic drugs, such as lithium, are better dosed based on the ideal body weight (IBW) rather than the total body weight. Furthermore, it can be predicted that lipophilic drugs could be better dosed based on total body weight (TBW), as they are more likely to deposit in adipose tissue, but it is not a generalized rule, and it is rather drug specific. Sufentanil has been shown to increase in the V_d as TBW increases, but remifentanil has shown a V_d similar to hydrophilic drugs, as its V_d positively correlates with IBW, rather than TBW [48].

The metabolism of drugs in obese patients can be altered as there are changes in concentrations and activities of CYP450 enzymes. Specifically, CYP3A4 has been shown to be downregulated in obesity, resulting in a decreased clearance of alprazolam, midazolam, fentanyl, carbamazepine, and cyclosporine. The enzyme CYP2E1 has been shown to be upregulated in obesity, resulting in increased metabolism of chlorzoxazone, enflurane, sevoflurane, halothane, and acetaminophen. The phase II conjugating enzyme UGT (uridine 5′-diphospho-glucuronosyltransferase) has been shown to be upregulated in obesity, resulting in increased glucuronidation of acetaminophen, oxazepam, and lorazepam [49]. Obesity is also associated with increased concentrations of pseudocholinesterase, resulting in increased metabolism of succinylcholine [1].

The changes in elimination of drugs in obesity are largely due to changes in kidney function. Initially, obesity is associated with an increase in glomerular filtration rate (GFR), which would result in an increase in clearance. However, obesity is an independent risk factor for focal and segmental glomerulosclerosis, which decreases GFR, thereby decreasing drug clearance. The changes in volume of distribution will also affect the renal elimination of a drug, as with an increase in V_d, there is less serum drug concentration, leading to decreased excretion [48].

17.4.4 Renal Insufficiency

Patients with renal insufficiency, whether it be acute or chronic, require special attention when it comes to drug dosing. The pharmacokinetic parameters that typically change in decreased kidney function include volume of distribution (V_d) and the clearance (CL). These changes are important because the therapeutic index window

can be easily exceeded when changes in V_d or CL are not taken into account in drug dosing, which could result in drug toxicity. Conversely, underdosing due to these changes, in an effort to protect from adverse reactions, could result in therapeutic failure, which is particularly important in acute situations, such as infection or immunosuppression [50].

Changes in the V_d are unpredictable in chronic kidney disease (CKD) and vary between drugs. Fluid expansion and edema, due to protein loss during CKD, could lead to increase in V_d, although some studies have shown no change at all. This change in fluid expansion most likely affects hydrophilic drugs rather than lipophilic drugs [51].

Initial dosing for acute kidney injury (AKI) and CKD depends on the type of drug as well as the condition it treats. A higher loading dose might be necessary if there is significant fluid overload as a result of the AKI or the CKD, especially if the drug is hydrophilic or if the disease that is being treated is a life-threatening infection [51]. The maintenance dosing of a drug will correlate with the degree of reduced kidney function. If kidney function is reduced <50%, maintenance dosing is not changed. If kidney function is reduced ≥50%, maintenance dosing is adjusted according to the eq. MD = CL * target concentration [51].

AKI poses high variability between patients, making drug dosing and maintenance difficult to achieve for clinicians in a short time. An increase in V_d is very common in AKI, resulting in many loading doses for antimicrobials to be insufficient for the sepsis it is attempting to combat. Methods used to quantify AKI are plasma creatinine concentration and urine output. Quantification of plasma creatinine can be deceiving as IV fluid and dialysis could act to slow the increase in concentration. Two other methods of measuring GFR during a single point in time are measuring creatinine clearance (CrCL) in patients without anuria over 2–12 hours or measuring GFR as an exogenous compound. AKI has also been associated with the decrease in function of certain CYP450 enzymes, specifically CYP3A4/5, with the substrate midazolam [51].

17.5 Implications of Incorrect Drug Dosing

17.5.1 Drugs with a Significant Risk of Overdose: Opioids

Opioids are powerful drugs that can dramatically reduce the acute or chronic pain a patient experiences both quickly and effectively. However, the statistics on the dangers of opioid prescriptions for long-term pain management are startling and worth addressing here. According to the Centers for Disease Control and Prevention [52], one out of every four patients receiving long-term opioid therapy, e.g., oxycodone, develops opioid substance use disorders (SUDs). In recent years, there have been 38 overdose deaths a day involving prescription opioid use [53].

Therefore, it is strongly recommended that opioid therapy be a last resort treatment option when other treatment options have not been effective in controlling chronic pain [54]. To mitigate the inherent risk of opioid use for patients, the CDC and other governmental and professional entities have outlined guidelines that medical providers should consider when determining the appropriateness of opioid prescriptions for patients. Table 17.1 is an adapted summary of the main points set forth by the CDC [54].

Morphine milligram equivalents (MME) were created to help providers keep track of patient opioid dosing and to help weigh the potential risks of a current treatment plan with the potential benefits [55]. It is recommended that physicians and providers should keep patients undergoing long-term opioid therapy for chronic pain below 50 MME/day [54]. According to the CDC, going above 50 MME/day has shown little additional efficacy in reducing pain but has shown great increases in patient odds for prescription opioid overdose and opioid SUD [54]. To calculate MME, multiply the total dosage of a given medication within a 24-hour period by that medication's relative strength which is its conversion factor (CF) [55]. Sum all values of MME together for each medication prescribed for the total MME for that patient:

$$\text{Total Dosage} \times CF = MME$$

$$MME^1 + MME^2 + MME^3 = \text{Total } MME$$

Table 17.2 contains a number of common opioids, their conversion factors, and example prescriptions:

Keep in mind that there are a number of mobile-based applications that can assist physicians in calculating and managing these values for patients.

Every patient undergoing long-term opioid therapy for chronic pain should also be given naloxone to help prevent accidental overdose [56, 57]. There are several different forms of naloxone available. For example, there are nasal sprays, auto-injectors, and syringes with ampules [58]. Each form of naloxone is straightforward and user-friendly; however, training (and maintenance training) should take place for both patients and physicians in anticipation of emergencies.

17.5.2 Drugs with a Significant Risk of Overdose: OTC Medications

There is an abundance of OTC medications that have high abuse potentials [59], for example, codeine-containing products, acetaminophen, antihistamines, pseudoephedrine, dextromethorphan, and laxatives to name a few [59, 60]. Abuse, misuse, and overdose trends for OTC medications appear to be based on both patient population demographics (e.g., age, sex, etc.) and geographic region (e.g., the USA compared to the UK) [60, 61]. Teens are more likely to abuse dextromethorphan or any OTC medication more than seniors [61]. Females are more likely to abuse barbiturates and sedatives more than males [61]. US patient populations are less likely to abuse codeine-containing products than patients in European countries [60].

Unfortunately, statistical studies on the prevalence of OTC drug overdose are still not as robust as the data available on opioid prescription overdoses [62].

Intentional OTC overdosing is done for various reasons. Aside from recreational use, some individuals do so to commit suicide through the ingestion of either diphenhydramine or acetaminophen [63], while others misuse medications like loperamide in an attempt to stave off opioid withdrawal symptoms and accidentally reach a toxic threshold [64, 65].

The below table contains commonly misused OTC medications, known or estimated lethal thresholds, and a non-exhaustive list of treatment options available (Table 17.3).

For treatment advice on OTC medication overdoses or poison-related emergencies, contact the national Poison Help hotline (USA) for assistance (1-800-222-1222).

Medical community awareness and action on medication abuse do have a positive impact on patient populations. For instance, abuse trends have declined significantly for drugs like dextromethorphan due in part to medical community advocacy, pharmacy retail policy changes, and FDA involvement [71].

Table 17.1 Summary of 12 CDC opioid recommendations 2016

1.	Use alternative and non-opioid Rx therapies first	5.	Start at the lowest effective dose. Stay well below 90 MME	9.	Review prescription drug monitoring program (PDMP) data at the start and then every 3 months
2.	Set realistic goals with patients and commit to reduction and discontinuation plans	6.	Acute pain opioid Rx should be less than 3 days	10.	Perform urine drug testing at the start and then annually to rule out illicit drug use
3.	Routinely go over patient and physician responsibilities	7.	Follow up within 3 weeks of start of opioid therapy and then every 3 months or less	11.	Avoid concurrent use of benzodiazepine and opioid drug prescriptions when possible
4.	Prescribe immediate-release opioids and avoid extended-release when possible	8.	Prescribe naloxone when at 50 MME, or if other risk factors are present	12.	If opioid SUD is determined, recommend behavioral therapy with buprenorphine or methadone treatment

Adapted from CDC Guideline for Prescribing Opioids for Chronic Pain — United States, 2016 [54]

Table 17.2 MME doses for commonly prescribed opioids and example prescriptions

CDC MME recommendation		Prescription and MME example		
Opioid	Conversion factor (CF)	Dosage	Frequency	MME example
Codeine	0.15	15 mg	1 tablet PO prn every 6 hours	9
Fentanyl transdermal	2.4	12.5 mcg/h	1 patch every 3 days	30
Hydrocodone	1	2.5 mg/325 mg (acetaminophen)	1 tablet PO prn every 4 hours	15
Hydromorphone	4	2 mg	1 tablet PO prn every 6 hours	32
Methadone	4	5 mg	3 tablets PO daily	60
Morphine	1	60 mg	1 tablet PO prn every 6 hours	60
Oxycodone	1.5	5 mg/325 mg (acetaminophen)	1 tablet daily PO q6hr	30
Oxymorphone	3	5 mg	1 tablet PO prn every 6 hours	60
Tapentadol	0.4	250 mg	1 tablet PO prn every 12 hours	120

Adapted from CDC Guideline for Prescribing Opioids for Chronic Pain — United States, 2016 [54]

Table 17.3 Commonly abused OTC drugs, estimated minimum toxic dose, and available treatment options

Drug name	Estimated minimum toxic dose	Emergency treatment options
Acetaminophen	Adults: 7 g–10 g Children: 150 mg/kg; 200 mg/kg in healthy children aged 1–6 years [66]	Activated charcoal N-Acetylcysteine (NAC) Stomach pump
Antihistamines	Adults: 20–40 mg/kg or 3–5 times usual dose Children: At or over 7.5 mg/kg [67, 68]	Activated charcoal IV fluid for tachycardia Urine catheterization Stomach pump
Pseudoephedrine	Not well characterized. One case study suggests 3–4 times therapeutic dose, or over 1 gram [69]	Activated charcoal Closely monitor vital signs Sudden drop in BP, tachycardia, febrile, and altered mental status may occur Stomach pump [67]
Dextromethorphan	Adults: 20–30 mg/kg Exceeding 300 mg can cause dissociative psychosis; >600 mg can cause comas and eventual death [69, 70]	Activated charcoal Benzodiazepines, e.g., lorazepam if patient becomes combative Cold IV, inspired oxygen Naloxone
Loperamide	Adults: >100 mg with median dose around 250 mg [64, 65]	ACLS for cardiac dysrhythmias Activated charcoal Naloxone

17.5.3 Overdose Prevention as Part of Drug Individualization

Individualization of drug prescription practices can have a dramatic effect on reducing overdose risk and as such is firmly rooted in the necessity of patient education and responsible prescription practices. Overdose has its origin in misuse of substances, poor patient understanding of medications, and socioeconomical disparities. It is influenced through individual perceptions of drugs' usage and role for treatments, social behavior behind drug usage, and lack of general knowledge about drugs due to lack of either edu-

cation in schools or education when receiving prescriptions [72].

One area where individualization is lacking within the pharmaceutical injury is with over-the-counter medications (OTC), as these come with "one size fits all" packaging. Because they are not as tightly regulated and do not require a prescription, patients will often take over-the-counter medications without consideration of their own individual genetics and comorbidities or the pharmacodynamics of those drugs. Thus, education about the associated risk of OTC medications is a necessity, as it can show patients how their own individual characteristics (such as renal failure, previous liver damage, a history of hypercoagulability) can be affected by those drugs. Patients might see OTC medications as safe to take as they do not require prescription or control by a medical professional and tend to form cognitive shortcuts in their understanding of medications' mechanisms (and adverse effects). One such shortcut of understanding is that medicine is seen as having a targeted approach, only treating the medical ailment of interest and not affecting other parts of the body [73]. For instance, a patient might believe that acetaminophen only treats their headache and might not consider that it will pass through and be metabolized by the liver, thus missing the critical side effect of potential liver damage. These misunderstandings persist through varied medication types and are compounded by the variation between brand names and off-brand medications that all have similar active ingredients.

Alternatively, patients may take concurrent OTC medications that have the same major active ingredient due to lack of medical understanding about those ingredients. This adverse event, termed "double dosing," is a common mistake. Patients may utilize pain relief medications at the same time as cold relief medications or alternatively may utilize similar drugs in the belief it will help relieve symptoms faster. Often, patients without medical training will not actively look at active ingredients listed on drug packaging; in stark contrast, those with medical training have the foreknowledge to consider the ingredients in various medications before taking them concurrently. Although drug packaging has changed to attempt to draw attention to those active ingredients, education in the form of public service announcements and warnings about common toxicities, such as acetaminophen poisoning, can help to reduce overdose risk [73].

Opioid misuse is another area where drug individualization is necessary to prevent overdose and toxicity. Individualizing how opioids are prescribed can have dramatic effects on decreasing the harmful effects of opioid overdose, as well as the associated elevated risk of developing blood-borne infections and the teratogenic effects on unborn fetuses [74]. Increased opioid dosage increases overdose risk; thus, one should try to limit the daily maximal dosage of opioids their patients take. In addition, patients on extended-release or long-acting opioids are more likely to overdose, as well as have depression, drug use disorder, or chronic pain [75]. Specific drugs are also associated with higher rates of opioid overdose specifically oxycodone, hydromorphone, and methadone. Another necessity is to cross-reference the patient's history for medications that may react with opioids or increase risk of drug misuse. Sedative hypnotics (especially benzodiazepines, e.g., diazepam) have increased risk of overdose due to their intrinsic CNS effects [75].

Another aspect of overdose that requires individualization is in the realm of intentional overdose, or attempted suicide by consumption of drugs. Opioid drugs, both illicitly obtained and prescribed, are commonly utilized in intentional overdoses. OTC drugs and controlled substances that were prescribed for mental health disorders are also used for intentional overdose. Opioids are the most often cause of death due to intentional overdose, followed by barbiturates, antidepressants, antidiabetics, and alcohol [76]. Notably, of these classes, alcohol is readily available with little to no regulation, and the other drugs are prescribed to patients with an increased risk of suicide (e.g., antidepressants, which are prescribed to lower depression's increased risk of suicide). Although both teenagers and adults attempt suicide, adults have more lethal drugs at

their disposal, meaning their success rate with intentional overdose is higher [76].

Alongside the access to these medications with known toxicities, physicians must focus on the risk of suicide in their individual patients. Alcohol and opioid use disorders significantly increase the risk for suicidal ideation, attempts, and deaths, with any substance use (including marijuana, cocaine, amphetamines) increasing the risk for suicide ideation [77]. Risk factors for suicide include job loss, divorce, mental illness, physical illness, chronic pain, stress, advanced age, and financial difficulties. Male and Caucasian patients also have a higher incidence of suicide attempts [78]. Prevention of overdose-related suicide, therefore, must be a multifaceted approach, focusing both on the suicidal ideation and the access to dangerous medications. Disposing properly of leftover drugs, locking up dangerous medications, and educating patients on which drugs might have dangerous interactions if in the hands of a person who has suicide risk factors can all help to reduce availability of drugs for suicide attempts. Design of drug packaging can also reduce risk, as blister packing will slow the ability of persons to open the lethal drugs, causing them to instead choose an easier to use drug (and often a less toxic drug) for the overdose in the moment [76].

17.6 Conclusion

Although adjusting drug dosing to individual patients' needs requires an understanding of complex topics such as pharmacokinetics, pharmacogenetics, and drug biochemistry, it provides an essential tool for effectively addressing patients' maladies. In addition, understanding how these factors differ between patients can prevent undesired toxicities, side effects, and overdose. As discussed in this chapter, a thorough understanding of the more common medications associated with differing pharmacokinetics and metabolism ensures therapeutic doses are prescribed accurately. As medicine becomes more personalized, these concepts enable the clinical utilization of prescriptions that are optimized to the patient and that also address comorbidities and social predeterminants of health.

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18 Models for Drug Individualization: Patient to Population Level

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Abstract

Multiple drug dosing models have been developed which try to address optimal drug therapy for the individual patient based on population data. Drug therapy individualization is based on many characteristics, such as ethnicity, age, gender, pregnancy, and renal function, all of which require a clinician's understanding of each factor. Drug dosing models such as pharmacokinetic/pharmacodynamic (PK/PD) and physiologically based pharmacokinetic modeling (PBPK) have been developed in order to gradually replace large and expensive clinical studies. By understanding how pharmacotherapy differs between individuals and populations, clinicians can begin to optimize drug therapy, taking the next step forward into personalized medicine which will optimize drug efficacy and safety.

Keywords

Therapeutic drug monitoring · Drug individualization · Pharmacogenetics · Pharmacokinetics/pharmacodynamics modeling · Physiologically based pharmacokinetic modeling

18.1 Historical Perspectives on Therapy Individualization

Drug therapy individualization is as old as medicine itself. Hippocrates himself purported the importance of understanding a patient's health and mind and how their individuality is influenced by the social and natural environment [1]. The Hippocratic tradition was, and still is, firmly rooted in exploring how psychological factors, mind, body, spirit, and social and natural environments influence one's health [1]. *Therapy individualization* is the process of adjusting dosage, medication type, drug packaging, drug delivery methods, and other aspects of medications to the patient's individual physical, emotional, and social needs. Individualization of drug therapy is multifaceted and must encompass the complexi-

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ties of medicinal prescription as well as social and personal norms of the patient. Individualization involves integrating pharmacology, physiology, and psychology, all of which can differ between different patients and patient populations. In addition, patients with a wide variety of comorbidities such as dementia, cerebral palsy, gastro-esophageal reflux, and even a history of myocardial infarction may encounter difficulties with taking medications on schedule, adjusting to the route of administration of that drug, following instructions on how to take those drugs, and avoiding drugs that will worsen their ongoing medical complaints.

At its core, therapy individualization is the backbone of prescribing medications and other treatments responsibly and successfully. As such, personalized medicine is rooted in a strong historical tradition. As early as 2600 B.C., the Egyptian physician Papyrus Ebers described how to treat individual patients for asthma, cancer, and a wide range of other maladies [2]. Testing the efficacy of individualized therapy appeared within the Bible's parable of Daniel testing Nebuchadnezzar's diet and adjusting it based on the king's responses [3]. In the eighteenth and nineteenth centuries, apothecary shops formed, mimicking modern-day pharmacies and bringing with them principles of changing prescription practices based on the patient's individual needs [2]. Physicians of the time would adjust medications based on their patient's "humors," participating in bloodletting and other practices based on their presentation and history. One great leap in medicine individualization came at the turn of the twenty-first century with the completion of the human genome project. Knowing the genetic code provided a basis from which to explore why certain drugs affected certain patients differently and therefore opened the door for true one-on-one prescription practices in medicine, tailored to a person's complete genetic profile [3].

In practice, drug individualization is also part of the stewardship of medications for future generations to use. *Resistance* is the process by which pathogens, tumors, and disease processes such as autoimmunity over time acclimate to a particular therapy and no longer respond as well or at all to that therapy. An all too harrowing lesson in drug resistance is seen with the history of antibiotic resistance to penicillin, as over time overprescription and inter-bacterial transfer of resistance genes against the antibiotic have led to significantly decreased utility of penicillin, the once so-called miracle drug. To address this resistance, antibacterial, antiviral, and antiprotozoal drugs require individualization based on the *minimum inhibitory concentration* (MIC) of that drug necessary to eliminate the pathogen of interest.

Utilization of a patient's history is also extremely helpful for individualizing prescription practices; patients may have a family or personal history of sensitivity to therapies. Adjusting to these factors can help ensure the drug is more effective and less dangerous to the patient. Side effects of medications change prescription practices; in HAART (highly active anti-retroviral therapy, a combination of anti-retroviral drugs with varying mechanisms) treatment of HIV, medications are often chosen based on the tolerability of side effects. While one patient may not be concerned about hair loss, another patient might prefer drugs that have more severe symptoms (such as nausea) in exchange for preventing that hair loss.

Within the patient history, drug individualization further requires an encompassing understanding of the patient's socioeconomic status. Patients may be at an increased risk of nonadherence for a drug based on their access to medical care and personal history, as well as the socioeconomic burden of that drug. Among patients with cardiovascular risk, higher education, greater income, less financial strain, and being employed were associated with better self-rated health, while financial strain was associated with poorer medication adherence [4]. For these circumstances, the prescribing clinician must adjust accordingly and consider other routes of prescription.

Due to the diverse implications of drug individualization, multiple models have been developed, addressing the different ways individuals may be affected by therapy from the individual level up to the population level. Drug therapy

requires individualization based on race, ethnicity, age, gender, pregnancy state, religious beliefs, and many more aspects, all of which require a physician's understanding of the patient as a whole. To aid this understanding, PK/PD, PBPK, and other diverse models have been developed in order to gradually replace in vivo studies with less ethically challenging systems of understanding drug utilization in the body. This chapter explores drug individualization from a patient level to the broader level of population-based modeling. By understanding how therapy differs between individuals and populations, clinicians can begin to optimize that therapy, taking the next step forward into the future of personalized medicine.

18.2 Patient Populations and Drug Individualization

When a patient presents to a provider, it is imperative to remember that they are influenced by broader factors that can directly influence their appropriate pharmacological treatment. Each individual may identify as being a part of many different patient populations, including their race and ethnicity, age, gender, pregnancy state, and more, that can impact the appropriate pharmacological strategy. Further, patients have unique comorbidities and manifestations of their diseases, as well as individual differences in the metabolism of drugs. It is the challenge of the clinician to combine the broader, biopsychosocial patient population influences with the unique physiology and desires of the patient sitting in front of them to create the most appropriate, individualized, and effective treatment strategy for each patient [5]. The following paragraphs discuss the influence patient population characteristics can have on treatment strategy.

18.2.1 Race and Ethnicity

Traditionally, race has been a relevant variable in the treatment algorithm of a variety of disorders. Individuals who self-identify as being of African-American descent can be low renin producers [6]. Renin is an important contributor to the renin-angiotensin-aldosterone system (RAAS). This system is designed to elevate blood pressure through regulation of blood volume and systemic vascular resistance. Due to low blood pressure, lower sodium concentrations detected in the distal convoluted tubule of the nephron, or beta-adrenergic receptor activation, the juxtaglomerular cells in the kidney are activated, leading to the cleavage of prorenin to active renin. While in the blood, renin cleaves angiotensinogen to angiotensin 1. Then, the enzyme angiotensin-converting enzyme (ACE) is responsible for converting inactive angiotensin 1 into active angiotensin II. Angiotensin II then acts on the kidneys, adrenal cortex, systemic arterioles, and the brain with the primary goal of increasing sodium and water to increase blood pressure [7]. ACE inhibitors or angiotensin II blockers (ARBs) are generally indicated as first-line treatment for hypertension and work by preventing the activity of this RAAS. However, patients who self-identify as being of African-American descent have been shown to be lower renin producers and, therefore, have lower activation of the RAAS pathway. Because of this, individuals who self-identify as being of African-American descent have negative feedback on their RAAS. Utilizing an ACE inhibitor or an ARB in a patient who self-identifies as being of African-American descent can lead to a reduced blood pressure response or even resistance to the effects of the medication [6]. Therefore, thiazide diuretics, such as chlorthalidone, or calcium channel blockers, such as amlodipine, are instead recommended as first-line monotherapy for this patient population [8].

Hepatitis C is another chronic disease in which incidence and treatment have traditionally been impacted by race. Unfortunately, those who self-identify as African-American are significantly more likely to suffer from this chronic disease. Despite only being 13% of the US population, 23% of HCV cases in the United States were in African-American individuals [9]. Further, race has been classically thought to influence differing immune responses and, therefore, differing

responses to pharmacological treatment. Specifically, those of African-American descent were found to have a higher prevalence of the non-CC interleukin IL28B genotype, which led to a decrease in effectiveness of pegylated interferon treatment. Thankfully, the newer treatments for hepatitis C were more effective in the African-American population than interferon treatment, but the disparity is still present. Researchers have found that patients who identify as African-American had a higher rate of relapse when treated with sofosbuvir/ledipasvir compared to those who did not identify as African-American. Therefore, race can be an important consideration in the treatment of hepatitis C [10].

The treatment of asthma is also classically associated with race and ethnicity differences. Unfortunately, there are also disparities in the prevalence of asthma, with the incidence being higher in those from those from African-American and Puerto Rican descent compared to those from European descent. Pharmacological treatment effectiveness also differs between patient populations. Short-acting β-agonists (SABAs) are commonly utilized in the treatment of asthma, working by activating the β2-adrenergic receptor in the respiratory tract. Through cAMP preventing intracellular calcium release and decreasing extracellular calcium entry, administration of SABAs leads to the overall effect of bronchodilation and relaxation of the airways, which can help attenuate an asthma attack. However, they were found to be less effective in African-American children and those of Puerto Rican descent [11]. Further, an inhaled corticosteroid in conjunction with a short-acting bronchodilator is often recommended for asthma. When looking at impact on children suffering from asthma, researchers found that this combination led to an increased bronchodilator response only among those who identified as Mexican American and not those who identified as African-American or Puerto Rican [12]. Therefore, when a patient presents with asthma, a clinician should consider how their race and ethnicity could influence their treatment.

It is important to emphasize that race has no biological definition, and therefore, the scientific community is moving away from including race as a prominent factor in clinical situations. In many instances, the factor of race fails to recognize social and environmental factors that also influence outcomes [13]. In addition, it must be noted that there are many differences between individuals of the same race. An example of this is showcased through allopurinol-induced severe cutaneous adverse reactions (SCARS), which have been shown to potentially be associated with the HLA-B*5801 allele. Since research has demonstrated that the allele prevalence varies among those of different races and ethnicities, the American College of Rheumatology recommends that those of Southeast Asian and African-American descent should be tested for this allele. However, this fails to consider the influence of geography within racial groups. In Switzerland, a country that is considered smaller and more homogenous, researchers found variability across the country. Residents in the city of Basel had a higher frequency of this allele than the entire US African-American population, demonstrating that within a relatively homogenous ethnic population, there was incredible variability based on geography. Further, it is recommended that those of Southeast Asian descent are screened. However, Japan, a country in Asia, has been shown to have an even more decreased prevalence of the HLA-B*5801 variation than Caucasian individuals from the United States [14]. It is evident that race is not the only factor a clinician must consider when prescribing pharmaceuticals, and clinicians must be sure to not generalize individuals into categories based solely on their race or ethnicity.

18.2.2 Age

When prescribing medications, it is important to keep in mind that drug regimens, dosages, and pharmacokinetics can differ based on the age of the patient. Therefore, to practice individualized medicine, a clinician must often adjust the dosage and even sometimes the medication recom-

mended for the age of the particular patient being treated. From birth to middle age to elderly adults, there can be significant differences in drug pharmacokinetics that are crucial for a physician to consider. When considering a drug with poor solubility or one that is slowly released over time, a clinician must consider drug absorption in the patient. Younger children have been shown to have decreased intestinal transit time, which can decrease drug absorption of drugs such as theophylline. For newborns, a clinician must consider the impact of gastric pH. At birth, it is thought that gastric pH is neutral, and then there is a decrease to acidic pH typically characteristic of gastric pH. For these newborn babies, this neutral pH can lead to higher concentrations of acid-labile drugs, such as penicillin, and lower concentrations of weakly basic drugs, such as itraconazole. Further, there are differences in drug distribution between children and adults based on their body composition. Infants tend to have higher levels of fat, and therefore lipophilic drugs like diazepam have a larger volume of distribution in these patients than in older children and adults. Further, extracellular water tends to decrease during development. Therefore, infants have a higher volume of distribution of water-soluble drugs and must be given higher doses of water-soluble drugs [15].

Drug metabolism can also differ based on age. The protein content within the liver changes throughout life, increasing from birth to a maximum in a 30-year-old adult, which can lead to a lower first-pass effect. However, hepatic clearance and blood flow through the liver are higher in children compared to adults, which can lead to a greater first-pass effect [16]. Therefore, it is important to look at the mechanism of each individual drug to properly individualize drug regimens for each stage of life [15].

Chloramphenicol, a broad-spectrum antibiotic, is an example of a drug in which understanding the lower enzyme concentrations in neonates is crucial. Chloramphenicol is typically metabolized by glucuronidation through the UDP-glucuronyltransferase enzyme and then renally excreted. Due to lower concentrations of this enzyme in the liver, premature neonates are at risk for toxicity due to the buildup of chloramphenicol. This can lead to the infamous "gray baby syndrome," which can lead to complications such as gray skin, abdominal distention, and respiratory and cardiovascular collapse [17]. Further, the composition of the intestinal bacteria is also believed to differ with age. This becomes clinically relevant with digoxin, a drug with a very narrow therapeutic index. Therefore, the correct dosing of digoxin is very important in order to avoid toxicity. As children age, the inactivation of digoxin leads to its excretion in the gut lumen to increase. Therefore, neonates require a higher loading dose than older patients of digoxin [15]. Neonatal dosing recommendations for several drugs are listed in Table 18.1.

Age is also an important consideration for drug metabolism when considering the elderly. The Beers Criteria for Potentially Inappropriate Medication Use in Older Adults was created by the American Geriatrics Society. The purpose of the Beers Criteria is to provide recommendations to clinicians in the formulation of an appropriate pharmacological plan for patients ages 65 years and older, as their age directly impacts their pharmacokinetics, side effects, and drug-related problems. As individuals age, the harm outweighs the benefit for many pharmacological interventions, and the Beers Criteria aims to mitigate this. An example of a drug recommended to be avoided in the elderly is that of chlorpropamide, a long-acting sulfonylurea. The half-life is extended in the elderly, which can lead to the adverse effects of SIADH and hypoglycemia [26]. It is essential to apply the Beers Criteria along with what would be best for the individual patient to truly practice drug individualization.

18.2.3 Sex

Sex, defined as the biological classification at birth due to anatomical and chromosomal differences, influences drug individualization. Even though the Institute of Medicine of the National Academy of Science advocated for the importance of analyzing sex differences in drug development, the FDA guidelines specifying that both

Table 18.1 Neonatal dosing recommendations for common drugs

Drug name	Indication	Dosing recommendations	Adverse effects
Ampicillin	Neonatal infections, especially Group B *Streptococcus* spp. and *Listeria* spp.	No specific dosing in neonates [18]	Rashes [19]
Cefotaxime	Sepsis, meningitis, 3rd-generation cephalosporin [19]	Lower dosage (4 mg/kg/day) in premature and full-term infants less than 1 week old; increase to 6–7.5 mg/kg/day at 1-week postnatal age [18]	Vomiting, diarrhea, rash [19]
Digoxin	Heart disease in children [19]	Increased dosing in younger children; decrease loading doses from 45 μg kg^{-1} in infants to 35 μg kg^{-1} in preschool children [15]	EKG changes, GI distress, CNS changes, arrhythmia [20]
Famotidine (Pepcid)	GERD (gastro-esophageal reflux) [21]	Utilize body size-based dosing [22]. A dosage of 0.5 mg/kg is typically followed [21]	Agitation, headache [21]
Furosemide	Fluid overload, management of PDA (patent ductus arteriosus), cardiac failure, pulmonary edema [19]	Decreased dosing in comparison to adults, as t1/2 of furosemide is greater in neonates [23]	Dehydration, hyponatremia, hypokalemia, rash, ototoxicity, nephrotoxicity, nephrocalcinosis [19]
Gentamicin	Gram-negative sepsis due to *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae,* and *Enterobacter* spp. [18]	Lower dosage (5 mg/kg/day) in premature and full-term infants less than 1 week old; increase to 7.5 mg/kg/day at 1-week postnatal age [18]	Hearing loss, reduced renal function and renal failure [19]
Ibuprofen	Closure of PDA, pain, fever [19]	In infants <6 months of age, ibuprofen should be prescribed based on body weight at 5–10 mg/kg [24]	Reduced urine output, platelet dysfunction [19]
Metronidazole	Necrotizing enterocolitis, anaerobic infections [19]	No specific dosing in neonates [18]	Gastrointestinal disturbances, peripheral neuropathy, neutropenia [19]
Phenytoin	Neonatal seizures unresponsive to phenobarbital [19]	10–20 mg/kg/day, greater than doses for adults [25]	Irritation at injection site, gastrointestinal distress, hypotension, coma, respiratory depression [19]

Adapted from Refs. [15, 18, 19, 21–25]

sexes be included in clinical trials, and the requirement to label drugs if there are differences noted between male and female patients, there is still a gap in knowledge about the differences in male and female pharmacology due partly to lack of inclusion of female patients in clinical trials. Pharmacokinetics and pharmacodynamics differ between men and women, influencing recommended drug individualization strategies for these patients [27]. The enzyme CYP3A4 is a crucial component of the cytochrome P450 family, which is essential for first-pass metabolism through the liver. In females, CYP3A4 expression and metabolic rate are thought to be significantly greater than in males. Therefore, drugs dependent on CYP3A4 can have a higher clearance in women than men [28]. Further, women have decreased concentrations of the MDR1 product, hepatic P-glycoprotein, in comparison to men. Verapamil, a non-dihydropyridine calcium channel blocker, is a substrate for P-glycoprotein and is metabolized by CYP3A4. Females can have a shorter half-life and mean residence time for intravenous verapamil compared to males, which is clinically relevant when considering the dosage [29]. Anatomical differ-

ences between males and females also can impact drug individualization. Women have higher levels of body fat compared to males, which is important when considering lipid-soluble drugs. Vecuronium, a skeletal muscle relaxant, was found to have a longer duration of action in women than men, and it is hypothesized to occur because of their higher fat content [27].

The physiology of women at different stages of life significantly influences how drugs affect these patients. Exogenous hormones are commonly prescribed in the form of oral contraceptives. Through competitive inhibition and/or estradiol downregulating expression, oral contraceptives lead to decreased concentrations of enzymes in the cytochrome P450 family [30]. Tizanidine, an α2-adrenergic agonist with significant first-pass metabolism, can have increased plasma concentrations in patients taking oral contraceptives. This is believed to occur through inhibition of CYP1A2 presystematic metabolism, and researchers found that it led to decreased systolic and diastolic blood pressures when compared to those not taking oral contraceptives. However, differences exist between women taking oral contraceptives containing gestodene and ethinyl estradiol, with some patients having greater reductions in blood pressure than others. This emphasizes the need to look at each aspect of individual patients, and not to generalize patients into one group [31].

Pregnancy leads to numerous changes that impact drug regimens. For one, some medications can be teratogenic and must be avoided in pregnant women to avoid adverse effects on the baby. An example of this is isotretinoin, a derivative of vitamin A utilized to treat severe acne. Isotretinoin is so teratogenic that two forms of birth control are recommended before prescribing it in women. It can lead to significant neurocognitive deficits and congenital deficits that can significantly harm the fetus [32]. Further, pregnancy leads to a number of physiological changes that impact drug pharmacokinetics. Pregnancy changes the composition of the body, leading to increased total body water, extracellular fluid, and plasma volume. Further, there is an increase in cardiac output and GFR. Pregnancy also tends to lead to an increase in the expression of certain hepatic enzymes, like CYP2D6. The addition of a placenta and fetus mean additional components are participating in metabolizing medications [33]. An example of a medication with known complications during pregnancy is lithium which is used to treat bipolar disorder. This medication is excreted primarily renally. In pregnancy, GFR and renal excretion increase, so lithium blood concentrations decrease. This can be dangerous, as it can lead to sub-therapeutic concentrations in pregnant women suffering from bipolar disorder [34]. Lithium has been described as a teratogen, with the potential to lead to cardiac concerns and increased weight in neonates [35]. Table 18.2 lists several drugs which can be teratogenic and/or toxic during pregnancy.

18.2.4 Other Considerations

As the past section demonstrated, it is crucial to consider the different patient populations and biophysical factors that influence patient health when considering drug individualization. However, it is essential to not broadly group patients into one category and forget that there are so many factors that go into the unique identity of each patient. If an African-American middle-aged pregnant woman presents to the clinic for blood pressure medication, the clinician must consider the implications of her sex, race, age, pregnancy state, and comorbid conditions on her drug regimens. The process of creating individualized pharmacological treatment strategies is difficult, but it is absolutely crucial so that each patient can have the most effective and safe care possible.

18.3 Models for Drug Individualization

18.3.1 Current Practices for Drug-Resistant Problems

Most simply put, drug dose individualization hinges on deciding upon the most efficient pharmacotherapy which both treats the disease to the

best of the clinician's ability, maximizing therapeutic effect while minimizing the risk of adverse events or side effects. A hugely prominent field in which pharmacotherapy individualization has immediate and tangible benefits is in the prescribing of antimicrobials.

With antimicrobial agents, the shift towards an individualized, efficient regimen is best achieved by expeditiously moving from empiric therapy to definitive therapy. One serious concern alleviated by moving patients to definitive therapy is apprehension regarding proliferation of antibiotic-resistant organisms. The current empiric therapy for acute uncomplicated cystitis is trimethoprim-sulfamethoxazole (TMP-SMX) as first-line treatment; however, resistance to TMP-SMX among uropathogens has been on the rise since the early 1990s [38]. This begs the question, at what point does resistance become too widespread, at what point do the empiric recommendations change? Research by Hooton et al. showed that there are already more and more physicians moving to fluoroquinolones as first-line empiric therapy despite the 95% clinical cure rate shown with TMP-SMX, a shift that may eventually encourage resistance to these essential antimicrobials.

It would be remiss to discuss antimicrobial therapy individualization without mentioning the challenges in that pursuit; namely, microbial data is often not available for 48–72 hours, and many cases are not even indicated for culture. However, there are clinically applicable suggestions allowing clinicians to move away from a one-size-fits-all empirical formula towards what David Paterson calls in his 2008 study on antibiotic resistance in Gram-negative bacilli the "individualizing initial empirical therapy" [39]. One important and often overlooked tool is surveillance programs; essentially, clinicians have the data to determine if they should deviate from the generically recommended first-line empiric drug

Table 18.2 Examples of teratogenic/toxic medications during pregnancy

Medication name	Medication type	Effects
Chloramphenicol	Antibiotic	Gray baby syndrome, bone marrow suppression [36]
Coumarin derivatives (e.g., warfarin)	Anticoagulant	Skeletal abnormalities, nasal hypoplasia, CNS malformations, intracranial hemorrhage in the fetus [36]
Diethylstilbestrol (DES)	Estrogen derivative for miscarriage prevention	Development of clear cell adenocarcinoma of the vagina and cervix in mothers; genital tract abnormalities in infants [36]
Glucocorticoids, e.g., prednisone	Anti-inflammatory, anti-autoimmunity steroids	Cleft palate [37]
Lisinopril, captopril	Angiotensin-converting enzyme inhibitor	Fetal hypotension, fetal kidney hypoperfusion, pulmonary hypoplasia, anuria [37]
Lithium	Treatment for bipolar disorder; mood stabilizer	Cardiac abnormalities, increased birth weight [35]
Losartan, valsartan	Angiotensin receptor blocker (ARB)	Neonatal oliguria, anuria, hypotension, renal tubular dysgenesis [37]
Misoprostol	Prostaglandin E_1 analog	Vascular disruptions (Moebius syndrome, terminal limb defects), induces abortions during the first and second trimesters. Safe during delivery to induce labor [37]
Quinolones and fluoroquinolones	Antimicrobials	Renal, cardiac, and CNS toxicity; organ agenesis and carcinogenesis in fetuses, articular cartilage damage [36]
Streptomycin	Antibiotics	Ototoxic, can damage CN VIII, leading to deafness in newborns
Tetracyclines	Antimicrobials	Liver necrosis, bone and teeth defects, suppression of skeletal bone growth and hypoplasia of tooth enamel [36]
Valproate/valproic acid	Antiepileptic	Cognitive defects, neural tube defects, cardiac abnormalities, fetal valproate syndrome [36]
Vitamin A (retinal/isotretinoin)	Vitamin supplement	Neural tube defects (exencephaly, spina bifida, hydrocephalus), thymic and cardiovascular abnormalities [36]

Adapted from Refs. [36, 37]

if resistance is suspected in their area. One example noted by Neuhauser et al. is rising levels of fluoroquinolone resistance among *Pseudomonas aeruginosa* [40]. As such, if fluoroquinolone resistance is demonstrated locally, greater pharmacotherapy individualization can be achieved by re-evaluating local empiric treatment. Paterson also notes that a clinical knowledge regarding the infection's port of entry can give hints guiding individualization of empiric treatment. In his research on Gram-negatives and antibiotic resistance, he delineates that regimens targeting Gram-negative infections must assess whether treating *Pseudomonas aeruginosa* is necessary [39]. Where clinical knowledge comes onto the field is knowing that *Pseudomonas aeruginosa* infection most commonly presents through ventilator-associated pneumonia and neutropenic fever and often in undifferentiated fever in the critically ill. All of these allow for individualizing empiric therapy before having knowledge directing to definitive therapy. Figure 18.1 summarizes stages in addressing microbial drug resistance.

18.3.2 Physiologically Based Pharmacokinetic (PBPK) Modeling

Pivotal in the development of drug individualization models was the advent of physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) modeling. This synthetic representation of drug performance relies on drug mechanism data combined with organ system physiology in a single organism to develop propositional concentrations over time within the respective compartments of an organ or tissue [41]. This is essential to drug therapy individualization because it can account for known differences in physiology not necessarily seen in drug trials, such as pregnancy, age, concurrent drugs, or genetic polymorphisms altering physiology or a combination of the above – all of which will be discussed in this chapter.

Due to fetal sensitivity and altered maternal physiology, pregnancy is not an ideal time to introduce pharmacological intervention and even less of an ideal time to evaluate drug efficacy. As such, patients are often prescribed based on protocols derived from studies excluding pregnant patients, which may lead to dosing below therapeutic concentrations [42]. To examine the physiologic variables that must be accounted for during pregnancy, a study can be done that

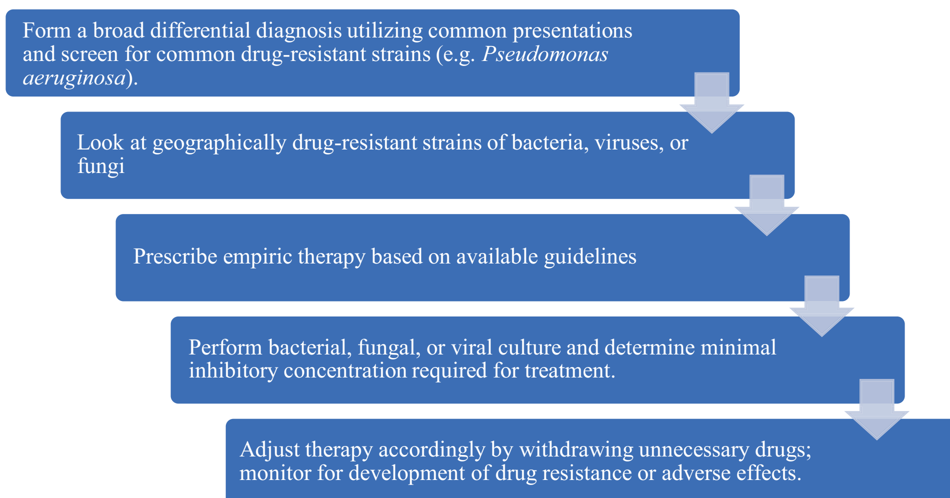

Fig. 18.1 Stages to addressing drug-resistant problems

devises a PBPK model for pregnant patients regarding the metabolism of a model compound bisphenol A (BPA). It alters endocrine function with pathological results, ranging from reproductive harm to increasing obesity risk to causing developmental delay [43]. As such, it is a compound of interest regarding maternal-fetal metabolism. BPA is also an excellent substance to make a maternal-fetal PBPK model after because there is already maternal BPA exposure, allowing for comparison between measured results in human subjects and the projected results based on a PBPK model. Ideally, this type of modeling could be used to devise dosing regimens for drugs whose metabolism is altered by pregnancy to better tailor dosing schemes. To begin individualizing drug dosing in pregnant patients based on a PBPK model, it is essential to know how the compound is metabolized in a healthy adult with no additional considerations. For the researchers that devised a maternal-fetal PBPK model for BPA, this meant modeling based on rapid hepatic and intestinal metabolism of BPA followed by excretion in the urine [43]. Adding the considerations for a pregnant patient meant accounting for transfer across the placenta, which was demonstrated by ex vivo studies to rapidly diffuse [44]. This was implemented by the researchers as a first-order kinetic following a simple diffusion process. Further concerns for a pregnant subject included in this model include changes in maternal plasma, hematocrit, fat, and amniotic fluid volume with further sub-compartments for the fetal liver, kidneys, plasma, and brain. These fetal sub-compartments are important to include because despite low exposure since some drugs may not diffuse or accumulate as readily, fetal metabolic pathways may not be adequately prepared to deal with them, regardless of concentrations in the fetal sub-compartments. One such example is gray baby syndrome in children whose UDP-glucuronyl transferase is not sufficiently developed to handle the toxicity of chloramphenicol. BPA is also metabolized by UDP-glucuronyl transferase [45].

The challenges in studying drug safety and efficacy in children are numerous. This is further compounded by a lack of interest on behalf of pharmaceutical companies, due to a combination of decreased finances in the pediatric drug market and difficulty recruiting children for these studies [46]. Consequently, dosing is often based on body size. As such, PBPK modeling to specify drug regimens in pediatric populations is a promising alternative. This was the approach of one team creating a PBPK model for theophylline and midazolam disposition from birth to adulthood. In order to build this model, the researchers relied on physiological data for neonates, 0.5-, 1-, 2-, 5-, 10-, and 15-year-old children. Additionally, volume and weight data were included, such as body weight, organ weight, extracellular fluid (ECF) and vascular volume, cardiac output, and glomerular filtration rate (GFR). All data was collected for both sexes. The goal was to determine the volume of distribution at steady state (Vdss), drug half-life (t1/2), and total and renal clearance of both drugs (CL & CL*R*) corresponding to age.

The results of the study correlated quite closely with the published data for the goal values the model predicted. Specifically, the Vdss of theophylline was reasonably close to the published values. However, there were no published values for term neonates; the Vdss for midazolam based on published results was more variable, but the Vdss predicted by the PBPK model was within the reported ranges for the ages studied. Since the organ volume/functionality and physiological data were collected to represent both sexes, the model was also able to predict a lower Vdss for theophylline and higher Vdss for midazolam in women as opposed to men, findings which are congruent with the literature [47, 48]. This was an excellent use of PBPK modeling because there was already existing literature against which to verify the results and test the validity of PBPK modeling.

The motivation behind studying drug-drug interactions (DDIs) is to determine potential reactions, from increasing potency through synergy to rendering one therapeutic ineffective. Potential DDIs are difficult to study in vivo in a human model, as there are differences in physiological environment between humans that can influence DDIs. As such, PBPK modeling is an

excellent alternative; with unparalleled safety due to the virtual study population, PBPK modeling can evaluate potential DDIs in the context of different genetic, demographic, or even ethnic populations. Accounting for interactions in all of the drugs currently prescribed in the context of all of these variables amounts to an implausible amount of in vivo studies. Therefore, the FDA states that data from PBPK modeling of potential DDIs can be used in lieu of prospective DDI studies in certain circumstances [49].

The goal of the 2019 study by Ji et al. was to study the DDIs between opioids and benzodiazepines. These two drug classes were selected because of the rise in opioid prescription rates in the past decade alongside the fact that benzodiazepines are rarely prescribed alone. This combination can be deadly due to their synergistic respiratory depression, but the exact mechanism was unknown. This is too dangerous to be studied in vivo in a human model. The study by Ji et al. was spurred by previous studies in animal models or in vitro studies that had attempted to evaluate pharmacokinetic interactions [50]. The research team chose to evaluate interactions between benzodiazepines, including alprazolam, diazepam, midazolam, and triazolam, and opioids, including fentanyl, oxycodone, and buprenorphine. Once the research team was able to make a working model for each individual drug, they evaluated their model against the literature for the area under the curve (AUC), Cmax, and tmax to verify their results. All of these were within acceptable limits except for the AUC and tmax of buprenorphine, which fell slightly below the lower reference value. Additionally, there was pharmacokinetic (PK) data available for fentanyl. In summation, the model was still deemed valid due to the model's projected data falling within the confidence interval of each drug's concentration-time curve. With a working model, the research team then used it to evaluate each of the drugs in combination. In an interesting prediction, the model showed no PK interactions between opioids and what the team deemed *normal* doses of benzodiazepines and only showed PK interactions when a normal dose of opioids was combined with a *severe* overdose of benzodiazepines. The observation was most true for fentanyl, which showed the greatest increase in AUC when combined with an overdose of benzodiazepines [50]. The team also found that the AUC of fentanyl was highest when combined with alprazolam, a prediction which explained why fentanyl and alprazolam made up the greatest proportion of drug-related deaths in which a benzodiazepine was combined with an opioid [51].

The last of the major uses for PBPK modeling is in determining potential drug outcomes based on genotype. This is very impactful in the realm of drug therapy individualization because genotypic variations in alleles responsible for drug metabolism can severely alter the necessary dosages. The goal of one study was to determine the pharmacokinetics of celecoxib in regard to genetic polymorphisms in CYP2C9, the major pathway of celecoxib metabolism, to reduce adverse events in celecoxib treatment. This study was carried out with the goal of determining how genetic differences impact drug dosing. This study conducted by Kim et al. first evaluated the metabolism of celecoxib in 39 individuals of known *CYP2C9*1/*1* or *CYP2C9*1/*3* genotypes. This data was then used to verify the data generated from a PBPK model. Once validated, the model was expanded to include *CYP2C9*1/*13* and *CYP2C9*3/*3* genotypes which could be validated based on data from other studies. Once completed, the PBPK model determined statistically significant differences in AUC0–48, AUCinf, Cmax, and CL/F (oral clearance) as confirmed by the group's in vivo pharmacokinetic study and the studies they found. The research team found a higher AUC0–48, AUCinf, and Cmax but lower CL/F in the *CYP2C9*1/*3* genotype [52] which paralleled their observations in their own pharmacokinetic study.

18.3.3 Pharmacogenetics

Just as a person's genes can impact the manifestations of a condition in an individual's body, genes can also play an essential role in the impact medications have on a person. Variations in the

genes could lead to differences in characteristics of a patient's conditions that could later impact their prognosis and how the drugs function in the body. Further, they can more directly impact how the drug works in the body, so it is crucial to understand exactly how it does so.

The patients' genes can affect the enzymes that break down and transport the drug, impacting how the drug leaves the body and the maintenance dose [53]. As a result of enzyme-substrate connections being extremely specific, the smallest change in the enzyme can cause a significant transformation in the function of the enzyme. If the drug is metabolized by the enzyme in a different manner than normal, it is essential to include that when figuring out the accurate dose for the patient to take. In terms of moving around the body, if the medication is not able to get to its intended target in the right amount of time or goes to the wrong place, the efficacy of the drug can be considerably altered.

A patient's genes can impact how that individual reacts to a particular drug, as genes can affect the proteins the drug comes into contact with [53]. Eventually, these interactions will cause a modification in whether more or less of the drug should be given at a time. Some examples of genes affecting drugs could be cytochrome genes like *CYP2C9* with irbesartan and celecoxib or *CYP2D6* with metoprolol [53]. In all of these examples, the genotypes affect how the drug leaves the body, which in turn can impact dosing [53].

Furthermore, genetics can be important in how drugs work with each other [53]. This can especially be of concern in patients with comorbidities and long-standing diseases for which they take many medications. For example, in the case of hypertension, a patient could be prescribed a combination of medications that work best together to help lower the patient's blood pressure, while being careful not to prescribe combinations that do not work as well. Drugs that do not interact well together can cause side effects and toxicities that can be avoided by understanding the individual pharmacogenetics of the patient. Even a seemingly small side effect continuing over a long period of time can be a significant concern to patients and decrease their overall quality of life. Disregarding this information can lead to dangerous effects on the patient. Table 18.3 lists common drug-metabolizing polymorphisms.

18.4 Challenges to Individualization

Despite the advantages offered by individualization, many challenges still exist. The following section explains the specifics of each type of challenge faced by various modes of drug individualization and discusses some options to address them.

18.4.1 Body Mass and Body Surface Area Dosing

Drug dosage models are generally prescribed on a fixed dosage or based on readily obtained physical parameters of the patient, including but not limited to age and weight. Such individualization methods include approaches such as body mass dosage and body surface area dosage [56]. As their names suggest, body mass dosing is prescribed on a basis of patient weight, whereas body surface area dosage is prescribed on the estimated surface area of a patient calculated through their height and weight. To date, these are the most commonly utilized individualized drug dosing methods with significant impacts on the current practice of drug individualization and will thus be the focus of discussion in this subsection.

Dosing based on body mass comes with inherent issues as weight may not accurately represent a patient's status. Research in the 1940s first indicated that body mass dosing suffers gross inaccuracies, particularly among younger populations [57] with frequently reported cases of overexposing patients to the drug in question [58]. To try and address the issues arising from such a unidimensional individualization approach, body surface area was proposed as an alternative [59] to incorporate additional factors such as height and

Table 18.3 Some of the common enzymatic genetic polymorphisms that affect common drugs

Enzyme	Important polymorphic variations	Drugs affected	Effects
N-Acetyltransferase	*NAT1* and *NAT2* (A and B)	Caffeine, isoniazid, nitrazepam, sulfonamides	Fast acetylators have only low concentrations of active drug; slow acetylators have higher concentrations of active drug
CYP2D6	80 genotypic variants, with autosomal recessive alleles corresponding to poor metabolizers. *CYP2D6*2×n CYP2D6*4, CYP2D6*5, CYP2D6*10, CYP2D6*17*	Over 70 drugs, including antidepressants, antiarrhythmic agents, beta-blockers, opioids, and neuroleptic agents	Extensive metabolizers (autosomal dominant wild-type gene) have increased breakdown of drugs; poor metabolizers have a 10- to 100-fold lower breakdown of drugs. Ultra-metabolizers have increased blood concentrations of morphine, as well as other opioid receptor agonists, resulting in greater risk for opioid-related adverse effects. In contrast, UMs require higher dosages of mirtazapine, a tetracyclic antidepressant, to achieve therapeutic effects
CYP2C9	*CYP2C9*2, CYP2C9*3*	NSAIDs, phenytoin, ARBs, sulfonylureas, warfarin	Increased warfarin activity in extensive metabolizers, leading to increased INR. Patients with *CYP2C9* as well as *VKORC1* have increased risk for major bleeding events
CYP2C8	*CYP2C8*3*	Arachidonic acid, paclitaxel, rosiglitazone, zopiclone	Inhibited by ketoconazole and gemfibrozil in extensive metabolizers
CYP2C19	*CYP2C19*2, CYP2C19*3, CYP2C19*17*	Diazepam, S-mephenytoin, moclobemide, PPIs, proguanil, SSRIs	Increased adverse drug reactions with utilization of prodrugs (drugs where the enzyme activates the drug); higher rates of eradication of *H. pylori* when utilizing triple therapy (PPIs + clarithromycin + amoxicillin). Carriers of the *CYP2C19*7* allele have improved effect with clopidogrel treatment, while *CYP2C19*2 and CYP2C19*3* carriers have reduced effects
CYP2B6	*CYP2B6*6*	Methadone	*CYP2B6*6* carriers have slower metabolic capacity, resulting in higher blood concentrations of methadone and efavirenz (a NNRTI for HIV treatment)
TPMT (thiopurine methyltransferase)	*TPMT*3A* *TPMT*3C*	Mercaptopurine, thioguanine azathioprine	In children with ALL, high TPMT activity causes a poor clinical response, while low TPMT is associated with severe to fatal myelosuppression
UGT1A1	*UGT1A1*28*	Irinotecan	*UGT1A1*28* carriers have increased risk of ADRs when treated for colon cancer with irinotecan

Adapted from Refs. [54, 55]

size of the patient. However, continued research suggests that dosing by body surface area frequently underexposes a patient to the prescribed drug [58]. Such data indicates both models display inaccuracies when extrapolated outside of the narrow patient population used in the original clinical tests to determine individualized drug dosing. This weakness of individualized drug dosing approaches becomes especially concerning when it comes to treating patient populations that may be prone to obesity or when a population's distribution density of BMI shifts over time, as clinicians may continue to use outdated dosing guidelines based on BMI ranges measured in a different era.

In light of the issues generated by the use of body mass or body surface area to estimate dosage, studies have assessed the benefits of incorporating additional patient parameters such as age and variations on body weight (ideal, adjusted, fat-free, and lean) into dosing estimations and comparing such individualized approaches against the usage of fixed dosages [56, 60]. Study results indicate that drug dosing may need to be adjusted based on differing parameters according to the drug in question, as different medications show ideal performance under different models. For example, for drugs such as hydrocortisone, vancomycin, linezolid, and aprotinin, weight-based dosing is recommended. For cyclosporine microemulsion, recombinant activated Factor VII, and epoetin α, fixed dosing shows superior results. For cases of atracurium and rocuronium, ideal body weight calculations are preferred. For intravenous busulfan and dalteparin, it has been found that age-based dosing is most appropriate [56]. Certain patterns that begin to emerge suggest that lean body mass may be a good predictor of kidney clearance [61], and total body weight is a better metric to use for lipophilic drugs [58]. Such data indicates that individualized drug dosing may need to be approached on a drug-based basis as clinical researchers come to understand the underlying mechanisms as to why certain parameters work better for dosing than others. However, all methods are ultimately approximations of patient individuality based on clinicians' assumptions towards what factors contribute most to drug toxicity and response. Therefore, all approaches suffer from the central issue stemming from unique or unexpected patient response due to special metabolisms or changing statuses. Dosing equations extrapolate poorly in pediatric situations [56] in the case of abacavir or acyclovir [62] and for pregnant women in the case of tinzaparin, whose manufacturer suggested weight-adjusted doses were not effective at anticoagulating most pregnant women against venous thromboembolisms [63]. Additionally, in special circumstances, factors such as kidney and liver functionality can also become as relevant to the dosage. Overall, due to varying success in application, not all clinicians will agree on the best model for each drug, and significant debate still exists across many clinical situations for the appropriate selection of individualized drug dosing approach [58].

A key advantage of body mass/body surface area dosing is its relative ease of use and wide applicability; however, it fails to accurately represent the unique variations of each patient's body and how they individually react to drugs. The inclusion of the extremes of weight subsets of the population into clinical drug testing [56] can build more accurate models for drug response outside of average weight ranges. This inclusion of diverse subsets of the populations can be applied for all "special populations" such as pediatrics, pregnant women, and special conditions relevant to the drug metabolism. Though ideal, this proposed inclusion of population subsets could provide significant challenge in the current process of bringing drugs to market: the necessity of recruiting adequate number (N) of patients to rule out the variations in personal response and testing drugs on sensitive populations such as those with extreme comorbidities, pregnant women, or pediatric populations. Such alternative chart creation is further complicated when more than one significant factor is present on the same patient. This is best illustrated by the decades-long debate between clinicians on how best to dose pediatric patients who are also obese [64–66].

A particular concern in generating individualization approaches is the standardization of methods used to make the measurements that establish body mass or body surface area parameters for the patient. Equipment differences, such as using manual scale in the clinic versus using weight-detecting gurneys, may create significant differences, especially in patients outside of average range. Though dosing based on measurable factors like body weight or body surface area is one of the least complex of the individualization models discussed, issues with standardization have resulted in numerous instances of non-compliance and non-standardization in real-life practice [56], such as in the vancomycin trial where the addition of electronic weight-based checking increased percentage in appropriate dosing in ED patients. Therefore, in the validation of individualization methods, it is not only important to ensure accurate prediction of dosage and drug response but is essential to ensure the basis of the methods is generated on reliable datasets.

18.4.2 PK/PD and PBPk Modeling

For drugs with extremely narrow therapeutic indexes, extreme care must be taken to dose patients accurately. Dosing strategies based on body mass and body surface area only apply to drugs that are affected significantly by weight with few additional limitations, such as significant interactions with other drugs. Thus, for drugs with highly specific therapeutic indexes, pharmacokinetic/pharmacodynamic models have been developed to provide more accurate dosing regimens.

PK/PD modeling seeks to mechanistically model the path across compartments (PK) and response (PD) to a drug. The data used to build PK/PD equations rely on administering the drug to a small group of participants from whom metabolites and drug concentrations are sampled to determine drug elimination rates [67, 68]. Although this method takes drug mechanisms and metabolism into consideration, ethical considerations exclude populations like children and pregnant women from initial testing. Such exclusions of sensitive populations may affect accurate prediction of drug dosage in relevant populations later in clinical settings. The use of indirect testing with population pharmacokinetics, allometric scaling, and in silico modeling to estimate the applicable dose to pediatric populations [67] has been proposed to circumvent this issue. Statistical tools such as population pharmacokinetics (PopPK) modeling analyze drug concentration variation in the wider population through meta-analysis of established clinical data. The inclusion of clinical data supplements the weaknesses of the PK/PD patient testing models; however, it requires long-term usage and detailed metabolite data collection among all patients receiving the drug over a long period of time. This exposes patients to subpar dosing regimens during the time enough data is generated to supplement the original model. Allometric scaling uses large populations of animal models to overcome the limitations of PopPK by replacing human patients with animal subjects. However, the high cost and limited equivalence of animal models to human patient response make such studies inferior to PopPK supplementation. In silico modeling utilizes computer models to replace in vitro experimentation. Though ideal, massive strides in computing and biological modeling must be made before such methods may be considered viable.

PK/PD modeling allows for rudimentary predictions of drug-drug interactions [69], mitigating the daunting task of creating dosing curves for all possible drug combinations. However, because PK/PD modeling relies heavily on a mechanistic understanding of drug metabolites, it cannot predict dosages and interactions with drugs whose mechanisms are unknown or unclear. The accuracy of PK/PD interaction modeling is dependent on understanding of metabolites including intermediates and their distribution in the body, as well as all receptors that interact with the drugs and their distribution across different compartments. Therefore, PK/PD in the present day suffers for being unable to account for interactions with common medications with unknown mechanisms, such as ketamine, lith-

ium, and general anesthetics [70–72]. These challenges are gradually being overcome as in vitro and in vivo experimentations using small to large models aim to elucidate drug actions at all levels of biology [73, 74].

Standardization of data collection also contributes to the complexity of PK/PD modeling and is vital to creating accurate and reliable mechanistic profiles of drugs. Recent PK/PD data of mass antiviral agent testing to combat the recent COVID-19 pandemic has highlighted many issues such as inconsistencies in methodology, lack of demographic data, or limitations in patient populations and controls due to co-administration of other agents [75]. Supplemental tools to PK/PD modeling such as PopPK rely heavily on accumulation of clinical data, making standardization of data collection and faithful reporting indubitably essential to actively improving PK/PD model accuracy and clinical relevance.

PK/PD modeling is highly specific and therefore may have issues with real-world complications when it comes to dosing in the clinical setting. The original model is unable to account for physiological realities such as changes in blood flow, which affects concentration of the drug to different compartments. Additional models such as physiologically based pharmacokinetic model (PBPK) are in development to provide a more granular version of PK/PD to account for additional factors and nested compartments that can predict drug flow across organelles. However, such methods may worsen the issues of complexity that already exist within PK/PD modeling, especially during stages of drug development where efficacy of a particular drug may not be certain [76]. Since PK/PD dosing calculations are so specific, real-world limitations of drug delivery can also influence patients' actual exposure to a drug. Variations in formulations of certain commercially available drugs, the need to compound drugs in house, or loss of formulation in IV or PICC lines can negate much of the work done in refining models to ensure adequate dosage.

18.4.3 TDM

Therapeutic drug monitoring (TDM) is the practice of monitoring concentrations or clinical responses of a drug (and/or its metabolites in the relevant compartments). This process is similar to the test done in a clinical trial for determining PK/PD models, but instead adjusts the patient's dose of the drug in real time to achieve the desired concentrations for optimal effect [77].

TDM circumvents many of the difficulties of other models: eliminating the need to consider drug loss during administration by focusing on final drug concentration and ignoring differences in patient metabolism by measuring drug concentration in the desired compartment (i.e., plasma). Clinical, biochemical, and plasma drug concentrations may all be valid options regarding the characteristics of the drug of interest. The difficulties in applying TDM successfully stem from deciding which measurement to use, as TDM was first developed based on blood pressure medications, where it is relatively easy to monitor and precisely titrate concentration of the drug over time to create the desired blood pressure. However, for other drugs, differences in patient response may not be due to differences in enzymatic concentrations or efficiencies in metabolizing the drug, but rather due to factors like differing receptors or secondary signaling, making the concentration of the drug irrelevant to the optimal therapeutic dose. This makes TDM less effective in cases where the physiological response is not as clear, immediate, and measurable as blood pressure.

The TDM requirement of measurement of drug concentration in relevant compartments may provide real-world difficulties, such as sampling from difficult to assay areas such as CSF, hepatic circulation, or intracellular concentration. Downstream difficulties in storage, transport, and consistent measurement in the laboratory provide further challenges in accurately measuring TDM. At this point in time, generalized methods of analysis include antibody-based and chromatography-based methods, which specialize in measuring protein-based and synthetic-based compounds, respectively. Further

assessment of drug interactions using TDM is reliant on understanding of the drug's PK and PD, such that that effective use of TDM must be based on previous work with PK/PD models. Therefore, TDM may serve to supplement rather than supplant PK/PD modeling, given its need of stringent patient monitoring requires personnel and clinical environments that can support the testing regimens.

18.5 Conclusion

Despite these growing areas of study and tools for pharmacotherapy, the practice of personalizing therapy is often left in the hands of the prescribing physician and clinical team. Existing data on the drug profiles of many medications is based on randomized control trials, which will use the same dosage across multiple people from differing backgrounds. Although this is helpful for blanket prescription practices, physicians are often left to fine-tune the medications to the patient's individual needs through adjusting dosage, delivery, or personal preferences of the patient for one drug over another [2]. Therapy individualization must encompass the patient's individual physical characteristics, such as age, weight, gender, or ethnicity; their medical history, including drug history, history of renal or hepatic failure, and comorbidities; genetic polymorphisms of the patient; family history; and sociocultural understandings. By having a core understanding of these factors, physicians can adjust to changing knowledge of drug prescription practices and most importantly prescribe medications not based on a formula, but rather on the individual needs of the patient.

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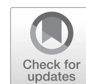

Toxicity Evaluation of Nanomedicine

19

Archna Panghal and Swaran Jeet Singh Flora

Abstract

Nanomedicine is the application of nanotechnology to achieve innovation in healthcare. It uses the properties developed by a material at its nanometric scale 10^{-9} m which often differ in terms of physics, chemistry, or biology from the same material at a bigger scale. Nanomedicine is understood to be a key enabling instrument for personalized, targeted and regenerative medicine by delivering the next level of new drugs, treatments and implantable devices to clinicians and patients, for real breakthroughs in medicine. Several areas of medical care are already benefiting from the advantages that nanotechnology can offer. Researchers are still fighting against numerous serious and complex illnesses like cancer, cardiovascular diseases, multiple sclerosis, Parkinson's disease and infectious diseases like human immunodeficiency virus (HIV). This chapter discusses the mechanisms and strategies for improving the safety of nanoparticles including important considerations for nanoparticle safety assessment.

Despite success of nanomedicines in biomedical sciences, there are several shortcomings/concerns due to associated toxicity. The unknown biodistribution pattern and undefined fate in biological system also contribute to their miscellaneous actions on organs. The small size of nanoparticles allows them to breach the biological barriers such as blood-brain barrier (BBB), blood-testis barrier (BTB), or other barriers resulting in organ toxicity such as neurotoxicity, reproductive toxicity and other toxicities. Although size and shape are strong determinants of toxicity of nanomedicines, other factors such as chemical composition, charge and structure also should not be ignored as they influence the interaction of nanomedicine with biological targets. Although the knowledge regarding molecular mechanisms of nanomedicine-induced toxicity is still in its infancy, mechanisms such as oxidative stress, inflammation, apoptosis and deoxyribonucleic acid (DNA) damage are considered key players in toxicity. Although nanomedicine toxicity has caught the attention of researchers in the last decade, the current knowledge is inadequate and requires extensive future studies. In our opinion we need to assess risk potential of nanomedicine, limitations of experimental methods and precise characterization of nanomaterials.

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Keywords

Nanomedicine · Nanoparticle · Nanosafety · Nanotoxicity · Organ toxicity · Safety · Toxicity

19.1 Introduction

Pharmaceutical applications of nanotechnology have largely transformed healthcare system in the twenty-first century. It includes the development of nanomedicines which deal with the biological systems in dimensions even less than 100 nm by multidisciplinary approaches. The term nanomedicine has been interchangeably used with nanopharmaceuticals since its advent [1]. Nanomedicines are synthesized by involving nanomaterials which provide them edge over conventional therapeutics by modulating several properties such as solubility and surface to volume ratio. Generally, they are nanomaterials which have noteworthy applications in biomedical science such as diagnosis, monitoring and therapeutic implications for a particular condition by targeting a specific organ or tissue [2]. Nano-size, enhanced permeation rate, site-specific active as well as passive targeting, economic suitability and higher safety and efficacy have revolutionized use of nanomedicines in biomedical field [1, 3]. In future, the practice of nanomedicines in healthcare system will flourish due to their breakthrough success and as an additional revenue-generating system for pharmaceutical players. However, there are few factors which need to be considered to improve current generation of nanomedicines. These factors include inculcating properties to evade innate defence system of body, traceability within biological system, effective pharmacokinetic profile, biodegradability and reduced toxicity [4].

Despite worth mentioning success of nanomedicines in biomedical system, still there are several concerns due to associated toxicity owing to their specific physico-chemical and pharmacokinetic profile [5]. Further, the unknown biodistribution pattern and undefined fate in biological system also contribute to their miscellaneous actions on organs. The small size allows them to breach the biological barriers such as blood-brain barrier (BBB), blood-testis barrier (BTB), or other barriers resulting in organ toxicity such as neurotoxicity, reproductive toxicity and other toxicities [6]. Although size and shape are strong determinants of toxicity of nanomedicines, other factors such as chemical composition, charge and structure also should not be ignored as they influence the interaction of nanomedicine with biological targets [7, 8]. For instance, metal ions such as silver (Ag), titanium (Ti) and cadmium (Cd) present in nanomedicines are inherently toxic, whereas metals such as iron (Fe) and zinc (Zn) are biologically tolerable, but might induce toxicity at concentration beyond physiological levels [9, 10]. Although the knowledge regarding molecular mechanisms of nanomedicine-induced toxicity is still in its infancy, mechanisms such as oxidative stress, inflammation, apoptosis and DNA damage are considered key players involved in toxicity [11]. Genotoxicity and cytotoxicity have been frequently reported with metal oxide and carbon-based nanomedicine [12]. Polymer- and silica-based nanomedicines have been found to be relatively less toxic and more biocompatible; however, ROS formation is considered to be involved in toxicity to some extent [13]. Although nanomedicine toxicity has caught the attention of researchers in the last decade, the current knowledge is mere a drop in the ocean and thus more needs to be explored to understand the toxicity of nanomedicines in depth. One of the most probable reasons of lagging behind in assessing risk potential of nanomedicine might be limitations of experimental methods and precise characterization of nanomaterials.

Identifying the toxicity potential of nanomedicine at an early stage has become a key issue in their wide applications. The limitations of nanomedicines need to be overcome by effective monitoring and defining their fate in biological system to ensure safety [14]. Since physico-chemical profile determines the toxicity of nanomedicine, thorough characterization is indispensable to understand their interaction with cellular targets. Further, multidisciplinary studies need to be carried out in addition to implementation of

advanced testing procedures to explore the toxicities of nanomedicines. Validated testing procedures such as in vitro and in vivo shall be upheld for benefit-risk assessment of nanomedicines. Further, adequate data generation on ADME profile and acute and chronic toxicity via in vivo studies might prove beneficial in limiting the toxicity of nanomedicine [15]. Although OECD guidelines provide elaborated protocols which need to be followed to determine toxicity of nanomedicines on regular basis, yet they need to be refined to focus more on hazards and biosafety of nanomaterials [16]. Sensors (such as biosensors and chemical sensors)-based approach for real time toxicity assessment and monitoring of nanomedicines might prove highly advantageous over conventional approaches. Additionally, implication of more advanced techniques including proteomics, genomics and metabolomics might be of critical interest to understand the risk potential more precisely [17]. This chapter focuses on risk potential of nanomedicine along with major molecular pathways involved in their toxicity. Further, conventional and novel approaches implemented in detection of toxicity due to nanomedicines also have been summarized.

19.2 Breakthrough Success of Nanomedicine

Implication of nanotechnology in the field of pharmaceuticals has been a breakthrough step for development of several therapeutics of interest. The roadmap for the use of nanomedicines for mankind is depicted in Fig. 19.1. With the advent of nanotechnology, several nanomedicines based on dendrimers, micelles, liposomes, protein-drug conjugates, nanoparticles (NPs) and hydrogels have been developed [18]. The first landmark achievement was the approval of liposomal formulation of doxorubicin (Doxil) by USFDA in 1995. More than 50 nanomedicines are in clinical use currently (Table 19.1). These marketed medications are used in several medical conditions such as cancer, neurodegenerative disorders, anaesthesia, rheumatoid arthritis, cardiovascular disease and rare genetic disorders [19]. Clinically used nanoformulations significantly enhanced biological half-life, improved bioavailability and limited the adverse effects as compared to conventional medicines. The statistics shows that not only the approval rate of nanomedicine is increasing, but new generation of nanomedicines is also making their way to the clinic [18]. Besides conventional molecules, nanomedicines also have potential to deliver DNA, siRNA, miRNA and peptides-based moiety. The approval of first siRNA-encapsulating nanodrug Onpattro for transthyretin amyloidosis in 2018 provided opportunities for nucleic acid delivery [20]. One of the most noteworthy accomplishments has been the development of first polymer-based biodegradable nanoparticle competent of targeting multidrug-resistant microbes by acting on bacterial cell walls and membranes [21]. Further, the most thrilling progress appears to be the in vivo application of DNA nanorobots in oncology for shrinking the tumours by cutting blood supplies [22].

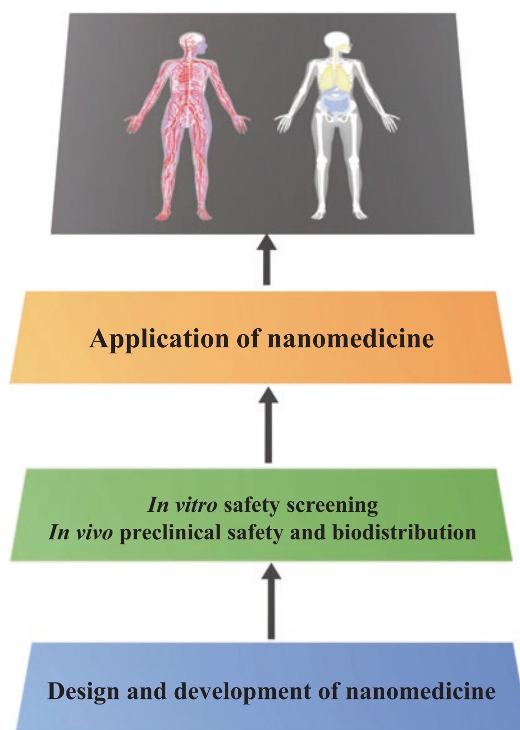

Fig. 19.1 A brief roadmap for nanomedicine-based application in humans

Table 19.1 FDA-approved nanomedicines [24, 25]

Drug name	Company	Applications/indications	Approval year
INFeD	Allergan	Iron-deficient anaemia	1992
Epaxal	Crucell, Berna Biotech	Hepatitis A	1993
Oncaspar	Servier Pharmaceuticals	Acute lymphoblastic leukaemia	1994
Doxil	Janssen	Kaposi sarcoma, ovarian cancer, multiple myeloma	1995
Abelcet	Sigma-Tau Pharmaceuticals	Fungal infections	1995
Amphotec	Ben Venue Laboratories Inc.	Fungal infections	1996
DaunoXome	Galen	Kaposi sarcoma	1996
Copaxone	Teva	Multiple sclerosis	1996
Dexferrum	American Regent	Iron-deficient anaemia	1996
AmBisome	Gilead Sciences	Fungal/protozoal infections	1997
Inflexal	Crucell, Berna Biotech	Influenza	1997
Ferrlecit	Sanofi	Iron deficiency in chronic kidney disease	1999
Curosurf	Chiesi USA	Respiratory distress syndrome	1999
DepoCyt	SkyePharma	Neoplastic meningitis	1999
Visudyne	Bausch and Lomb	Macular degeneration, myopia, ocular histoplasmosis	2000
Myocet	Elan Pharmaceuticals	Metastatic breast cancer	2000
Venofer	American Regent	Iron deficiency in chronic kidney disease	2000
Peglntron	Merck	Hepatitis C infection	2001
Neulasta	Amgen	Neutropenia	2002
Eligard	Tolmar	Prostate cancer	2002
Emend	Merck	Antiemetic	2003
Mepact	Takeda Pharmaceutical Ltd.	Non-metastatic osteosarcoma	2004
DepoDur	SkyPharma Inc.	Pain management	2004
Tricor	Lupin Atlantis	Hyperlipidaemia	2004
Abraxane	Celgene	Lung cancer, metastatic breast cancer, metastatic pancreatic cancer	2005
Focalin XR	Novartis	Psychostimulant	2005
Cimzia	UCB	Crohn’s disease, rheumatoid arthritis, ankylosing spondylitis	2008
Feraheme	AMAG	Iron deficiency in chronic kidney disease	2009
Exparel	Pacira Pharmaceuticals Inc.	Pain management	2011
Marqibo	Acrotech Biopharma	Acute lymphoblastic leukaemia	2012
Injectafer	American Regent	Iron deficiency anaemia	2013
Plegridy	Biogen	Multiple sclerosis	2014
Onivyde	Ipsen	Metastatic pancreatic cancer	2015
ADYNOVATE	Takeda	Haemophilia	2015
Vyxeos	Jazz Pharmaceuticals	Acute myeloid leukaemia	2017
Onpattro	Alnylam Pharmaceuticals	Transthyretin-mediated amyloidosis	2018

The impact of nanotechnology in the biomedical field is evident by the fact that more than 247 new clinical trials started in the beginning of 2018. Further, a quest for developing lipid-based nanomedicines of COVID-19 vaccines already started in May 2020 with the initiation of three clinical trials [23]. Taken together, landmark escalation in the entry of nanomedicines in the clinical trials followed by clinics is itself proof of breakthrough success in improving mankind health.

19.3 Benefit-Risk Assessment of Nanotherapeutics

Emergence of nanomedicine has provided new opportunities for improving the poor drug solubility and drug targeting. Along with the better safety and efficacy, nanoformulations also allow dose reduction which improves patient compliance and reduces the toxic effects owing to accumulation of high volume. Nanomedicines improve dissolution rate, solubility and intracellular uptake of drugs by increasing surface area. Multifunctional nanomedicines have high therapeutic index due to better efficacy of drug delivery and release at the specific site [26]. Nanoformulations have potential to deliver the drugs across physiological barriers such as BBB, blood-lung barrier and BTB by facilitating the active as well as passive transport across tight junctions [27, 28].

Besides the widespread use, knowledge on the safety and toxicity profile of many nanoformulations is still lacking. The most often reported adverse effect after injecting nanotherapeutics is hypersensitivity reaction which perhaps may be due to activation of the immune system [29]. Increased surface area improves solubility on one side, whereas it creates problems like interparticular friction, sticking and varied physical and chemical interactions on another side. Further, their small size contributes to high clearance rate from the body which restricts their use for therapeutic purposes. Another major downside of nanomedicines is that they do not have common properties other than their size; therefore, each nanomedicine has to be evaluated individually [30]. Nanoformulations elevate ROS level and oxidative stress which are major culprit factor for inducing several disorders such as metabolic, cardiovascular and neurological disorders [31]. Further, NPs have tendency to cause inflammatory responses and immune and systemic effects.

19.4 Toxicity Profile of Nanomedicine

In biomedical context, nanotoxicology deals with the negative impact of nanomedicines on biological system due to their unique surface modification. Route of administration is also a contributing factor in toxicity profile of nanomedicines. Increased endocytosis rate due to distinct physico-chemical properties of nanomedicines initiates inflammatory and pro-oxidant responses which further trigger the activation of different molecular pathways leading to toxicity at organ, tissue, cellular and molecular levels.

19.4.1 Hepatotoxicity

The liver has been recognized as a vital organ where >90% nanomaterials are accumulated from systemic circulation. Meanwhile, accumulated nanomaterials interact with the hepatic cells and, therefore, lead to the structural and functional perturbations in the liver. Kupffer cells are resident macrophages in the liver which elicit immune response; however, they are not efficient enough to prevent the nanomaterials which get access through open sinusoidal fenestrations [32, 33]. Recently, it was reported that cerium oxide (CeO_2) and titanium oxide (TiO_2) NPs injected into mice were detected into sinusoids and Kupffer cells up to 6 months post-exposure [34]. NPs may induce hepatic toxicity by activating the inflammatory signalling as evident by the elevated pro-inflammatory and reduced anti-inflammatory cytokine level in response to nickel oxide (NiO) NPs [35]. Serum parameters such as total bilirubin, alkaline phosphatase and aspartate aminotransferase serve as good biomarkers to identify toxicity potential of nanomedicines [35, 36]. NPs induce hepatic toxicity via different mechanisms depending upon their size, chemical composition and physical characteristics. For instance, Ag NPs manifest their hepatic toxicity by inducing necrosis and haemorrhage in hepatocytes and endothelial damage in portal vein [37]. TiO_2 NPs trigger infiltration of inflammatory cells, increase collagen deposition and induce fibrosis [38]. Gold (Au) nanorods elicit hepatic toxicity by activating hepatic macrophages which subsequently aggravate hepatitis and liver damage [39]. Poly-amidoamine (PAMAM) dendrimers have been reported to cause hepatic toxicity by inhibiting cell growth and inducing mitochondrial injury, apoptosis and Akt/mTOR-

mediated autophagy [40]. Although the role of inflammation, apoptosis and oxidative stress has been elucidated in nanomedicine-induced hepatic toxicity, in-depth understanding of metabolic changes in terms of energy, protein and lipid metabolism in in vivo and in vitro system is still lacking.

19.4.2 Reproductive and Developmental Toxicity

The available data on reproductive nanotoxicity is inadequate to make a conclusive remark on nanomedicine-associated risk potential to gonadal health. The scarcity of the research in this field can be understood by the fact that out of more than 60 countries having their own nanotechnology research programmes, just 29 countries have undertaken research activities in this domain [41]. The potential of nanostructures to breach the BTB has raised concern about harmful effects of nanotherapeutics on male reproductive system [42]. Metallic NPs made up of Ti, Ag, Zn and Au have been frequently reported to hamper normal reproductive functions in both male and female rodent models [43–45]. Decreased sperm counts, defective oogenesis, hormonal dysfunctions and perturbations in hypothalamus-pituitary-gonadal axis are few of the adverse effects of nanotherapeutics observed in in vivo models [45, 46]. The complex interplay between oxidative stress, inflammation and apoptosis is the most accepted mechanism for nanomedicine-induced reproductive toxicity. For instance, long-term TiO_2 NPs (10 mg/kg) exposure in female mice induced inflammation and oxidative stress, upregulated Cyp17a1 gene expression and down-regulated apoptosis regulating genes as well as also [47]. Further, exposure of male mice to 50, 150 and 450 mg/kg ZnO NPs for 14 days resulted in apoptosis and endoplasmic reticulum stress-mediated azoospermia and testicular toxicity in a dose-dependent manner [48]. Besides action on the germ cells, nanomaterials may also induce toxicity by affecting other cells such as Leydig cells and Sertoli cells. Ag NPs have been reported to cause shedding of epithelial cells, Leydig cell dysfunctions and germ cell apoptosis in in vivo study [49, 50].

Several developmental toxicities such as early miscarriages, retarded embryo and neonatal growth rate have been identified against nanotherapeutics exposure. Since foetus has not developed adequate defence system to counter the toxic insults, therefore it is more susceptible for nanomedicine-induced malformations [51]. Being small in size, nanomedicines have inherent properties of crossing the placenta which is considered as the main reason behind the nanotherapeutics-induced developmental toxicity in embryo and foetus. The toxicity pattern involving direct interaction of nanomaterials with the developing embryo is regarded as direct effects of nanomedicines. Besides this, the concept of indirect effect has also emerged in recent years where the nanomaterials trigger the release of mediators without their translocation across the placenta which results in toxicity [52, 53]. Besides physico-chemical factors, gestational stage also affects nanomedicine-associated developmental toxicity which makes it complicated to predict the adverse effects [54]. For instance, oral administration of zinc NPs in mice during gestational day (GD) 7–16 decreased foetal viability and growth, whereas no such serious effects except slight change in placenta weight were noticed when administered during GD 1–10 [55]. Further, nano-engineered formulations interfere with uterine contractility and placental vasculature [56, 57]. Previous findings suggest that nanomedicines accumulate inside tissue and, therefore, alter the transport of crucial micronutrients across the placenta as evident by the findings of Blum et al. [58]. Upon accumulation in tissues, nanomedicines may activate oxidative stress and inflammation which in turn trigger the release of inflammatory cytokines in systemic circulation which are transported to the tissues relevant to the pregnancy such as uterus, placenta and foetus. These cytokines may trigger the immune activation in target organs which leads to the developmental toxicity [59].

19.4.3 Pulmonary Toxicity

Lungs are one of the most common routes of entry of NPs in the body and therefore have high chances of accumulation of nanomaterials. After inhalation, NPs enter into interstitial spaces followed by their infiltration to alveoli. NPs ranging in the size of approximately 20 nm are more likely to get deposited in alveoli as compared to smaller and larger size particles [60]. Airway inflammation in response to inhalation of nanomaterials is one of the most common adverse effects. The degree of inflammatory response of airways differs depending upon the type of nanomaterials. For instance, single-walled carbon nanotubes induced higher interstitial inflammation in mice lungs as compared to carbon black and quartz NPs after 7 and 90 days of intratracheal administration [61]. NPs elicit their toxic effects on lungs via initiation of several cellular and molecular cascades such as activation of ER stress, mitogen-activated protein kinases (MAPK), nuclear factor-kB (NF-kB), tyrosine kinases and numerous other inflammatory and fibrotic genes [62, 63]. Dokka et al. reported that the polyvalent cationic liposomes-induced pulmonary toxicity is mediated by reactive oxygen species and that toxicity potential owes to the cationic charge and not to the liposome [64]. Further, cationic PAMAM dendrimers have been reported to induce acute lung injury in mice by inducing Akt-TSC2-mTOR-mediated autophagy as evident by amelioration of lung injury in response to autophagy inhibitor 3-methyladenin [65]. Intraperitoneal administration of mesoporous silica NPs at 25, 50, 100 and 200 mg/kg dose in Wistar rats induced pulmonary toxicity by attenuating redox imbalance and TNF-α activation [66].

19.4.4 Nephrotoxicity

After the liver, kidneys are other prominent targets for nanomaterials-induced toxicity as they are involved in their clearance from body. Additionally, kidneys are highly vascular organs and able to concentrate nanomaterials reaching to them via systemic circulation and therefore are highly susceptible to nanotoxicity [67]. Nanomedicines elicit their toxic response in kidneys in terms of glomerular swelling, necrosis of epithelial cells and basal membrane degeneration which are more frequently correlated with oxidative stress, increased apoptosis, inflammation and DNA damage [68]. Several studies suggested harmful effects of carbon nanotubes (CNTs) on kidneys as evident by glomerular degeneration and tubular necrosis. Reddy et al. reported cytotoxicity in human embryonic kidney cell line (HEK293) due to oxidative stress induced by carbon nanotubes [69]. Carbon NPs such as fullerenes and CNTs exhibit potentially deleterious effects on kidneys by inducing alterations in trans-epithelial electrical resistance and expression of barrier proteins [70]. Long-term Ag NPs exposure leads to inflammation and necrosis-mediated ultrastructural changes in the kidney [71]. Copper NPs were found to cause redox imbalance which ultimately leads to ROS production, poor podocyte activity and apoptosis [72]. Further, ZnO NPs also have been reported to induce renal toxicity by inducing hypoxia inducing factor-1α (HIF-1α), apoptosis and autophagy in in vitro and in vivo models [73]. Although liposomal amphotericin B is less toxic as compared to conventional formulations, yet it has been associated with several side effects out of which nephrotoxicity is more pronounced. Approximately, 43% of persons consuming 15 mg/kg/day liposomal amphotericin B have been reported to suffer from renal toxicity [74]. In a recent study, authors reported that intravenous injection of iron (Fe) NPs (encoated in polymeric micelles) at dose 5 and 15 mg/kg in mice induces nephrotoxicity by inducing oxidative stress [75]. Further, Yousef et al. found synergistic effects of aluminium oxide (Al_2O_3) and ZnO NPs on hepato-renal toxicity in terms of increased DNA damage and pro-oxidant levels along with decrease in antioxidants levels in male Wistar rats [76].

19.4.5 Neurotoxicity

Nanomedicines get access to the central nervous system (CNS) by surpassing the BBB. Additionally, nanotherapeutics may also get entry into the brain via trigeminal nerve and olfactory nerve post-inhalation [77]. Upon their entry into the brain, nanomaterials have potential to get accumulated in several regions such as olfactory lobes, striatum and cerebral cortex [78]. Nanomedicines interact with native cells of the brain including neurons and glial cells which initiates a cascade of disrupted events eventually leading to the neurotoxicity [79]. Neurological disturbances induced by nanomaterials are highly dependent on their physiochemical properties such as size, shape, charge and surface coating as reflected in several in vivo and in vitro studies [78, 80]. The most common mechanisms involved in nanomedicines-induced neurotoxicity include oxidative stress, glial cell activation, inflammation, apoptosis and autophagy. Further, neurotoxic response may be elicited in terms of neuronal structural and physiological changes and neurotransmitters dysbalance [81]. Conventionally used evaluation methods of neurotoxicity are not much efficient and reliable to fully understand the molecular mechanisms of nano-neurotoxicity. In an in vivo study, polysorbate-encapsulated chitosan NPs have been found to induce neurotoxicity by increasing inflammation and oxidative stress which owes to their accumulation in the frontal cortex and cerebellum [82]. Liposomal formulations have been reported to possess intrinsic neurotoxic properties in the form of neuroinflammation and necrosis as proved by Huo et al. in glioma cells and rats [83]. Cationic dendrimers exert toxic effects on human neural progenitor cells by altering mitochondrial activity, apoptosis, cell differentiation and redox balance; however, these effects are influenced by surface group charge and density [84]. Further, the intranasal administration of NPs leads to their accumulation in the brain which subsequently causes cognitive impairment, plasticity loss, neurotransmitter imbalance and synaptic changes [85].

19.4.6 Cardiotoxicity

In recent years, the potential of nanotherapeutics to exert adverse effects on the cardiovascular system (CVS) has gained attention of research community. Although the accumulating evidences indicate ability of nanomaterials to cause pathophysiological changes in CVS, the precise molecular mechanisms involved in cardiovascular toxicity are not fully elucidated [86]. Translocation of NPs into systemic circulation, induction of oxidative/inflammatory response and activation of neurogenic pathways are considered most fundamental mechanisms involved in nanotherapeutics-induced cardiovascular dysfunctions [87]. TiO_2 NPs have been reported to cause arrhythmia, myocardial fibre disarrangement, decreased cardiac output, heart fragmentation and hypertrophy of cardiomyocytes in several in vitro and in vivo studies [88, 89]. Further, Feng et al. reported systolic and sarcomeric disorders, interstitial oedema and myocardial apoptosis in response to acute SiO_2 NPs treatment in rats [90]. Furthermore, exposure of Ag NPs in mice via nasal route induced oxidative stress and pulmonary as well as systemic circulation. In addition, NPs got access to systemic circulation followed by their accumulation in the heart subsequently causing lipid peroxidation, thrombosis and apoptosis of cardiomyocytes [90]. Thompson et al. reported the role of adrenergic signalling and mitochondrial membrane permeability in CNTs-induced cardiac injury in mice model [91]. In the view of current scientific literature, it can be concluded that high dose and chronic exposure to nano-sized particles seem to cause more cardiac damage as compared to acute exposure. Extensive research to determine cardiovascular toxicity against chronic exposure to different-sized NPs might be helpful to understand the pattern of toxicity.

19.4.7 Genotoxicity

In recent years, NPs-induced genotoxicity has gained the interest of researchers. It has been reported that cationic liposomes, dendrimers and

supermagnetic iron oxide NPs cause genotoxicity even at non-cytotoxic doses which renders scientific community to ponder on their risk-benefit profile [92]. The need of determining the genotoxicity potential of nanomedicines has led to the arising of nanogenotoxicology branch which specifically deals with the assessment of impact of nanotherapeutics on DNA. Contrary to cytotoxicity, genotoxicity of nanodrugs has been studied in limited research works [93, 94]. Determination of genotoxicity potential of nanocarriers meant for delivery of non-cytotoxic drugs is of utmost importance to design safer nanocarriers. The physico-chemical properties of nanocarriers such as size, charge, composition and molecular weight are the key determinants for their genotoxicity profile as reported by Shah et al. They reported that cationic nanocarriers induced more micronucleus formation in cells as compared to anionic nanocarriers [92]. Further, Bahadori et al. investigated DNA-damaging potential of lipid-based and polymeric micelles and reported that later one was more genotoxic as compared to lipid-based micelles owing to its synthetic nature [95]. Cationic micelles and liposomes have been found to cause DNA damage as well as alter the gene expressions in the spleen, lungs and liver as reported by Knudsen et al. [96].

19.5 Insights Into Mechanisms of Toxicity

Understanding of the molecular mechanisms involved in nanotherapeutic toxicity is most crucial for their safe designing to reduce the adverse biological impact. The synthetic strategies need to be oriented towards minimizing the side effects considering the mechanisms of toxicity. The detailed mechanisms of cytotoxicity, genotoxicity and immunotoxicity of nanomedicines have not been fully elucidated yet. Here, we focus on some of the most important mechanisms such as oxidative stress, inflammation, apoptosis, necrosis, autophagy and DNA damage which contribute to nanomedicine toxicity profile and have been depicted in Fig. 19.2.

19.5.1 Oxidative Stress

Nanomedicines-associated toxicity is more closely related to extensive generation of ROS in cellular environment as evident by several studies conducted in vivo and in vitro. Elevated ROS level disrupts redox homeostasis leading to oxidative stress which is highly deleterious for cellular functioning [97]. Nanodrugs-mediated excessive ROS generation is attributed to the presence of reactive functional groups on their surface which get involved in interaction with cellular targets. Mild oxidative stress increases biosynthesis of antioxidant enzymes via upregulation of Nrf2 expression. Moderate oxidative stress leads to pro-inflammatory response by triggering activation of mitogen-activated protein kinase (MAPK) and nuclear factor kappa B (NF-κB). Further, extreme oxidative stress causes mitochondrial membrane damage and electron chain perturbations which in turn lead to apoptotic cell death [98]. Several NPs have been reported to induce biological toxicity by elevating ROS levels [31]. The oxidative stress has been found to play key role in CuO, ZnO, Ag_2O_3, SiO_2 and TiO_2 NPs-induced biological toxicity [99–102]. Terada et al. reported that cationic lipids induced cytotoxicity in mouse macrophage cell line RAW264 via oxidative stress and apoptosis; therefore, oxidative stress may be involved in toxicity of lipid-based nanodelivery systems such as liposomes and micelles [103]. Further, the complex interplay between oxidative stress and autophagy has been found culprit in PAMAM dendrimers-induced neuronal cytotoxicity as proved by Li et al. [104]. Furthermore, Mukherjee et al. also reported the role of oxidative stress, glutathione, apoptosis and inflammation in PAMAM dendrimers-induced cytotoxicity in human keratinocytes cells [105]. Findings of Rasras et al. suggest that CNTs can cause mitochondrial dysfunctions by generation of ROS as evident by increased malondialdehyde levels and decreased antioxidants level [106].

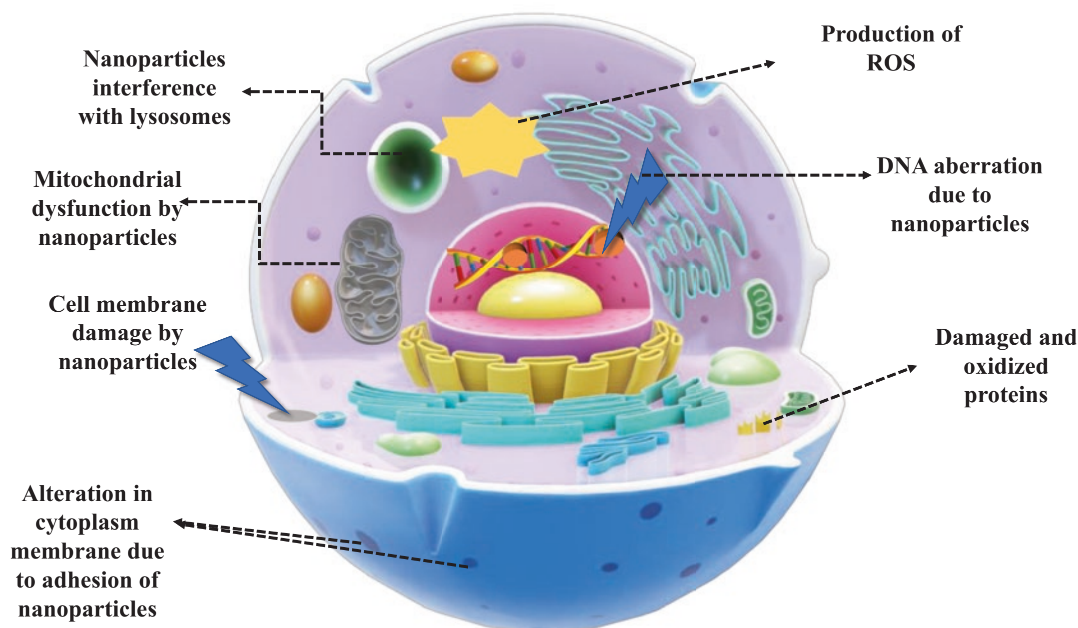

Fig. 19.2 An illustrative representation of various mechanisms of cellular toxicity

19.5.2 Inflammation

Several studies have demonstrated the induction of inflammatory response with nanomedicine exposure. For instance, carbon-based nanotherapeutics (CNTs and fullerenes) trigger inflammatory response in various cells and tissues such as pulmonary airways, human keratinocytes and macrophages [63, 107]. Recent computational studies suggest that innate immune response which is associated with the release of chemokines and interleukins is activated with the exposure of NPs as they are considered as foreign invaders by the Toll-like receptors [108]. Further, liposomes and other lipid-based nanoformulations have been reported to induce inflammatory response by activation of complement signalling pathways [109]. Recently, Hu et al. proved the crucial role of ROS in triggering inflammation and apoptosis in keratinocytes with the therapy with PEGylated liposomal doxorubicin [110]. Fascinatingly, oxidative stress may also direct the induction of inflammatory response by promoting the NF-κB- and MAPK-mediated release of pro-inflammatory cytokines [111]. Metal oxide NPs have been frequently reported to induce in vivo and in vitro inflammatory response via ROS-mediated NF-κB activation which leads to release of TNF-α, IL-8, IL-2 and IL-6 [102, 112]. Further, TiO_2 NPs have been found to cause bronchial inflammation via p38/ERK MAPK pathway-mediated release IL-8 [113]. Interestingly, certain surface properties, steric effects and shape of nanocarriers are key determinants of immunogenic response of the formulations. For instance, rod-shaped NPs have shown high potential to induce macrophagic inflammation due to their high uptake as compared to spherical- and star-shaped NPs [114].

19.5.3 Apoptosis

NPs-induced apoptosis is dependent on physicochemical profile of NPs. For instance, metal NPs such as TiO_2, Ag_2O_3, ZnO and CuO NPs have diverse apoptotic potency [115]. Nanotherapeutics can induce apoptosis either by mitochondrial pathways or by activating the ER stress signalling. Excessive ROS generation triggered by

nanomedicines activates intrinsic and extrinsic apoptotic pathways in mitochondria [116, 117]. Mitochondria perturbations-mediated apoptosis is the most important pathway that contributes to toxic effects of several metal NPs [118, 119]. Ag NPs have been reported to induce apoptosis by activation of TP53 protein which in turn upregulates BAX and BAD expression and downregulates BCL2 family protein expression causing alterations in mitochondrial membrane permeability, release of cytochrome c and cell blebbing [120]. Moreover, Ag NPs trigger apoptosis via ROS-mediated activation of JUN/c-JNK-dependent pathways [116]. Recently, Hanna et al. reported role of mitochondria-mediated apoptosis in ovarian cancer cell cytotoxicity with exposure to folic acid-coated TiO_2 NPs [121]. Elevated ROS level is involved in disruption of calcium homeostasis which triggers ER stress. Recent scientific evidences suggest long-term ER stress-mediated misfolded protein accumulation as an important aspect involved in nanomaterials-induced apoptosis [122]. ER stress-mediated apoptosis has been found with the exposure to several NPs such as ZnO NPs exposure to human umbilical vein endothelial cells (HUVECs) and PLGA and gold NPs exposure to leukaemia cells [123–125].

19.5.4 Lysosomal Dysfunction and Autophagy

Since NPs are sequestered in lysosomes, lysosomal dysfunction has been recognised as one of the outcomes of NP exposure [126]. Alterations in the permeability of lysosomal membrane potential have been associated with the toxicity of CNTs and cationic nanospheres in human fibroblasts and macrophages, respectively [127, 128]. Further, PAMAM dendrimers have been reported to cause lysosomal membrane permeabilization leading to mitochondrial dysfunctions as evident by altered mitochondrial membrane potential and apoptosis [129]. ZnO NPs-induced lung injury in rat has also been credited to lysosomal dysfunctions and uptake by macrophages [130]. Autophagy induced by nanomedicines has both beneficial and detrimental role; therefore, it contributes to the therapeutic as well as toxic effects of nanodrugs. Multifaceted interaction of autophagy with other molecular pathways renders it difficult to understand the individual effects of autophagy in vitro and in vivo [131]. Autophagy has been implicated in multiple organ toxicity such as neurotoxicity, hepatotoxicity, nephrotoxicity and pulmonary toxicity with the exposure to nanomaterials [132–135]. The accumulating evidences suggest that inhibition of pro-survival mechanisms of autophagy is the most probable cause of nanomaterials-induced cytotoxicity as evident by the disrupted autophagic flux and accumulation of autophagosomes [126]. For instance, acute exposure of rats to polyalkylsulfonated fullerene caused lysosomal accumulation along with blockade of lysosomal trafficking [136].

19.5.5 Necrosis

Necrosis is one of the most frequently encountered cell death modalities in response to nanomaterial exposure. The necrotic effects of NPs are conflicting because on one side loss of cell viability without deep focus on the exact mechanism of cell death is reported, whereas on the other side apoptosis leading to necrosis has been reported in plenty of literature [137]. Size, structure and surface charge of the NPs are the most crucial properties to determine the cell death mode with their exposure. For instance, anatase-shaped TiO_2 NPs induced necrosis, whereas rutile-shaped NPs resulted in apoptosis in mouse keratinocytes [138]. Charged gold NPs induced apoptotic cell death, whereas neutral NPs elicited response via necrosis as reported by Schaeublin et al. [139]. In the preview of current knowledge, it is evident that the more accurate and precise studies are required to fully delineate the role of necrosis in nanomedicines-induced cytotoxicity [140, 141]. Till now, pro-oxidants-mediated oxidative stress is considered as the main reason for the nanomedicines-induced necrosis. Excessive ROS reduces mitochondrial membrane potential and ultimately leads to the necrotic cell

death [142]. Cationic nanocarriers have been reported to induce necrosis via impairment of Na^+/K^+ ATPase pump which subsequently resulted in inflammatory response [143]. Further, the release of von Willebrand factor and release of pro-inflammatory cytokines are the most potential mechanisms of endothelial cell necrosis in response to nanodelivery systems [142]. Another most potential mechanism involved in nanomedicines-induced necrosis is the rapid lysosomal degradation of NPs followed by destabilization of lysosomes and the cytosolic release of toxic mediators [144].

19.5.6 DNA Damage

NPs have been recognised as genotoxic agents since long back. They have been reported to induce DNA damage in terms of chromosomal fragmentation, DNA strand breakages and the gene mutations. Nanogenotoxicology is a field which is specifically dedicated to the study of genotoxic potential of nanomaterials including nanodelivery systems and nanomedicines [93]. Nanomedicines might be inducing genotoxicity by acting at two fronts either by directly inducing DNA damage or by inhibiting the DNA repair mechanisms [145]. For instance, Demir et al. reported alterations in DNA repair system in human lymphoblastoid cell line with ZnO NPs exposure [146]. Further, impairment of DNA repair mechanisms was observed by Kruszewski et al. in human cell lines exposed to Ag NPs [147]. The previous literature has suggested that metal NPs induce DNA fragmentation and formation of oxidation-induced DNA adducts [148]. Nanotherapeutics can induce genotoxicity either by direct mechanisms or by indirect mechanisms. The direct mechanisms involve direct interaction of nanomedicines with the genetic material post their diffusion through nuclear membrane, whereas indirect mechanisms are triggered with inflammatory response involving macrophages and interleukins [145]. Although the mechanisms of nanomedicines-induced genotoxicity are known to an extent, the knowledge regarding interaction of nanomedicines with genetic material is still in its infancy. Additionally, nanomedicines have been reported to induce epigenetic changes in genetic material such as DNA methylation and histone modifications; however, the knowledge of their mechanisms is still lacking. The in vitro and in vivo evidences suggest that NPs can induce the following epigenetic changes: (i) alterations in DNA methylation in non-specific and gene-specific manner; (ii) increased phosphorylation of histone proteins; (iii) increased acetylation of H3 and H4 histones; and (iv) elevated HDAC2 protein levels and decreased levels of HDAC1 and HDAC6 proteins [149–151]. The lack of deep information about the impact of nanomedicines on epigenome is also a limiting factor to fully elucidate the genotoxicity potential of nanotherapeutics.

19.6 Evaluation Techniques of Nanomedicine Toxicity

The propensity of nanomedicines to induce several toxic effects paves the way for the need to evaluate their toxicity potential. The combination of various conventional in vitro and in vivo techniques along with the new emerging techniques such as sensors-based tests is employed to hunt for the nanomedicines-induced specific toxicity biomarkers as well as to determine the toxicity profile of new nanotherapeutics. The assay methods, advantages and disadvantages of several nanotoxicity detection techniques are summarized in Table 19.2.

19.6.1 In Vitro Approaches for Toxicity Evaluation

In vitro toxicity assessment is an effective method to understand the toxic mechanisms of nanotherapeutics-induced cytotoxicity; however, these methods are not complete substitutes for in vivo methods of toxicity evaluation. The most common assays carried out to determine toxicity potential of nanomedicines include alamar blue assay, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay, lactate dehy-

Table 19.2 Advantages and disadvantages of several approaches which are usually carried out for nanotoxicity evaluation [152, 157–159]

Assays/techniques	Methods	Advantages	Disadvantages
In vitro			
Cell viability	MTT, MTS, XTT, alamar blue assay	Fast, sensitive	NPs may interfere with signal transduction
Cell death (apoptosis, necrosis and autophagy)	Trypan blue assay, LDH assay, caspase 3/7 activation, FACS, ELISA, Annexin-V assay, comet assay, TUNEL assay, immunoblotting, immunofluorescence	Differs between different modes of cell death	Cross-reaction, non-specificity, non-definitive for individual protein expression and activity
Oxidative stress	ROS assay, MDA assay, oxidants/antioxidants ratio	Fast and reliable for studying basic mechanisms of toxicity	Non-specific, properties of NPs may interfere with the results
Genotoxicity	Comet assay, micronuclei presence, TUNEL assay	Rapid, facile and reliable to identify genotoxic agents	Need to be performed under stringent conditions to prevent exogenous DNA damage
In vivo			
Pharmacokinetic	Biodistribution and clearance assays	Characterize NPs in terms of uptake, localization and biodistribution	Not sensitive enough to detect extremely low levels of drugs, rigorous compartmental modelling
Histopathology	H & E staining, PAS staining, PSR staining, Masson trichrome staining	Provides cell-specific toxicity effects of NPs on an organ	Rigorous procedure, false-positive and false-negative outcomes are encountered
Sensors			
Chemical sensors	Ion-selective electrodes, optodes, fluorescent sensors, anodic stripping voltammetry	Detection limit is low (in ppb) Rapid and selective discrimination among metal NPs	Non-ion specificity
Biosensors	FRET, electrical impedance sensing	Monitor cellular dynamics at single cell level, real-time monitoring in heterogenous cell population	High cost, less specificity and resolution, immunogenic
Nanochemical sensors	CNTs-coated interdigitated electrode, NPs-coated ion electrodes and fluorescent sensors	Highly sensitive and selective, monitor drug translocation in real manner, able to interact with target via multiple interaction sites	Labelling process can modify surface and functions of NPs, difficulty in finding the adequate labelling techniques
Nanobiosensors	NPs-coated FRET and electrical impedance sensing	Portable and highly sensitive, able to interact with target via multiple interaction sites	Immunogenic, sometimes biomolecules lose their properties due to labelling
Omics			
Genomics/miRNomics	miRNA profiling	Better platform to study genetic changes, rapid screening	Lack of correlation to in vivo findings

(continued)

drogenase (LDH) leakage assay, apoptotic and necrotic test, endotoxin signalling and oxidative stress assays [152].

Determination of NPs' potential to breach and interact with cellular barriers is an important aspect to evaluate their toxicity. Assays used to

Table 19.2 (continued)

Assays/techniques	Methods	Advantages	Disadvantages
Transcriptomics	DNA microarray, NGS, subtraction hybridization	Only one type of biomolecules has to be extracted and analysed	Lack of direct correlation between changes in mRNA expression and phenotypic changes
Proteomics	Shotgun method, LC-MS, MALDI-TOF, protein microarray, gel electrophoresis, NMR and X-ray crystallography	Determine protein expression and characterize expressed proteins	Different types of protocols have to follow for different types of proteins, and samples are prone to contamination
Metabolomics	NMR, LC-MS, GC-MS, HRMS	In-expensive analysis, non-invasive sampling	Organism non-specific metabolites, different detection techniques required for different metabolites
Computational			
QSAR	Hansch analysis model, 3D-QSAR, nano-QSAR (1D, 2D and 3D)	Rapid, in-expensive, less resources required	Large dataset required, non-specific due to unknown exact structure of NPs
Molecular docking	Conformational search algorithm such as GA, MC, IC	Rapid, in-expensive, less resources required, provides insight into the drug-biomolecule	Lack of quality database, standardization and scoring functions, less correlation to in vivo results due to target flexibility

Abbreviations: *MTT* 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide, *MTS* (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium), *XTT* 2,3-bis (2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino) carbonyl] -2H-tetrazolium hydroxide, *LDH* lactate dehydrogenase, *FACS* fluorescence-activated cell sorting, *ELISA* enzyme-linked immunosorbent assay, *TUNEL* terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling, *MDA* malondialdehyde, *H & E* haematoxylin and eosin, *PAS* periodic acid-Schiff, *PSR* picrosirius red, *FRET* fluorescence resonance energy transfer, *miRNA* microRNA, *NGS* next-generation sequencing, *LC-MS* liquid chromatography-mass spectrometry, *MALDI-TOF* matrix-assisted laser desorption/ionization-time of flight, *NMR* nuclear magnetic resonance, *GC-MS* gas chromatography-mass spectrometry, *HRMS* high-resolution mass spectrometry, *QSAR* quantitative structure activity relationship, *GA* genetic algorithm, *MC* Monte Carlo, *IC* incremental construction

carry out for determining cellular infiltration are required for quantitative analysis of particles within cells and qualitative identification of nanomaterial's destination. Nanotherapeutics tagged with fluorescent tags such as fluorescein isothiocyanate (FITC), Alexa dyes with fluorescence-activated cell sorting (FACS), confocal laser scanning microscopy (CLSM), and imaging flow cytometry (IFC) serves the need for quantitative and qualitative determination of nanomaterials inside cellular system [152]. Most of the conventional cytotoxicity assays involve chemical reagents for the evaluation of cellular metabolic disruption. These chemical reagents and cell culture media interact with the NPs under test and therefore can influence the test results and give false-positive or false-negative outcomes.

Although analysis of caspases by immunofluorescence, immunoblotting and affinity tests with attached reported moieties offers new approaches for determination of apoptotic activities of nanotherapeutics, however none of these caspase-based assays are suitable for estimating activities of individual caspases. The ability of caspases to cleave multiple substrates is the main limitation for establishing apoptotic potential [153]. Further, assays such as Annexin-V assay, comet assay, TdT-mediated dUTP-biotin nick end labelling (TUNEL) assay, and DNA laddering assay efficiently serve for the purpose of identification of apoptotic cell death [15].

Additionally, quantification of oxidative stress and inflammatory biomarkers such as ROS, lipid peroxidation, antioxidant levels and inflamma-

tory cytokines reveals the in vitro cytotoxicity-inducing potential of nanomedicines. Quantitative luciferase assays along with inflammatory cytokine estimation and ROS determining assays are a wholesome approach for recognizing the in vitro cytotoxicity of nanomedicines [154].

Endotoxin-based detection approaches exploit the use of Limulus amoebocyte lysate (LAL) assay which constitutes the gel clot assay, the coagulogen-based (turbidity) assay and the chromogenic assay [155]. Remarkably, the arising literature suggests that LAL assay-based approaches are not accurate for the toxicity assessment of NPs. Advanced approaches take consideration of toll-like receptor (TLR) 4 as a reporter protein which has come to way as HEK-Blue™ commercial kits [156].

19.6.2 In Vivo Approaches for Toxicity Evaluation

Accomplishing preclinical studies to evaluate the in vivo toxicity is a pre-requisite for any new chemical entity and nanomedicine to make their way to clinical trials [160]. In vivo studies endow with the information about the lethal dose (LD_{50}), maximum tolerated dose (MTD), no observed adverse effect level (NOAEL) and acute and chronic toxicity profile of the drug candidate and should be carried out in rodents as well as non-rodents [161, 162]. Taking consideration of current literature, invertebrates such as *Caenorhabditis elegans* and *Drosophila melanogaster*, zebrafish, rodents and non-human primates have been employed for the toxicity assessment of nanotherapeutics [163]. In vivo toxicity assessment-based approaches stress upon the determination of pharmacokinetic as well as pharmacodynamic profile of the test chemical. A thorough and quantitative pharmacokinetic investigation of the nanostructure, i.e. absorption, metabolism, distribution and excretion, can lead to advances in the nanostructure's design for therapeutic and diagnostic applications. For instance, Baati et al. explored the pharmacokinetic, biodistribution and toxicity profile of titanate nanotubes in mice [164]. The toxicity of nanotherapeutics is highly dependent on the exposure conditions and dose and duration of treatment. The toxicity potential of nanomedicines curtails their use in single and low dosages, and therefore, chronic application of nanomedicines is still a dream to come true. Fonseca-Gomes and coworkers studied the biodistribution and toxicity potential of polymeric and lipid-based NPs in chronic disease in vivo model and interestingly reported that repeated dose exposure of NPs was not associated with side effects [165]. The effects of chronic exposure to PEGylated copper indium sulphide/zinc sulphide quantum dots were evaluated in BALB/c mice to determine their in vivo biocompatibility [166]. The in vivo approach of toxicity evaluation has an edge over the in vitro method in terms of reproducibility and clinical relevance. Since the physiology of animal models is closer to humans, the data obtained by in vivo assessment approaches is more realistic and relevant as compared to in vitro methods [167]. Another major advantage of in vivo methods is that they provide wholesome picture of toxic effects of nanomedicines on an organ which can be observed by histopathological evaluations [168].

19.6.3 Sensor-Based Techniques

Sensor (chemical sensors, biosensors and nanosensors)-based approaches of toxicity assessment are a hot topic in the field of monitoring and risk assessment of nanomedicines. Sensor-based techniques are able enough to identify the selective interaction between the sensor entity and the cellular markers and thus eliminate the limitations of the currently followed endpoint evaluation methods.

Numerous chemical sensors such as ion-selective electrodes and fluorescence sensors have been used for detecting Ag ions and thus can be explored for assessment of nanotoxicity of Ag-based nanomedicines [169]. Although researchers used atomic absorption spectroscopy (AAS), plasma emission spectroscopy and anodic stripping voltametric techniques for Ag ion detection, these techniques rarely have been imple-

mented for evaluation of Ag NPs toxicity [170]. Chatterjee et al. used rhodamine-based fluorogenic and chromogenic probes for detection of Ag ions and NPs in aqueous media [171]. The implementation of chemical sensors for metallic nanomedicines risk assessment is restricted due to complicated coordination properties and poorly defined stereo-chemical profile.

Biosensors are devices which are deemed to detect and record the physiological changes. They are usually made by coupling bioentities (proteins, antibodies, microorganisms, DNA and cells) with the signal transducers such as electrodes, optical transducers and semiconductors [172]. These devices have profound several applications including the field of nanomedicine. Cell-based biosensors sense live cells' physiological changes induced by internal or external stimuli [173]. EIS biosensors are able to provide rapid and real information about the cytotoxicity with response to nanomaterial exposure. Further, chip-based biosensors are also a promising tool for the evaluation of nanomedicines' toxicity. Chip-based approach has been tested by Kim et al. where they used lithographic chip coupled with gold-sensing electrode to evaluate the size-dependent toxicity of SiO_2 NPs [174]. Further, neural cell chip fabricated on the conductive surfaces modified with nanostructured ligands also serves as the purpose for risk assessment of nanomaterials [175]. Furthermore, FRET technology has ability to monitor the stimuli-triggered hazards at cellular level in real-time manner and therefore might be a promising technique for assessment of nanomedicines' toxicity [176].

The major advantage of nanosensors is that they are highly sensitive due to their small size and high surface to volume ratio which enables them to interact with the target at multiple sites. The ability of nanosensors to monitor cellular changes induced by nanomaterials has led to development of "nano assessing nano" approach for the assessment of nanotherapeutics-induced toxicity [177]. Geng et al. employed Ag NPs-based nanosensors coupled with the green fluorescent protein to perceive the early cellular changes induced by low exposure to Ag NPs and reported the morphological changes in cells even at nanomolar concentrations [177]. Nanosensors can be made by coupling nanostructured ligands either with a chemical moiety (nanochemical sensors) or with a biological component (nanobiosensors). Nanobiosensors are more developed in comparison to nanochemical sensors in terms of their use for monitoring the nanotherapeutics. Nanochemical sensors have nanostructure which is radio-labelled or tagged with fluorescence moieties which aid in live tracking of the test compound in the biological system. In the scenario where NPs labelling is difficult, tracking the NPs by electron microscopy is a promising approach to monitor the electron-rich nanomedicines in the diverse organs.

19.6.4 Omics Techniques

Omics technologies such as genomics, proteomics, transcriptomics and metabolomics are emerging techniques which have promising ability to evaluate the in vitro and in vivo toxicity of nanomedicines. Omics techniques are superior over other conventional techniques in terms of less interference with the NPs properties due to which the probability of false-positive and false-negative results is low. Notably, omics technology provides an insight in the molecular interaction and cellular/tissue/organ responses to the nanotherapeutics exposure [178]. Numerous pathways such as oxidative stress, inflammation, apoptosis and proliferation have been found to be involved in NPs toxicity by using transcriptomics and proteomics. Risk assessment of NPs by using transcriptomics and proteomics is higher as compared to metabolomics and genomic. Further, implementation of omics technology is more prominent in in vitro assessment of NPs as compared to in vivo assessment. Additionally, Ag, SiO_2 and ZnO NPs have been mainly assessed by transcriptomics, whereas effects of Au and CNTs have been studied by proteomics as reviewed by Frohlich et al. [158]. Transcriptomics has been successfully implicated for the evaluation of nanotoxicity of CuO, TiO_2, ZnO and SiO_2 NPs and cadmium quantum dots [179–181]. Further, genomics approach has been exploited

for the risk assessment of CNTs in invertebrates [182, 183]. The evolution in the technological field has influenced the implication of proteomics in nanotoxicity evaluation. Ability of proteomics to evaluate composition, charge and size of proteins renders it more applicable for evaluation of toxicity of NPs of varying sizes [184, 185].

Metabolic profiling by mass spectroscopy is another efficient approach for the nanotoxicity evaluation and provides evidences of alterations in the cellular signalling with the exposure to nanomaterials, as in the case of SiO_2 NPs [179, 186].

19.7 Conclusion

Nanomedicines differ from conventional medicines in their physiochemical properties such as size, and surface to volume ratio. Nanomedicines contribute to drug targeting as well as efficient drug delivery; and this affords them some edge over conventional medicines. However, these properties also have been associated with potential toxicity of nanomedicines. Early recognition and in-depth understanding of the molecular mechanisms involved in nanomedicines-induced toxicity are critical for safe use of nanomedicines in public health. Several in vitro, in vivo, sensors- and omics-based approaches are currently being used for the risk assessment of nanomedicines.

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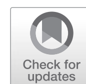

20 Biochemical Indices of Drug Toxicity

Emmanuel Kwaku Ofori

Abstract

Generally, drugs or chemicals have the potential to trigger unpleasant responses when taken in excess. Changes in biochemical markers provide information on the mechanism of toxicity, the functional state of important organ systems, and the identification of target tissues (liver, kidney, hematopoietic and immune systems, etc.). This is because variations in the levels of enzymes and non-enzymes in the serum signal tissue or cellular damage, which results in an abnormal release of intracellular components into circulation. It is, thus, important to understand the biochemical indices involved in drug toxicity to enable rapid response, which may include withdrawal of toxic agent. The biochemical indicators of drug toxicity, as well as the basis behind the measurement of enzymes or proteins implicated in drug toxicity, are covered in this chapter. This chapter will detail the kidney, liver, and cardiac function tests used, as well as the interpretation of results. We'll also go through what we know about novel toxicity biomarkers and how they are being applied to human populations after being studied in animal models.

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20.1 Introduction

The use of drugs and the adverse or toxic consequences that arise is a public health problem. The pharmaceutical industry's primary focus continues to be on reducing toxicity of drugs to the greatest extent possible. It has been proposed that a variety of biochemical markers be tracked to investigate the potentially detrimental effects of chemical compounds, including plant extracts. The term "biochemical marker" refers to a measurable biological observation that may be used to substitute for and, in some cases, anticipate a clinically meaningful endpoint that is more difficult to detect [1]. It is important to note that biochemical markers are used in toxicology for three primary purposes: they can be used to confirm the presence of a harmful agent, they can be used to monitor individual susceptibility to a toxicant, and they can be used to quantitatively assess the deleterious effects of a toxicant on an organism or an individual [1, 2]. These biochemical markers are also used for a variety of other purposes, including the prediction and treatment of adverse medication responses, the identification of cell types, pharmacodynamics, and dose-response

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studies [3]. These techniques may also be applied in the context of health and disease states for chemical screening during drug discovery, diagnosis, and characterization, as well as for monitoring prognostic markers and designing tailored treatments [4, 5].

Ideally, good biochemical markers should be measurable with little or no systematic variability, should improve when the condition improves and vice versa, and should be able to distinguish clinically significant changes over time from background values such as biological fluctuations and measurement errors [6–8]. The measurement of enzyme and non-enzyme activity is extremely important in detecting metabolic changes early in the course of a disease as well as in detecting organ-specific consequences of a medication's action, determining toxic processes, and establishing pharmacological effects. To use enzyme levels as a marker of cellular or tissue damage, the basic principle relies on a comparison of the changes in activity in serum or plasma enzymes, which are primarily present in the intracellular environment and are only released into the serum in very low concentrations [1, 4, 9]. It follows that fluctuation of these enzymes in serum is indicative of tissue or cellular injury, which results in an aberrant release of intracellular components into the bloodstream. Blood serum or plasma is a particularly useful protein pool for repeated biomarker analysis throughout time because it contains a large number of proteins. It is also highly significant in toxicological investigations to analyze urine for the presence of kidney involvement, which might be difficult to detect without it. Urinalysis can provide an early indication of whether or not the kidney is working normally or whether or not its functionality has been impaired. The pH, specific gravity, glucose, ketones, protein, nitrites (including bilirubin), urobilinogen, and cellular microscopy of urine are all measured in addition to the color and appearance.

Table 20.1 contains a collection of biochemical indicators that are commonly used in drug and organ toxicity investigations. The enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), 5′ nucleotidase, and creatine kinase (CK) are the most routinely assessed biochemical markers in drug toxicity. Among the non-enzymatic parameters that are commonly investigated in toxicological studies are the measurements of bilirubin, creatinine, blood urea nitrogen (BUN), malondialdehyde (MDA), glucose (including fasting levels), total protein (including albumin and globulin levels), B-type natriuretic protein (BNP), troponins, cholesterol, and triglyceride levels. In medication toxicity research, certain common electrolytes and minerals are being studied extensively. These include sodium, potassium, phosphate, magnesium, calcium, and chloride, to name a few. It has been possible to test these parameters using standard biochemical procedures, automated clinical chemistry analyzer techniques, and commercial kits [10, 11]. It is the purpose of this chapter to offer a concise summary of various conventional and novel biochemical indicators that are associated with organ damage and medication toxicity.

20.2 Kidneys

The kidney is one of the organs that is commonly evaluated in the pharmaceutical business because it plays a critical role in drug excretion and detoxification [12, 13]. The glomerulus is responsible for the filtration of the blood in the nephron, which is the beginning of renal excretion. After passing through this filtering barrier, the primary urine enters the tubular system for processing. It then passes via the proximal tubule, where essential components are reabsorbed, and onto the loop of Henle, where urine concentration is determined, the distal tubule, and lastly the collecting duct, where the final urinary concentration is adjusted and the urine is discharged [13, 14] In addition, the kidneys receive about 25% of the cardiac output and play a substantial role in the biotransformation of medicines into hazardous metabolites [15, 16], making them particularly vulnerable to tubular epithelial cell injury [17].

Waste materials and extra fluid can accumulate in the body if the ability of the kidneys to filter blood is significantly diminished. It has

Table 20.1 Biochemical markers of drug toxicity and organ damage

Enzymes	Non-enzymes	Electrolytes
GGT (gamma-glutamyltransferase)	Total bilirubin	Sodium
ALT (alanine aminotransferase)	Total cholesterol	Potassium
AP (alkaline phosphatase)	Triglycerides	Chloride
AST (aspartate aminotransferase)	Total protein	Calcium
LDH (lactate dehydrogenase)	Albumin	Phosphorus
CK (creatine kinase)	Glucose	Magnesium
Carbonyl transferase	Bile acids	Iron
Malate dehydrogenase	Creatinine	Copper
Sorbitol dehydrogenase	BUN (blood urea nitrogen)	
Paraoxonase	Glutathione	
5′ nucleotidase	Troponins	
Lipase	Malondialdehyde	
Amylase	BNP (B-type natriuretic protein)	

been shown that a loss in the kidneys' ability to reabsorb endogenous components such as small proteins, glucose, or metabolites following a renal injury might increase the amount of these components excreted in the urine. Injury repair and remission rely on the severity of the injury and entail the migration, proliferation, and differentiation of epithelial cells, which help the body restore its structure and function after it has been damaged. Biomarkers with improved sensitivity and specificity are required in drug development risk assessment studies as well as in predicted toxicological screenings for chemical substances and the evaluation of therapeutic agents for the activation of kidney regeneration [18].

It has been shown that acute kidney injury (AKI), also known as "acute renal failure," is linked with a significant mortality rate of up to 80% in some studies [19, 20]. Blood and/or protein in the urine, high blood pressure, frequent and/or painful urination, puffiness around the eyes, and edema of the hands and feet are some of the traditional warning symptoms of kidney disease [8–21]. Irreversible damage to the kidney results in a decrease in the glomerular filtration rate (GFR), which compromises kidney function and ultimately leads to renal failure and mortality. GFR decreases as a result of irreversible kidney injury. Patient monitoring for renal damage has traditionally relied on serum creatinine, blood urea nitrogen (BUN), other waste products, and the detection of urine components, among other things. Nonetheless, there are certain limits to their diagnostic capacities when it comes to the diagnosis of kidney disease. The levels of serum creatinine and BUN are not completely predictive of GFR. They are generic and insensitive when it comes to distinguishing between various phases of cellular damage or the consequences of cellular injury [22, 23]. These significant disadvantages have resulted in clinical outcomes that have been delayed. As a result, the discovery and validation of biomarkers for the early identification of AKI are an absolute requirement. This article provides a concise summary of various classic and developing biomarkers for the early diagnosis of kidney disease, as well as potential clinical applications.

20.2.1 Serum Creatinine and Urea

The serum creatinine and blood urea nitrogen (BUN) levels are the most routinely utilized markers for the detection and monitoring of renal function. Muscle catabolism results in the production of creatinine, which is a chemical waste molecule [24, 25]. Phosphocreatine, a molecule engaged in energy generation in muscles, undergoes spontaneous cyclization, which results in the formation of creatine and inorganic phosphorus and is catalyzed by creatine kinase (CK) [9, 26]. Phosphocreatine is a molecule involved in energy production in muscles. Further spontaneous breakdown of creatine into creatinine happens at a very regular and uniform pace during each day. The level of creatinine in the blood is a reasonably good predictor of intrinsic kidney

function. The glomerulus filters creatinine freely, and the clearance of creatinine from the plasma to the urine may be used to estimate the glomerular filtration rate (GFR) to a reasonable degree of accuracy [27]. A little quantity of creatinine is released by the proximal tubules of the kidney; however, unlike urea, no creatinine is resorbed by the tubules in the same way. Additional studies have shown that the use of creatinine clearance might result in artificially high GFR values, particularly in persons with chronic renal failure [28, 29].

If the kidneys become compromised for whatever reason, the quantity of creatinine in the blood will rise as a result of inefficient or decreased clearance of creatinine by the kidneys, which will cause the blood to become acidic. High creatinine levels are indicative of compromised renal function or kidney disease. When comparing blood creatinine concentration to urea concentration, it has been shown that the former is impacted by muscle mass while the latter is essentially unaffected by dietary impacts and protein catabolism [30, 31]. The blood creatinine level is also affected by factors such as age, gender, and weight [32, 33]. The presence of gastrointestinal bleeding has also been observed to raise serum creatinine concentrations, with no apparent adverse effect on the kidney [34, 35].

In humans, urea is the most important end product of nitrogen metabolism. It is produced by the liver's cells from ammonia produced by the breakdown of amino acids derived either from exogenous protein digestion or from endogenous tissue proteins [36]. Ammonia is generated by the breakdown of amino acids derived either from exogenous protein digestion or from endogenous tissue proteins [37]. Urea is excreted by several organs, including the kidneys, the gut, the saliva, and the perspiration.

Because the kidney is the most significant route of urea elimination, urea has long been employed as a marker of renal function. Like creatinine, urine is freely filtered by the glomerulus; however, minor quantities are reabsorbed by the renal tubules and released by the proximal tubules throughout the filtration process [38, 39]. When the body is functioning normally, urea arrives in the glomerular filtrate in the same concentration as it does in the bloodstream. Urea concentrations in the blood rise as a result of decreased glomerular filtration [13, 28]. The concentration of serum urea may also be determined as urea nitrogen, and this test is referred to as blood urea nitrogen (BUN). Uremia (increased BUN levels) can develop in patients with renal failure (both acute and chronic), according to the literature [40].

A variety of disorders can harm the kidneys, resulting in inefficient urine production and excretion. Congestive heart failure can result in low blood pressure, elevated urea levels, and a resulting drop in glomerular filtration rate (GFR) [41, 42]. Urinary tract infections, on the other hand, might result in elevated urea levels [39]. When there is an increase in protein catabolism, such as during gastrointestinal bleeding, clinical dehydration, hypovolemia, shock, congestive heart failure, pre-eclampsia, or rhabdomyolysis, urea concentrations might rise or fall [13, 43]. Hemodialysis is done in severe instances to eliminate soluble urea and other waste products from the bloodstream. The measurements of urea and creatinine are used in combination to determine renal function. They are, on the other hand, relatively impervious to even little renal damage due to infection. Changes in creatinine concentrations in the blood tend to correspond to changes in urea concentrations in the blood when there is renal toxicity. However, as compared to urea, the concentration of creatinine varies more slowly over time. Even though it is not conclusive, the urea to creatinine ratio can be useful in making a differential diagnosis of azotemia.

20.2.2 The Glomerular Filtration Rate (GFR) and Clearance

To represent the flow rate of filtered fluid via the kidney, a computed value is used [44, 45]. Renal blood flow and pressure are both influenced by the clearance concept, and GFR is intimately connected to both [46]. It is possible to define clearance as the amount of blood that can be cleared of the drug under consideration in a par-

ticular length of time. It is the assessment of how much a certain item may be removed from a given volume of blood in a specified amount of time by the kidneys. If the item being examined is to be relevant as a biomarker for clearance investigations, it must be eliminated only by passive filtration at the glomerulus. For the tubules to function properly, there must be almost no reabsorption or additional secretion. The substance must also exist in the same biochemical form in both the blood and the urine, which necessitates the absence of any metabolic change before urinary excretion may take place. Inulin (a plant polysaccharide) has been shown to satisfy this need in experiments; but, in reality, creatinine is utilized since it is naturally available in plasma and urine as a byproduct of muscle metabolism and does not need to be injected into the body. Thus, creatinine clearance is defined as the volume of plasma that is cleared of creatinine in a certain amount of time per unit of time. Several blood samples are obtained together with a timed urine collection to evaluate this parameter (usually a 24 h collection). The data can be used to determine how well the kidneys are doing in terms of excretory function. As a whole, these computed numbers can be used to track the evolution of kidney damage that has already occurred. Early identification of a growing injury, on the other hand, is not achievable [47, 48]. As a result, there is an urgent need for novel biomarkers that are better, cheaper, and more efficient than the ones now available.

20.2.3 Blood Cystatin C

It has been discovered that cystatin C in the bloodstream is a 13-kDa peptide of low molecular weight that is widely dispersed in nucleated cells of the human body [49]. Cystatin C is a protein that is readily filtered by the glomerulus, completely reabsorbed by the kidneys, and not released by the tubules, making it a promising marker for glomerular function (GFR). Additionally, the severity of acute and end-stage renal damage, diabetes, and cardiovascular risk is all associated with this endogenous marker of renal dysfunction [50–52]. Cystatin C levels in urine can be used to detect drug-induced renal damage [53] and are related to a reduction in tubular reabsorption as a result of the injury. Furthermore, biological parameters such as gender, age, ethnicity, and muscle mass have no significant effect on cystatin C levels in the blood. Cystatin C appears to be a more sensitive biomarker of reduced GFR than creatinine, although it has not been well tested to the point where it can be recommended for frequent usage.

20.2.4 Fibrinogen

Fibrinogen is a protein that is essential to the clotting process and is found in large quantities in the blood [54]. As an acute response protein, it has been shown to have an important function in inflammatory illnesses [55, 56]. When there is AKI, the fibrinogen protein gene is activated in the kidneys, and the fibrinogen protein is expelled more in the urine [57]. In patients with and without AKI, urinary fibrinogen may be used to successfully discriminate between the two groups, with the excreted protein reaching an AUC-ROC of 0.98, which is comparable to other recognized biomarkers of renal damage [18, 57]. Consequently, fibrinogen is developing as a novel translational biomarker for the diagnosis of AKI, and it has the potential to be examined further as a therapeutic target to treat AKI.

20.2.5 Kidney Injury Molecule-1

Kidney injury molecule-1 (KIM-1) is a type 1 cell membrane glycoprotein of 104 kDa that contains immunoglobulin-like domains and is not detectable in normal kidney tissue or urine [58]. Because KIM-1 protein is produced by tubule epithelial cells in response to damage, it is an excellent biomarker for renal tubular function [59]. In addition, KIM-1 is very sensitive to interferences from unrelated kidney problems [60–62], as well as precise and specific. It is also extremely stable in urine and is unaffected by interferences from other kidney disorders. Kim-1

has superior diagnostic capabilities than the usual indicators of renal injury (serum urea and creatinine), and it is capable of detecting subtle types of tubular damage [63].

20.2.6 Neutrophil Gelatinase-Associated Lipocalin

A tiny 25 kDa secreted protein of the lipocalin superfamily, neutrophil gelatinase-associated lipocalin (NGAL) has been identified as a potential marker of renal tubular damage [64]. NGAL was first discovered in innately activated neutrophils and has now been identified in other cell types [65]. As a result of acute kidney injury (AKI), NGAL expression is significantly expressed in the renal tubules, ultimately resulting in elevated plasma and urine NGAL concentrations [64, 66]. This means that NGAL can be used as a valuable early indication of AKI in both the serum and urine. In humans, the protein expression levels of NGAL are increased rapidly in the epithelial cells of the lungs, colon, and liver within a few hours of the onset of cellular injury [67, 68]. In addition, the protein expression levels of NGAL are increased rapidly in the epithelial cells of the intestine [67, 68]. NGAL levels in urine 1 day after kidney transplantation were found to be predictive of recipients who would have delayed graft function and those who would require dialysis [69, 70].

20.2.7 N-Acetyl-beta-glucosaminidase

The protein N-acetyl-glucosaminidase (NAG) is a lysosomal enzyme that is mostly present in proximal tubule cells [71]. In addition, because NAG is a big protein that is difficult to filter by the glomerulus, its excretion into urine is associated with greater tubular cell damage [19, 72]. As a result, it is a sensitive and specific urinary marker of renal injury. Increases in blood NAG levels are frequently reported before increases in serum creatinine and BUN levels are observed [73]. Because NAG excretion is increased in other glomerular disorders, such as nephrotic syndrome [19, 74], the use of NAG is still limited in clinical practice.

20.2.8 Beta-2-Microglobulin

Circulating β2-microglobulin in circulation is a single polypeptide chain with a molecular weight of 12 kDa, which is easily filtered by the glomeruli and virtually fully reabsorbed and degraded by the renal tubules [75]. The production of β2-microglobulin in persons who appear to be in good health (normal) is rather consistent [76]. Because of a decrease in their absorption in the renal tubules, there is an increase in the concentration of β2-microglobulin in urine [77]. Treatment with some medicines, such as antiretroviral treatments in HIV patients [78] and cisplatin therapies [79], has been shown to significantly reduce tubular reabsorption of β2-microglobulin.

20.2.9 Electrolytes

Electrolytes are ions in solution that are both positively and negatively charged, and they may be found in all bodily fluids. These ions can conduct electrical currents. When evaluating the safety of medicine and, to a significant degree, the function of the kidneys, one of the most widely employed biochemical markers is serum electrolyte concentration. Sodium, potassium, and chloride are among the electrolytes that are most frequently monitored in toxicology studies. Iron, magnesium, copper, calcium, and phosphate are all checked to a certain extent as well. Electrolyte imbalances can arise as a result of vomiting or diarrhea in the gastrointestinal system [80]. Aside from electrolyte loss or gain, acid-base disorders, intrinsic kidney disease, and stimulants in the respiratory center can all cause electrolyte loss or gain [81, 82].

20.2.10 Sodium

Sodium is the most prevalent cation in the body and the most abundant cation in the extracellular fluid. Ionized sodium (Na^+) is necessary for the maintenance of normal osmotic pressure and fluid volume, as well as the excitability of neuromuscular fibers [83, 84]. At the glomerulus, plasma sodium (Na^+) is loosely filtrated and reabsorbed in both the proximal tubules and the loop of Henle, where it is combined with chloride ions and water. Aldosterone controls sodium reabsorption in the distal convoluted tubules, and sodium ions are exchanged at the cost of a univalent cation (e.g., potassium and hydrogen ions) [85, 86]. It is possible to discriminate between pre-renal and intrinsic acute renal failure with high accuracy by utilizing fractional sodium excretion [87]. Changes in body water and plasma volume can have an impact on serum sodium concentrations, either directly or indirectly. The increase in sodium causes a decrease in water retention, which increases the extracellular fluid (ECF) volume [88, 89]. The loss of sodium results in the loss of water and the shrinking of the ECF volume. The extracellular fluid compartment's volume is therefore controlled by the sodium concentration of ECF [90]. A wide range of xenobiotics affects sodium metabolism, causing it to be retained or depleted [90]. Controlling bodily water is performed by the measurement of plasma osmolality, blood volume, and sodium concentrations, which are all interconnected. Osmo- and baro-regulations are used to accomplish this.

20.2.11 Potassium

In the intracellular environment, potassium is the most abundant intracellular cation and is responsible for the maintenance of electrochemical gradients and the transmission of impulses [91, 92]. The resting potential of excitable cells such as neurons and muscle, including the myocardium, is determined by potassium (K^+), even though it is present in low amounts in ECF. Almost all of the total body K^+ (98%) is found within cells, with the remaining 2% found in the extracellular fluid (ECF) being distributed across the interstitial and plasma compartments [93, 94]. Maintaining the intracellular potassium gradient is accomplished by an ATP-structured energetic pumping of sodium, which is balanced by the calcium-mediated intracellular pumping of potassium and hydrogen ions into the cells [83, 95]. Potassium may also play important roles in the regulation of intracellular volume, clinical enzymology, protein synthesis, and glucose metabolism, among other functions [95]. The amount of potassium in the blood does not alter appreciably in response to water loss or retention. Factors that produce even a slight or unexpected alteration in intracellular K^+ concentrations will result in a significant shift in extracellular K+ concentrations. Insulin stimulates the uptake of potassium by the cells. When the pH of a solution is acidic, hydrogen ions (H+) flow into cells (to be buffered) in exchange for potassium ions (K^+).

20.2.12 Chloride

With sodium, chloride is a major anion in extracellular fluid, and the two of them work together to maintain electro-neutrality and osmolality in the body. In the ECF, it is the most abundant anion linked with sodium [96, 97]. After passing through the glomerulus, chloride is passively reabsorbed in the proximal convoluted tubules and actively reabsorbed with sodium in the loop of Henle and the distal tubules after being concentrated in the kidney. The ability of the kidneys to vary daily chloride excretion allows total body chloride levels to remain relatively constant and serum chloride concentrations to remain within a restricted range despite significant fluctuations in daily intake. The measurement of serum Cl^- concentration seldom provides additional information to that acquired from the measurement of Na^+ concentration. Patients who vomit or have unusual chloride-losing episodes may benefit from measuring serum Cl^-, which may be used to calculate the "anion gap" and, as a result, may be able to be diagnosed with various acid-base disorders.

20.2.13 Calcium

When it comes to minerals, calcium is the most prevalent, accounting for around 1.5% of total body mass [97]. The bones and teeth include hydroxyapatite [Ca_{10} $(PO_4)_6(OH)_2$], which accounts for about 99% of the body's calcium. The remainder is primarily concentrated in the ECF compartment, with around 9 mmol in the plasma. Calcium is essential in the physiology and biochemistry of animals and cells, as well as in the biochemistry of cells.

Calcium has a role in the release of neurotransmitters, the contraction of all muscle cell types, coagulation, cell growth, membrane transport processes, fertilization, and signal transduction pathways, where it serves as a second messenger [98]. This cation is also involved in the generation of cardiac action potentials and the functioning of pacemakers, as well as the contraction of cardiac, skeletal, and smooth muscle, which has implications for myocardial infarction and pharmaceutical treatment [98]. Coagulation experiments have shown that calcium ions are necessary as a cofactor [99].

Total serum calcium is composed of three major forms: ionized calcium (which accounts for approximately 50% of total), protein-bound calcium (which accounts for 40% of total), and the remainder, which is complexed with anions such as bicarbonate, citrate, lactate, and phosphate (which accounts for approximately 10% of total) [100]. The majority of the calcium that is bound to proteins is linked to albumin. Calcium in the form of ions, or free calcium, is the metabolically active form of the mineral. Two variables have a significant impact on plasma calcium levels: albumin levels and pH [101]. Changes in plasma albumin affect total calcium, regardless of whether or not ionized fractions are present. Laboratory technicians compute "adjusted" or "corrected" calcium when plasma albumin is much higher or lower than normal to correct for this. When examining calcium, it is critical to consider the relationship between ionized calcium and the acid-base state. Acidosis produces a rise in plasma ionized calcium concentrations, whereas alkalosis causes a drop in plasma ionized calcium concentrations as a result of the effects of pH on the extracellular fluid (ECF) or protein binding. Under the influence of vitamin D, calcium and phosphate are absorbed in the gut together with other nutrients. Calcium is stored in the bones and excreted through the kidneys by the body as a waste product. The hormone parathyroid hormone, vitamin D, and the hormone calcitonin all affect renal excretion of calcium [102].

20.2.14 Magnesium

Magnesium is the second most important intracellular cation after sodium. Magnesium is a mineral nutrient that is found in every cell type in every organism. An adult weighing 70 kg possesses around 25 g magnesium (1000 mmol). The bones and teeth contain half of this total quantity. Of the remaining half, 98% is found in cells where magnesium is the second most abundant intracellular cation after potassium. The remainder (1–2%) is found in the extracellular fluid (ECF), with plasma magnesium concentrations averaging around 0.7 mmol/L. When numerous enzyme systems, particularly those involved in energy metabolism, are activated, magnesium is required to ensure that they function properly. As previously stated, it is required for the binding of macromolecules to organelles (e.g., the binding of mRNA to ribosomes). Magnesium plays a vital role in the regulation of calcium entrance into cells as well as calcium activity inside cells [103, 104].

Because magnesium is a component of chlorophyll, green vegetables, cereals, and animal meat are all excellent suppliers of the mineral. The amount of magnesium reabsorption in the tubules is enhanced by parathyroid hormone (PTH). Inhibition of $Mg2^+$ tubular reabsorption is observed when aldosterone activity is elevated, but $Mg2^+$ cellular uptake appears to be promoted when thyroxine is administered. One may distinguish between three different fractions of serum magnesium: protein-bound, ionized (the physiologically active form), and complexed with anions such as phosphate, bicarbonate, and citrate

[105, 106]. Magnesium homeostasis is determined by the balance between the absorption of magnesium by the small intestine and the excretion of magnesium by the renal system.

20.2.15 Phosphates

Phosphates, a widely distributed element, are a key intracellular anion in mammals and serve a variety of activities [107]. Phosphate may be present in a variety of tissues and fluids throughout the body, including plasma, extracellular fluid, cell membrane structures, intracellular fluid, collagen, and bone [108]. Organic and inorganic phosphates account for approximately 700 g of phosphorus per 70 kg of body weight in an adult. The majority of the phosphate in the body (80–85%) is found in the bones and teeth as the mineral hydroxyapatite [Ca_{10} $(PO_4)_6(OH)_2$]. The remainder (15–20%) is primarily found within cells as organic phosphate compounds, which are toxic to cells (AMP, ADP, ATP). Organic and inorganic phosphates are both present in serum phosphate levels. Phospholipids, phosphate esters, phosphoproteins, nucleic acids, and other organic compounds contain organic phosphate. In the environment outside of cells, phosphate is primarily inorganic, occurring as a combination of HPO_4^{2-} (80%) and $H_2PO_4^-$ (20%) at physiological pH levels.

Phosphate is a significant component of phospholipid membranes, RNAs, nicotinamide diphosphate (an enzyme cofactor), cyclic adenine, guanine nucleotides (second messengers), and phosphoproteins, and it is essential for the intracellular metabolism of proteins, lipids, and carbohydrates. All of the variables that promote glucose absorption in the body including glucose and fructose, alkalosis, insulin, adrenergic stimulation, and anabolism work together to promote glucose uptake. Many of the variables that influence blood calcium concentrations also have an impact on serum phosphate concentrations, either directly or through indirect means. As a result, laboratory readings for calcium and phosphate should be evaluated in conjunction with one another [109, 110]. The kidney continues to be the most important organ in the regulation of phosphate homeostasis. A considerable change in phosphate levels is therefore thought to be the outcome of renal disease.

20.2.16 Iron

Iron, a trace element, in addition to DNA synthesis and oxygen transport, is necessary for cellular development and defense as well as the energy generation [111]. Iron is an essential trace element for all of life's main cell functions. Iron is essential to life [112] because of its extraordinary flexibility in functioning as both an electron donor and an electron acceptor. Ferritin is formed in the intestinal mucosa when the iron is absorbed into apoferritin and stored by the mucosal cells. Alternatively, iron is transferred through the intestinal mucosa to the circulation, where it binds with transferrin. The liver contains the majority of the iron reserves, with a small amount also present in the bone marrow and spleen [113, 114]. Iron concentrations are tested in serum or plasma, and it is most typically employed as a marker of iron status (deficiency or excess) and inflammation. Iron has the potential to be poisonous as well. It can accelerate the transformation of hydrogen peroxide into free radicals or the formation of insoluble salts. A broad range of cellular structures can be damaged by free radicals, which can eventually lead to the death of the cell [115, 116].

20.3 Liver

The liver, which is positioned in the upper right region of the abdominal cavity behind the diaphragm and is responsible for the metabolism of almost every foreign material, is a vital organ in the body's defense against infection. The most prevalent symptom of drug toxicity is liver damage induced by drugs or chemicals [117, 118], which is responsible for more than half of all cases of acute liver failure [119] and is the most common cause of death from drug overdose [120].

Chronic hepatic damage is the major hurdle to drug research, and it is also the most prevalent reason for medication recalls from the marketplace [120]. Drugs become more hydrophilic as a result of biochemical activity in the hepatocyte, which results in water-soluble molecules that are excreted in the urine or bile [121]. Activation of oxidative pathways in the liver, particularly the cytochrome P-450 enzyme system, is required for biotransformation [122]. In the presence of transport proteins on the hepatocyte membrane, the hydrophilic product is exported into the bloodstream or bile, and it is then expelled from the body via the kidney or the gastrointestinal tract after undergoing a series of metabolic steps, which typically include conjugation to an amino acid, a glucuronide, a sulfate, or glutathione. Assessing liver damage in basic toxicological research and toxicity testing is often done using serum biochemical measures, which are then confirmed by histopathology after the first assessment.

The most common clinical patterns of liver injury in humans are intrinsic hepatocellular (affecting hepatocytes), cholestatic (affecting the biliary system), and mixed hepatocellular/cholestatic (affecting both hepatocytes and the biliary system) [123, 124]. Intrinsic hepatocellular (affecting hepatocytes) and cholestatic (affecting the biliary system) are the most common clinical patterns of liver injury in humans. Examples of liver injury indicators include a variety of enzymes and peripheral proteins that are produced in reaction to cellular damage, as well as proteins that have undergone significant alteration within the liver. Because many severe liver ailments are accompanied by normal levels and because abnormal levels can be detected in asymptomatic healthy persons, a single liver function test is of limited value in the screening for liver disease and other chronic diseases. The use of a battery of liver function tests, on the other hand, is a very sensitive procedure. The amount of false negatives is reduced as a result of this method. The use of a battery of liver tests is also associated with a high level of specificity, which is especially important when a large number of tests are abnormal. When the pattern of enzyme abnormalities is interpreted in the context of the patient's features, it may be possible to influence the course of subsequent diagnostic investigation.

20.3.1 Aminotransferases

Drug-induced liver damage can be detected by elevated levels of the enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) [125]. Indications of hepatocellular damage include the presence of aminotransferases (also known as transaminases), which are the most prevalent and specific. AST and ALT, which were originally called serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT), respectively, transfer aspartate and alanine to the keto group of ketoglutaric acids [126, 127]. The quantity of this enzyme present in the blood is determined by an AST test. The presence of AST in the blood is generally insignificant. When bodily tissues or organs such as the heart or liver become ill or injured, more AST is released into the bloodstream to compensate. According to the intensity of tissue injury, the amount of AST in the blood is proportional to that severity. The AST test and the ALT test can both be conducted at the same time in the same lab. According to [128], the AST/ALT ratio can indicate if the liver or another organ has been injured. Both enzymes are highly active in tissues, with the liver, heart, and muscles being among the most active sites. Any damage or injury to the cells of these tissues may result in the release of these enzymes into the circulation, boosting the activity of these enzymes in the serum. If you are trying to figure out whether your liver or another organ has been harmed, the AST to ALT ratio might be helpful. The significance of elevations in serum AST and ALT levels is often proportional to the number of hepatocytes that have been injured.

Even though ALT is commonly located in the liver, AST may be found in a variety of other organs as well. In preclinical research and clinical surveillance of adverse effects, these blood indicators of hepatocyte damage have been used

for several decades. When it comes to liver injury or illness, ALT is a valid screening test, whereas AST is found mostly in skeletal muscle and the heart and is most often associated with damage to these organs. Even though AST is not highly specific, elevated levels indicate liver cell destruction. The levels of AST and ALT are raised in almost all liver illnesses to some degree or another. Extreme viral hepatitis, drug- or toxin-induced liver necrosis, and circulatory shock are the conditions with the highest levels of aminotransaminases [129, 130]. Even though enzyme levels might indicate the degree of hepatocellular damage, they do not necessarily correspond to the outcome of the test. Falling AST and ALT values in patients with fulminant hepatic failure may indicate either a favorable prognosis or a bleak outlook [131]. ALT grows more than AST when there is liver damage or illness, and the quantity of ALT increase is larger than the amount of AST.

20.3.2 Cholestatic Enzymes

When the biliary system is physically or functionally blocked, either within or outside of the liver, it is referred to as cholestasis (or bile flow blockage). Cholestasis is characterized by an increase in the levels of alkaline phosphatase, gamma-glutamyl transferase, and bilirubin in the blood. When compared to only measuring blood bilirubin levels, ALP and GGT have higher sensitivity for identifying this abnormality in the bloodstream. However, because ALP activity is influenced by a wide range of diverse factors, it is not specifically designed for this use.

20.3.3 Alkaline Phosphatase

Alkaline phosphatase (AP) is a multi-isoenzyme complex that hydrolyzes organic phosphate ester links, releasing an organic radical and inorganic phosphate [132]. It is produced by the hydrolysis of organic phosphate ester linkages. Cholestasis and hepatobiliary damage are the most common markers [133] for this condition. Different isoforms of AP are found in the liver, bone, stomach, placenta, and kidney, among other tissues [132]. Predominant forms can also be found in bone tissues, where they stimulate the activity of osteoblasts, as can be seen in broken bones. Low levels of alkaline phosphatase are caused by a variety of conditions including hypothyroidism, pernicious anemia, and zinc deficiency [129, 134].

20.3.4 Gamma-Glutamyl Transpeptidase

Gamma-glutamyl transpeptidase (GGT), also known as gamma-glutamyltransferase, is a protein present in the cell membranes of several organs, including the kidney, bile duct, pancreas, gallbladder, spleen, heart, brain, and seminal vesicles [135, 136]. Gamma-glutamyl transpeptidase (GGT) is a hepatobiliary damage biomarker characterized by cholestasis and biliary repercussions. Birth weight and baby GGT levels are high during the first year of life and continue to climb for the next 60 years [137]. Men have greater GGT concentrations in their serum than women [138]. When a person has liver disease, GGT activity and alkaline phosphatase levels are significantly connected. Pharmacological agents such as phenobarbitone, phenytoin, paracetamol, and tricyclic antidepressants can cause GGT to be elevated. Anticonvulsant medicines have been shown to boost GGT and AP activities in people even when there is no evidence of a liver injury [139]. Several illnesses, including diabetes mellitus, acute pancreatitis, and myocardial infarction, are associated with elevated GGT levels [140, 141]. Because GGT is not found in bone, the majority of its diagnostic applications are limited to the exclusion of bone disease.

20.3.5 Blood Bilirubin

In normal red blood cell hemoglobin disintegration, bilirubin is a yellow breakdown product that is used to identify liver impairment [142, 143]. Heme oxygenase is a catalytic enzyme that converts heme to biliverdin. Biliverdin reductase

then converts biliverdin to unconjugated bilirubin (UCB), which is excreted in the urine. This sort of bilirubin is referred to as indirect bilirubin, which is a phrase used to characterize it. Nonpolar and somewhat insoluble in water, UCB is a molecule that binds to albumin and is transported to the liver, where it is conjugated with glucuronic acid by the action of the enzyme glucuronyl transferase. The conjugation phase makes bilirubin more water-soluble, which makes it simpler to eliminate from the body. A substance known as conjugated bilirubin (CB) is produced in the bile, which travels to the duodenum. The activity of bacteria in the gut causes it to be converted into urobilinogen in the intestine. Urobilinogen is reabsorbed and circulated through the enterohepatic system before being ejected by the kidneys in a proportionately large amount.

Detectable quantities of conjugated bilirubin in the blood are only seen in patients suffering from hepatobiliary disease [129]. Because light can induce changes in bilirubin levels, serum and plasma samples must be stored in the dark before being tested. However, whereas high total bilirubin levels in the blood are an excellent early diagnostic of cholestasis, they may not be a particularly sensitive indicator of liver malfunction or illness prognosis in the long term. As a result of hepatotoxicity, the amount of urobilinogen in the urine may increase [144]. Following alcoholic liver damage and hemolysis, it has also been discovered that urinary urobilinogen levels increase. Hyperbilirubinemia can be caused by a variety of factors, including increased bilirubin synthesis, reduced liver absorption or conjugation, and impaired biliary elimination [145]. The presence of urobilinogen in the urine is a sensitive indicator of hepatocellular failure. If you have this symptom, you most likely have alcoholic liver damage, cirrhosis, or malignant liver disease. When a person has cholestatic jaundice, the protein urobilinogen vanishes from their urine. Ehrlich's aldehyde reagent becomes purple when it comes into contact with urobilinogen. This reagent is packaged in a dipstick, which allows for fast semi-qualitative testing using freshly voided urine in a short period.

20.3.6 Glutamate Dehydrogenase

In the mitochondria, the enzyme glutamate dehydrogenase (GDH) is responsible for catalyzing the conversion of glutamate to ketoglutarate [146]. GDH is found in high concentrations in the liver tissue of humans and most mammalian species, and it is a sensitive and specific marker of liver disease in these animals [147]. Besides the kidney and stomach, it may be present in a variety of other human tissues including muscle and the salivary gland [148]. Given the fact that this enzyme is situated in the mitochondria of cells, it must be disturbed before it can be released in significant quantities into the bloodstream. As a result, any significant increase in GDH levels in the serum is considered an indication of hepatic necrosis.

20.3.7 Sorbitol Dehydrogenase

Sorbitol dehydrogenase (SD) has been discovered in several different human and animal tissues over time. Hepatic, renal, and seminal vesicle mitochondria are likely to be the most common locations for this enzyme [128]. A sensitive enzyme marker for liver necrosis (levels are generally low, but spike following acute bouts of liver damage), however, it should be used in combination with other enzyme measurements such as ALT or other hepatic enzymes.

20.3.8 Plasma Proteins

Total proteins are made up of all of the protein species that have been measured combined. The glomerulus does not filter big or highly charged proteins, but small proteins pass readily across the glomerular barrier and are reabsorbed by the proximal tubules after passing through the glomerular barrier. Proteins are excreted in the urine as a result of damaged nephrons [149]. Among other things, proteinuria is characterized by an abnormally high protein excretion in the urine, which is a prognostic sign of moderate to severe kidney damage and a reliable predictor of

progressive renal function loss in many clinical situations [150, 151]. However, the liver does not manufacture globulins, but it does produce the proteins as well as albumin and blood-clotting factors [152, 153]. The total protein content in the blood is generally proportional to the concentration of albumin in the blood unless otherwise stated. Total protein changes are often associated with decreased synthesis (liver) or increased loss (muscle) (kidney).

Albumin is the most abundant protein in plasma produced by the liver in terms of amount, and it may be used as a biomarker of hepatic function to monitor liver function. Albumin in serum has a half-life of around 21 days, which is rather short. Patients suffering from cirrhosis and ascites frequently have reduced serum albumin levels [154]. Because albumin detects both glomerular and tubular damage, it is the protein that is most likely to be detected at high levels early in a variety of renal diseases. In addition to nutritional conditions, hormonal balance, and osmotic pressure [155], albumin production is controlled by a range of other variables as well. Ceruloplasmin, an acute-phase protein produced by the liver, is another product of the liver. It has been shown that infections, rheumatoid arthritis, pregnancy, and obstructive jaundice can result in a rise in plasma concentration [156]. A low plasma level of ceruloplasmin is usually observed in patients with Wilson's sickness, and this is a critical diagnostic indicator [157]. Low levels of ceruloplasmin can be caused by a variety of conditions including neonates, kwashiorkor, marasmus, protein-losing enteropathy, copper deficiency, and aceruloplasminemia [129, 158].

20.3.9 Other Enzymes of Toxicological Relevance

In addition to the enzymes indicated above, numerous more enzymes are used as markers to identify drug-induced damage in addition to the enzymes stated above. Among the enzymes involved in the urea cycle, ornithine transcarbamylase (OTC) is present almost exclusively in the mitochondria of animal liver cells [159]. Both acute and chronic liver diseases increase the activity of these enzymes. The fact that OTC is categorized as a "liver-specific" enzyme is owing to its high concentration in hepatic tissue when compared to other tissues; nonetheless, the technical requirements for its testing frequently prevent it from being used to its full potential.

Malondialdehyde (MDA) is a three-carbon dialdehyde that is produced as a byproduct of the metabolism of fatty acids [160]. MDA is a biomarker for oxidative stress and lipid peroxidation in general [161], as well as for lipid peroxidation in particular. It has been shown that lipid peroxidation plays a role in the pathophysiology of several different types of tissue injuries, including tissue damage produced by a range of toxic substances. Because MDA is produced as a result of lipid peroxidation, it can be used as a biomarker to detect cell membrane damage. It has been demonstrated that there is a possible relationship between kidney lipid peroxidation levels and renal damage [162].

20.3.10 Other Non-enzymes of Toxicological Relevance

20.3.10.1 Triglycerides

Triglycerides (TGs), also known as neutral fats, triacylglycerols, or triacylglycerides, are lipids that are composed of three long-chain fatty acids esterified to a glycerol. Triglycerides (TGs) are a type of lipid that is found in a variety of foods. Exogenous (chylomicrons) and endogenous (pre-lipoproteins) transport of these substances to target cells has been demonstrated [163]. While endogenous triglycerides are synthesized and stored in the liver, exogenous triglycerides are obtained from the food [164]. TGs are the body's principal source of energy and also serve as the human body's primary and most dependable energy reserves, according to the World Health Organization. It has been shown that TG plays a role in metabolic pathways that regulate the rate of fatty acid oxidation as well as the fate of lipoproteins in the body [165]. When TG is stored in the cytoplasm of a cell (e.g., muscle), it is surrounded by a monolayer of phospholipids and

hydrophobic proteins, which is known as the TG storage complex [166].

Only trace levels of triglycerides are normally allowed in the blood. To establish whether or not there are high triglyceride levels in the blood, the serum triglyceride concentration is determined [167]. Increased serum TG levels produce an increase in blood viscosity as well as platelet aggregation, which results in a reduction in vascular flow. An increase in total lipid (TG) levels in the blood is usually coupled with a decrease in HDL cholesterol levels in diabetes patients, and both are risk factors for cardiovascular disease (CAD [168]). According to toxicity studies [169], there may be a variety of associated lipoprotein metabolism abnormalities.

20.3.10.2 Cholesterol

Cholesterol, a steroid oil hydrocarbon with a four-link structure, is a substance that plays an essential role in the function of membranes and the metabolism of lipids [170]. In addition to being a precursor to steroid hormone biosynthesis (including glucocorticoids, estrogens, progesterone, androgens, and mineralocorticoids), cholesterol is also a bile acid and vitamin D producer [171]. Aside from these functions, cholesterol is also involved in signaling and sperm production [172]. Lipoproteins, which are protein bundles, are responsible for transporting cholesterol through the body. A lipid profile includes measurements of total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides, among other things. Chronic hypercholesterolemia (hyperlipidemia) is a primary cause of coronary artery disease and stroke [173].

20.3.10.3 Bile Acids

Bile acids are the byproducts of cholesterol metabolism in animals, and their principal functions in the intestines are to act as powerful detergents or emulsifying agents to aid in the digestion and absorption of dietary fats [174, 175]. They are produced by the liver, and they are stored in the gallbladder. Feeding causes the gallbladder to contract, allowing bile acids to pass into the gut through the digestive system. When there is liver damage or dysfunction, total bile acids are elevated because they are engaged in a wide number of signaling pathways. Cholesterol catabolism and elimination, as well as regulation of pancreatic secretions and the release of gastrointestinal peptides [176], are all functions of bile acids. The small intestine is also aided in the digestion and absorption of dietary fat (and, indirectly, fat-soluble vitamins). In this way, bile acid concentrations can be employed as a valid biomarker of hepatobiliary function in the laboratory setting.

20.3.10.4 Glucose

Glucose is the most important simple sugar (monosaccharide) in mammalian metabolism because it is the most readily available. It is a carbohydrate molecule with six carbon atoms. Glucose is a critical component of cellular respiration and one of the primary products of photosynthesis, and it is produced in large quantities by plants. Cells employ glucose as an energy source and as a metabolic intermediate supply material for a wide range of biosynthetic actions [177]. There are several pathways involved in the metabolism of glucose in the body. These include glycolysis, gluconeogenesis, glycogenolysis, glycogenesis, the pentose-phosphate pathway, and the citric acid cycle [178, 179]. The primary purpose of testing blood glucose levels is to determine how well the body is handling carbohydrates. Normal plasma glucose levels can only be maintained by maintaining a perfect match between glucose consumption and endogenous glucose production or dietary glucose supply (or both).

20.3.10.5 Cytokines

The use of blood cytokines as toxicity markers has lately gained prominence [180, 181, 183]. The use of cytokines as biomarkers, on the other hand, is problematic because of their short circulation half-life, low baseline levels, and lack of tissue-specific expressions. Interleukin-18 (IL-18) is a pro-inflammatory cytokine that is generated by macrophages and other cells and belongs to the IL-1 superfamily, according to the National Institutes of Health [182]. Plasma IL-18 levels are elevated in patients suffering from inflammatory arthritis, inflammatory bowel dis-

ease (IBD), systemic lupus erythematosus (SLE), psoriasis, hepatitis, and multiple sclerosis (MS) [183–185]. Furthermore, in individuals with acute renal damage, urine interleukin-18 (IL-18) levels are significantly raised before increases in serum creatinine levels [186, 187]. Although this IL-18 appears to be a promising potential biomarker in the context of AKI, its pro-inflammatory properties and upregulation machinery under inflammatory settings may limit its sensitivity and specificity when used in clinical applications.

Inflammatory peptide C-reactive peptide (CRP) is an acute protein that has been proposed as a biochemical biomarker for the risk of coronary heart disease. CRP levels in the blood are used to demonstrate the presence of active inflammatory cells in plaques [188, 189].

20.4 Muscle

It is becoming increasingly usual to encounter muscle toxicity as a concern in drug development, and research into methods to predict skeletal muscle injury is becoming more prevalent. Toxicologically significant are traditional muscle injury signs such as elevations in aminotransferase (AST), creatine kinase (CK), and lactate dehydrogenase (LDH) [190]. These conventional identifiers are not without their downsides, though (specificity and sensitivity). Several proteins, including skeletal troponin I and T, creatine kinase protein M, myosin light chain 3, fatty acid-binding protein 3, aldolase A, and myoglobin, are sensitive and specific indicators of drug-induced skeletal muscle injury [190, 191] and have shown promise as sensitive and specific indicators of drug-induced skeletal muscle injury.

20.5 Cardiac

The list of indicators used to measure cardiotoxicity includes markers for structural and functional alterations, as well as markers for oxidative stress. Inflammatory or recent muscular injury or damage is indicated by elevated blood levels of creatine kinase (CK), an enzyme that leaks into the bloodstream. Lactate dehydrogenase (LDH) is a tetrameric protein that catalyzes the reversible conversion of pyruvate to lactate in the presence of oxygen [192]. LDH is an intracellular enzyme that is widely distributed throughout the body and is particularly abundant in tissues that utilize glucose for energy [193] such as the skeletal and brain muscles, cardiac muscle, kidneys, and liver [194]. As a result of this distribution, a rise in LDH can cause damage to a wide range of tissues as a result of its elevation. The majority of LDH isoenzymes are distributed in a tissue-specific manner.

Troponins are the protein filaments that are responsible for contractile muscle contraction in the heart and skeletal muscles [194]. Troponin T's role is to provide a connection between the troponin complex and the tropomyosin strand of the actin thin filament. Troponin I suppresses the activity of the actinomycin ATPase enzyme. Troponin I is found in a variety of plants. Troponin C regulates contraction by forming a complex with four calcium ions. Cardiomyocyte troponins I and T have amino acid sequences that differ from those found in skeletal muscle, and these differences can be used to distinguish between the two tissue types [195, 196]. An increase in cardiac troponin levels in the blood might indicate the presence of heart illness, the most frequent of which is myocardial infarction [196]. As a result of the release of the enzyme into the circulation when the heart is wounded, increased troponin levels improve diagnostic sensitivity and specificity in identifying cardiac muscle cell death in the patient.

During times of ventricular stress, cardiomyocytes secrete the peptide hormone B-type natriuretic protein (BNP), which has anti-arrhythmic properties [197]. BNP (brain-derived neurotrophic factor) is a cardiac-specific hormone that detects mechanical stress inside the myocardium [198]. It has been proposed as a biomarker to detect cardiac pressure and volume overload when pathological symptoms are present. BNP is elevated when these symptoms are present. It is a powerful "negative predictor," since a low result implies that the volume parameters are within

normal limits. Increased BNP levels are an independent predictor of death in patients suffering from acute coronary syndromes [199, 200].

20.6 Recent Developments and Future Perspectives

Currently, available early toxicity indicators are ineffective in effectively addressing pharmaceutical toxicity concerns. Molecular epidemiology and genomic studies have received a great deal of attention in recent decades to identify specific biomarkers for nephrotoxicity and hepatotoxicity, as well as for learning more about the underlying mechanisms of various kidney and liver insults [201]. It will be easier to gain a comprehensive understanding of how toxic insults affect biological systems with the introduction of these "omic" technologies (genomics, proteomics, metabolomics, and cytomics) in the future, which will lead to more accurate toxicity prediction models that can be used in drug development, as well as more effective drug development. When it comes to biomarker discovery and characterization, one of the more recent breakthroughs in the quest for biomarkers has been the detection of plasma microRNAs (miRNAs), which are small RNAs that are found in the plasma of healthy people. Endogenous RNA molecules of 22 nucleotides in length, microRNAs are produced in the nucleus and processed in the cytoplasm before being recruited to silencing complexes, where they block the translation of certain target mRNA transcripts. DNA microarrays allow for the simultaneous monitoring of the expression of hundreds or thousands of genes using a single sample of DNA. A great deal of work has been made in understanding the beneficial effects of microRNAs in cancer, cardiovascular disease, and other organ damage illnesses.

Analytical techniques such as nuclear magnetic resonance (NMR), gas chromatography, high-performance liquid chromatography (HPLC), and mass spectrometry (MS) can be employed to investigate metabolite expression patterns in biological matrices. NMR and mass spectrometry (MS) are powerful analytical methods for generating multivariate metabolic data. Because it is non-destructive and acceptable for intact biomaterials, nuclear magnetic resonance (NMR) has the benefit of providing intrinsically more information-rich results in terms of molecular structure identification. Even though MS is more analytically sensitive than NMR, it requires the use of extraction and derivatization processes before the experiment. The successful application of NMR- and MS-based metabonomics in toxicological and clinical research will aid us in our quest to get a better understanding of pharmaceutical toxicity.

20.7 Conclusion

Variations in serum biochemical parameters that occur during toxicity have been discussed. The working state of important organ systems such as the liver, renal, cardiac, hematopoietic, and immunological systems may be determined using biochemical markers. In toxicological research, enzymes are frequently used to identify and evaluate cell damage, and they are also used to assess the severity of the damage. The use of biomarkers in drug development, illness, and monitoring of favorable effects of therapeutic interventions is beneficial in a number of ways. It is also vital to conduct research and discover early and sensitive biomarkers that will enable the creation of prompt detection of toxicity. Several emerging biomarkers, including urine protein biomarkers, microRNAs, proteomics, metabolomics, and targeted mass spectrometry assays, have the potential to exceed traditional indicators in mammalian models in terms of sensitivity and predictive capacity.

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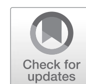

21 Therapeutic Drug Monitoring and Clinical Toxicology: Challenges and Future Directions

Seth Kwabena Amponsah and Yashwant V. Pathak

Abstract

Therapeutic drug monitoring (TDM) is the clinical practice of measuring levels of drugs in the plasma, serum, or blood at predetermined times or intervals in an effort to maintain blood concentration of a drug within optimum range. Drugs that are usually monitored are those with low therapeutic indices, drugs that have irreversible adverse effects, as well as drugs used in the treatment of diseases whose symptoms are similar to the toxic effects of the drug. TDM is useful in detecting compliance and non-compliance to drug therapy in patients. It also provides a means of detecting treatment failure. Clinical toxicology is the study of the physiological effects of toxic agents, their mechanism of action, and ways of managing these effects. Furthermore, clinical toxicology helps in the identification of chemicals, drugs, or toxins that may affect patients.

Even though TDM and clinical toxicology are useful, they come with some limitations. Some of the assays used in TDM and clinical toxicology lack sensitivity. Taking samples at the right time is another challenge associated with TDM and clinical toxicology. Inaccuracies from sampling site and handling of samples prior to analysis can affect results. Some drugs also have active metabolites that might not be detected by TDM and clinical toxicological assays. Additionally, TDM and clinical toxicology are expensive to undertake. Indeed, the significance of TDM and clinical toxicology in clinical practice cannot be overemphasized. Nonetheless, more research can be done on alternate sampling matrices such as saliva and dried blood spot. These matrices would make TDM and clinical toxicology more convenient and easy to do. There is the need for better interpretation of results obtained from TDM and clinical toxicological assays. Hence, health professionals need to be trained and re-trained on appropriate interpretation of TDM and clinical toxicological results so patients are managed appropriately.

Keywords

Blood concentration · Challenges · Clinical interpretation · Sampling · Sensitivity

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21.1 Introduction

Therapeutic drug monitoring (TDM) is a means of ensuring safety and efficacy of drug therapy by regular and consistent monitoring of blood concentration of the drug. It is a means of ensuring that blood concentrations of certain drugs remain within a given range in order to maximize the effectiveness of drugs as well as minimize side effects. The initial focus of therapeutic drug monitoring was mainly on adverse drug reactions [1]. As a result, the drugs that were mainly monitored were drugs of narrow therapeutic indices such as digoxin, phenytoin, lithium, theophylline, and some of the aminoglycoside antibiotics [2]. Later on, clinical pharmacokinetic monitoring was introduced in therapeutic drug monitoring. Here, the concentration of the drug in the blood is related to the responses that the drug produces. Indeed, TDM is not merely the measurement of drug levels in blood sample. TDM involves expert clinical interpretation of drug levels which would ultimately help to optimize a patient's treatment. Therefore, drugs that do not offer any meaningful clinical information when levels are assayed in blood often do not require TDM.

The concept of TDM is based on two assumptions; there is a relationship between the administered dose and the concentration of the drug in the blood. This is also affected by the time of measuring. The second is there is a relationship between the plasma drug concentration and therapeutic or physiological effects [3, 4]. In the population, there may be individuals who may possess unique pharmacokinetic and pharmacodynamic characteristics which may affect drug concentration at steady state. Hence, TDM is very important in individualizing drug dose, which invariably would aid in the attainment of optimal plasma drug concentration needed to maximize efficacy and reduce toxicity.

Clinical toxicology is the study of the physiological effects of toxic agents, their mechanism of action, and ways of managing their adverse effects [5]. In cases of toxicity during the administration of therapeutic agents, TDM can be used to determine the level of toxicity by quantifying the amount of the toxic substance in the blood. However, clinical toxicology would seek to identify and manage toxicity. Therefore, TDM and clinical toxicology can be complementary.

21.2 Reasons for TDM

TDM is recommended or requested for only when absolutely necessary. This is because it can increase healthcare cost for patients. Drugs with low therapeutic indices and drugs whose toxicity causes irreversible organ damage or death are usually monitored. Drugs that do not produce enough clinical efficacy to determine whether there are therapeutic or toxic effects are also monitored [2]. Patient factors such as poor response to treatment and suspected noncompliance to treatment can also be a reason for the requisition of TDM [6]. TDM would also be requested if the manifestations of the disease condition and the toxic effects of a drug are similar [7]. Drugs such as digoxin have toxic symptoms that are very similar to symptoms of the heart disease for which it is used. Aronson and Hardman report that monitoring the plasma levels of digoxin helps to point out the actual source of the patient's symptoms [8]. A similar phenomenon is observed with aminoglycoside antibiotics. Additionally, TDM aids in monitoring compliance to drugs used for prophylaxis. A typical example is the use of phenytoin to prevent episodes of seizures. In most cases, patients who take drugs for prophylaxis lack the awareness of the need to comply with treatment and, hence, are likely to be nonadherent. In some cases, a patient may not be showing significant response to treatment despite therapeutic concentrations of the drug (at steady state) in the blood. This may likely be as a result of treatment failure. TDM therefore offers clinicians any easy way to detect treatment failure. According to Molden, TDM can be used in assessing different measures of treatment failure [9].

21.3 Challenges with TDM

21.3.1 Drug in Circulation May Not Correspond to Amount at Site of Action

A limited number of drugs can be monitored; and subsequent relevant clinical interpretations are made. Aside from concentration of a drug in circulation, the amount of drug present at the site of action is often difficult to determine. Pichini has suggested the analysis of drugs in areas such as bronchial secretions, peritoneal fluid, interstitial fluid, tears, and nails [10]. This is because these areas may help show the concentrations of the drug at the site of action. Even with this, it may be difficult to measure the amount of drug at the actual site of action which is the receptor site. This is partly because receptors of a drug may not be localized in one part of the body. As a result, the most convenient physiological fluid used in therapeutic drug monitoring is the blood (plasma). For TDM of a drug to be beneficial, the desired effect and adverse effect of the drug should have a correlation with the plasma concentration [11]. A limited number of drugs have this correlation; and these include amikacin, gentamicin, phenytoin, lithium, vancomycin, methotrexate, cyclosporine, digoxin, phenobarbital, carbamazepine, and valproate [12].

21.3.2 Low Sensitivity of Some of the Assays Used in TDM

Lack of sensitivity of some drug assay methods is a limitation of TDM. Analytical methods that can be used to assay drugs in biological samples could be gas chromatography, high performance liquid chromatography, or ultra-performance liquid chromatography coupled to ultraviolet, mass, or fluorescence spectrophotometry. Other methods, which are non-specific, include radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), or enzyme-linked turbidimetric inhibition assay (PETINIA). The non-specific assays, for example immunoassays, could overestimate plasma concentrations of a drug, and this may be as a result of cross-reactivity of assay material and many inactive metabolites of the drug [13]. More often, there exists high variation between results obtained from different sample batches and sometimes within the same run. Chromatographic methods are more accurate in quantifying plasma drug concentration. However, these methods are expensive and technically demanding; hence, immunoassays are still used in the analysis of several drugs such as theophylline, methotrexate, cyclosporine, gentamicin, and amikacin. Chromatographic and immunoassay methods normally measure total concentration of the drug in the blood. This includes both protein-bound drug and free drug. For drugs that are highly protein-bound (for instance, phenytoin which is about 90% protein bound), total concentration obtained from the assay methods may not always correlate with what pertains in systemic circulation [14]. It is well documented that some endogenous substances cross-react and interfere with serum concentration of drugs measured. In the immunoassay of digoxin, digoxin-like immunoreactive substances (DLIS) alter serum concentration measurement by cross-reacting with the antidigoxin antibodies used in the assay [15]. This may lead to false low digoxin levels measured. DLIS levels are normally clinically insignificant in healthy persons, but levels may rise significantly in conditions such as hypertension, liver disease, uremia, and congestive heart failure. This can be dangerous since it might lead to the tendency for an increased dose adjustment of digoxin. Given the narrow therapeutic range of digoxin, this might be detrimental to the patient.

21.3.3 Difficulties in Sampling at the Right Time

Another challenge associated with TDM is selecting the right sampling time. A key to obtaining useful and accurate measurement of blood concentration is taking samples at the right time. Determining the right time to take a biological sample can be quite challenging. The pharmacokinetic properties of drugs play an important role in determining the sampling times. Others factors

such as age, liver and renal function, drug interactions, and some genetic factors may also affect drug levels and the time it takes to reach steady state. Therefore all these factors should be considered in sampling times for TDM. TDM is expensive; hence, it is imperative to determine appropriate sampling times in order to obtain clinically relevant data. In normal practice, sampling usually takes place just before the next dose (trough concentration of the drug). In other cases, the sample is taken immediately after drug administration (peak plasma concentration). Knowledge of the sampling time is of clinical importance in the interpretation of information obtained from drug monitoring. For some drugs that require TDM, there exist possible sampling times; and these are presented in Table 21.1.

Aside from these times, sampling can be done anytime toxicity is suspected or when the patient is showing no response to ongoing treatment [16].

Table 21.1 Sampling times for some common drugs that require monitoring

Drug	Appropriate sampling time
Gentamicin	At steady state; after at least 4 half-lives (half-life = 2–3 hours) Trough samples; 30 minutes before next dose Peak samples; 30 minutes after IV infusion or IV bolus
Digoxin	At steady state; 8 days Trough sample; before next dose Peak sample; at least 6 hours after last dose
Carbamazepine	At steady state; 2–4 weeks after initiation Trough sample; within 2 hours before next dose
Phenobarbital	At steady state; 2–3 weeks after initiation Trough sample; within 2 hours before next dose Peak sample; at least 3 hours after last dose
Phenytoin	At steady state; 5 to 10 days after initiation Trough sample; within 2 hours before next dose
Valproic acid	At steady state; 2–4 days after initiation Trough sample; within 2 hours before next dose

21.3.4 Inability to Assay Some Active Metabolites of Drug

The presence of active metabolites of a drug in circulation may also contribute to the therapeutic effect of the drug. Some of the drugs that are routinely monitored may have some active metabolites which are often or may not usually be measured [6]. These metabolites are sometimes equally as active or more active than the parent drug. For instance, the active metabolite of clozapine, norclozapine, has been shown to be as active as the parent drug [17].

In the determination of plasma concentration of a drug, the measurement obtained is usually a total of both the parent drug and the active metabolite. Other assay methods are also able to measure the parent drug but not the active metabolites. Some reports suggest that desethylamiodarone which is an active metabolite of amiodarone is not measured and accounted for in the therapeutic range of amiodarone during TDM [18]. Since the metabolite is active and accounts for part of the therapeutic activity, the practice of measuring the parent compound only may be inaccurate.

21.3.5 Inaccuracies in Detecting Medication Compliance

TDM has been used over the years in assessing drug adherence (compliance) in patients. However, TDM is only valuable in detecting short-term compliance or non-compliance. TDM has also shown very little benefit in improving compliance to drug in cases of prophylaxis. This is especially true in non-hospitalized patients or outpatients. Patients are likely to take a dose of their treatment prior to blood sampling for analysis or before medical appointment [19]. Drug plasma levels alone may not be entirely accurate in detecting compliance. Additionally, individuals who are fast metabolizers may also record low plasma drug levels on certain occasions [11]. This may be falsely attributed to medication non-compliance. The concomitant administration of drugs that are enzyme inducers or inhibitors may

also affect plasma drug concentration [20, 21]. For instance, it has been shown that the concomitant administration of clozapine and carbamazepine significantly decreases serum clozapine concentrations [22]. This can be attributed to potent liver enzyme-inducing ability of carbamazepine.

21.3.6 TDM Is Expensive

One of the shortcomings of TDM is the cost involved in analyzing samples. TDM can be expensive since it involves repeated testing. Two main strategies for TDM exist: reactive TDM where monitoring is done if toxicity or treatment failure is suspected, and the other is proactive TDM, which is routine repeated testing done at specific times. Even though it can be argued that TDM makes overall healthcare cost-effective, there is the likelihood that regular TDM could be additional cost for patient. A study conducted by Campbell et al. showed that out-of-pocket costs lead to a decrease in the willingness of patients to participate in TDM [23]. This means TDM may not be affordable to patients especially if there is no external support. Some analytical procedures such as liquid chromatography-mass spectrometry are costly; hence if these methods are employed in TDM, patients might find this procedure expensive [24]. One of the main benefits of TDM is dosage adjustment. Some centers do their adjustments using "trial and error" method of different dosages till an optimal serum concentration is achieved. This approach in TDM has been shown to lead to unnecessary healthcare cost for the patient [25].

21.3.7 Challenges with Sampling and Sample Handling Before Analysis

The process of drawing samples and handling of specimen before analysis are critical in TDM and, hence, have to be done properly. It requires taking extra precautions at the site of sampling to avoid contamination [26]. In the analysis of some local anesthetics like bupivacaine, blood samples from central veins and arteries have been shown to be more reliable than peripheral venous samples [27]. Cyclosporine administered intravenously is known to have high concentrations in samples from central venous catheter than peripheral venous samples [28]. After blood samples are collected, they are normally stored in tubes before analysis. The purpose of these collection tubes is to preserve the blood sample with its content of drug and metabolite(s) till assay [29–31]. These collection tubes may contain anticoagulants such as ethylenediamine tetraacetic acid (EDTA), heparin, and trisodium citrate (TSC) and sometimes no anticoagulants at all [32]. These anticoagulants and barrier gels found in the collection tubes can affect drug concentration in the blood (plasma or serum) that will be measured. Lithium heparin tubes have been reported to be ideal for TDM [33, 34]. One the contrary, for monitoring serum lithium levels, lithium heparin tubes have been found to interfere with test results. This is because the lithium in the tube can react with porphyrin compound in the blood to cause a falsely elevated serum lithium concentration [35]. There are a number of systematic reviews and studies that have reported cases of false elevated lithium levels as a result of the use of lithium heparin tubes [35].

21.4 Future Directions

21.4.1 Alternate Sampling Matrices

In analysis of biological samples during TDM, the most common body fluid used is venous blood. The traditional invasive venous blood sampling comes with its challenges. It presents a lot of discomfort especially in pediatric patients. Alternate matrices with corresponding highly sensitive assays for TDM are recommended. Saliva has been utilized in monitoring drugs such as carbamazepine, primidone, phenytoin, and ethosuximide [36]. Saliva is a suitable matrix for analyzing these drugs because the concentrations of these drugs in saliva are directly proportional or can be used to extrapolate concentrations of

the drug in serum [37]. Even though there are a number of drugs, especially anticonvulsants, that can be assayed in saliva, some drugs such as valproic acid cannot be analyzed using saliva [36]. Saliva may be advantageous because, comparing this with the traditional venous blood sampling, it is noninvasive, has high patient preference (because of the ease of taking samples especially in children), there is no need for a professional phlebotomist in taking samples, and its cost-effectiveness [37, 38].

Sara Capiau and colleagues report in a study that an ideal sample collection method should have the following properties: noninvasive, allows for self-sampling, requires little sample volume, applicable to everyone, robust, and economic [25]. Dried blood spot sampling is a method where capillary blood is collected from a finger or heel prick onto a filter paper [39, 40]. This method meets the criteria specified by Sara Capiau et al. [25]. Dried blood spot sampling is minimally invasive and requires a very small amount of blood (5–35 μL) [25]. This method facilitates home sample collection, especially for children and patients with psychological disorders. It also helps in dealing with one of the major challenges in TDM, which is sampling at the right time. In most cases, trough level samples can be taken immediately before the next dose. This is often done early morning or late evening when the clinics and laboratories may not be working. Dried blood spot sampling will make sampling possible at these times for a patient who will be at home. Dried blood spot sampling has also been shown to be more stable than frozen blood samples and hence makes it more convenient for storage and later transport [39]. Bioanalytical methods for TDM could be validated to utilize dried blood spot samples.

21.4.2 Individualized Therapeutic Concentration

Individualized therapeutic concentration in TDM is a principle that when used would bring great improvement and benefit to patients. Usually in TDM, serum and plasma drug concentrations are compared to therapeutic reference ranges. The therapeutic reference ranges are serum/plasma drug concentrations that are expected to produce desired therapeutic effect [40]. These reference ranges may vary from one laboratory to the laboratory. They may also not be universally favorable to all patients. The purpose of TDM is to manage the disease of a patient appropriately and not merely obtaining and recording values. A lot of factors can alter effective therapeutic concentrations of different drugs in different patients. These include, but are not limited to, genetic variables, concomitant administration of drugs, age, and pharmacokinetic characteristics of patient. Some patients may achieve maximum benefits of the drug at lower concentrations than the suggested optimal therapeutic ranges, while others may experience adverse effect within the suggested ranges [40]. In the management of seizures with phenytoin, one reference range for all patients may not be applicable [41]. The seizure type, severity of the illness, and genetic abnormalities may affect optimal drug concentration needed by the patient. Therefore, establishing an individualized therapeutic concentration range for each patient is the most appropriate approach in TDM.

21.4.3 Appropriate Clinical Interpretation of TDM Results

TDM is not only concerned with mere measurement of drug concentration in the blood [12] but also making appropriate clinical interpretation of the data. Dosage adjustment normally follows analysis of drug levels. Before dose adjustment is done, expert clinical interpretation has to be made in order to derive meaningful results from the procedure [42]. Most centers, especially in developing countries, have TDM limited to assay only, instead of both assay and clinical interpretation [42]. Ideally, there should be well-established clinical pharmacology and toxicology units in hospitals that will make recommendations based on TDM data. The need to improve understanding of pharmacokinetic principles and related TDM data among health professionals cannot be

overemphasized [43]. Current health professionals should therefore be trained in appropriate clinical interpretation of TDM data obtained from laboratories.

21.4.4 Merging Target Concentration Intervention (TCI) with TDM

One area that can be explored is merging target concentration intervention (TCI) with TDM [21]. TCI is a method that makes use of pharmacokinetic and pharmacodynamic principles to estimate how patients would reach target concentration when given a specific dose of a drug. Some of the parameters examined include clearance (Cl), maximum effect of the drug at high concentrations (E_{max}), and concentration that produces 50% of E_{max} (EC_{50}) [44]. It is ideal to use TCI when the effect of a treatment being administered is difficult to quantify. It is therefore used to maintain drug concentration of a target drug which has been shown to be effective in majority of populations [45].

Another reason for using TCI is when a group-based dosing system (such as dosing based on weight) does not reduce variability between different members [45]. Even though TCI and TDM are different principles that are practiced separately, merging the two may be of more benefit than practicing them separately. Merging these two means TDM is now going to consider pharmacogenomics. Hence, monitoring of serum/plasma drug concentrations would not be aimed at only dosage adjustment but rather reaching individual target concentration [44].

21.4.5 Computer-Assisted TDM

Finally, computed-assisted TDM is another area that can be studied in terms of improving the practice of TDM. Computerized clinical decision support systems may have the potential of improving the benefits of drugs that are normally monitored. During dosage adjustment, some models are used in the calculation of the right dose to be administered. The Bayesian algorithm is considered the gold standard for dosage adjustment calculations. Timing of when samples should be taken is often challenging. The Bayesian model helps to solve this problem by comparing a random concentration to a population average concentration-time curve [46, 47]. Pharmacokinetic models can be applied to predict specific individualized pharmacokinetic profiles in order to make room for inter-patient variability [48]. More research and validation need to be done on the computer-assisted method of TDM as this can lead to significant advancement in patient therapy.

21.5 Conclusion

There are a number of challenges associated with the TDM and clinical toxicology, and these include low sensitivity of some of the assays, difficulties in sampling at the right time, and inability to assay some active metabolites of drug, among others. Despite these challenges, TDM and clinical toxicology play important roles in patient therapy (care). Nonetheless, the practice can be improved by using alternate sampling matrices (like saliva and dried blood spot), individualized therapeutic concentrations, appropriate clinical interpretation of results, and use of computer-assisted TDM.

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